Human Retroplacental Serum Polyamine Oxidase

VOLUME II

Human Retroplacental Serum Polyamine Oxidase

Purification and Characterization

A thesis submitted to
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by

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ABBREVIATIONS

Ø (column) diameter
ΔAB$^+$ human AB$^+$ serum from healthy nonpregnant donors, heated to 56 °C for 30 min
ΔFBS fetal bovine serum, heated to 56 °C for 30 min to inactivate complement proteins
2D-PAGE two-dimensional polyacrylamide gel electrophoresis
aa amino acid
AA arachadonic acid
Ab antibody
ABP amiloride binding protein
ABP_HUKI.s human kidney ABP/DAO sequence [M55602]
ABP_HUMN.s human diamine oxidase sequence [EMBL 78212]
ABP_HUMAN human amiloride-binding protein precursor
ABP_RATC.s rat colon/lung ABP/DAO sequence [X73911]
ABTS 2,2[1]-azino-bis-(3-ethylbenzthiazoline-6-sulphonate)
ALL acute lymphoblastic leukemia
AML acute myoblastic leukemia
AMO_ECOL.s *E. coli* amine oxidase (maoA) sequence [L47571]
AMO_HANS.s *Hansenula polymorpha* AO sequence [X15111]
anh. anhydrous
AO amine oxidase (EC 1.4.3 or 1.5.3)
AO_ANTHR.s *Anthrobactr* methylamine oxidase (maoxII) sequence [L12990]
AO_BOVIN.s bovine serum/liver copper amine oxidase sequence [S69583]
AO_KAERO.s *K. aerogenes* AO sequence [D10208]
AO_LENSC.s lentil seedling AO sequence [X64201]
AO_PISUM.s pea seedling AO sequence [L39931]
APS ammonium persulphate
BAO benzylamine oxidase
BCA bicinchoninic acid (4,4′-dicarboxy-2,2′-biquinoline)
BHK baby hamster kidney
BIS *N,N′*-bis-methylene acrylamide
BPB bromophenol blue
BSA bovine serum albumin
CAPS 3-[cyclohexylamino]-1-propanesulphonic acid
CBB Coomassie Brilliant Blue
CF cystic fibrosis
CHO chinese hamster ovary
CI Colour Index
CK-2 casein kinase II
con A concanavalin A
CTC copper-tartrate-carbonate
DAO diamine oxidase = histaminase (EC 1.4.3.6 although may have EC 1.5.3 activity)
DEAE diethylaminoethyl
DFMO α-difluoromethylornithine
DMPTU dimethylphenylthiourea
DMSO dimethylsulphoxide
DNS dansyl
DOC deoxycholate, sodium
DPM disintergrations per minute
DPTU diphenylthiourea

DPU diphenylurea
DTT dithiothreitol
EBI European Bioinformatics Institute
EC Enzyme Commission (nomenclature recommended by NC-IUBMB *q.v.*)
EIA enzyme immunoassay
ELISA enzyme linked immunoassay
EMBL European Molecular Biology Laboratory
EPR electron paramagnetic (or spin) resonance
FAD flavin–adenine dinucleotide
FBS fetal bovine serum
fMLP *N*-formyl-methionyl-leucylphenylalanine (peptide)
ftp file transfer protocol
GABA γ-aminobutyric acid (γ-aminobutyrate)
G6PD glucose-6-phosphate dehydrogenase
GSH reduced glutathione
HAT hypoxanthine/aminopterin/thymidine
HECS human endothelial cell supernatant
HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid
HES hybridoma enhancing supplement
HGPRT hypoxanthine guanine phosphoribosyl transferase
HIC hydrophobic interaction chromatography
HMP hexose monophosphate
HMT histamine methyltransferase (EC 2.1.1.8)
HPLC high-pressure (-performance) liquid chromatography
HRPO horseradish peroxidase
HT hypoxanthine/thymidine
HVA homovanillic acid
IBM-PC/DOS IBM personal computer/disk operating system
IEF isoelectric focusing
Ig immunoglobulin
IL-2 interleukin-2
IUdR iododeoxyuridine
LPS lipopolysaccharide
LT leukotriene
mAb monoclonal antibody
MAO monoamine oxidase (EC 1.4.3.4 although may have a 1.4.3.6 phenotype)
MAOx methylamine oxidase (EC 1.4.3)
MGBG methylglyoxyl-bis(guanylhydrazone)
MLR mixed lymphocyte (culture) reaction
MS-Windows Microsoft® Windows
NC-IUBNB Nomenclature Committee of the International Union of Biochemistry
NMDA *N*-methyl-D-aspartate
ODC ornithine decarboxylase
ONPG *o*-nitrophenol-β-D-galactopyranoside
OPA *o*-phthalaldehyde
PA polyacrylamide (gel)
PAGE polyacrylamide-gel electrophoresis
PAO polyamine oxidase (EC 1.5.3.11, EC 1.4.3.6, or EC 1.4.3.4)
PBS Dulbecco's phosphate buffered saline without Ca^{2+} or Mg^{2+}
PBS-T PBS containing 0.05% Tween 20

PCR polymerase chain reaction
PCZ procarbazine (*N*-isopropyl-α-(2-methyl hydrazino)-*p*-toluamide hydrochloride)
PDB (Brookhaven) Protein Data Base
PEAO 2-phenylethylamine oxidase (EC 1.4.3.-)
PEG polyethylene glycol
Pg prostaglandin
PHA phytohaemagglutinin
PHYLIP phylogeny inference package
PITC 1,4-phenylenediisothiothiocyanate
Plasma AO plasma amine oxidase = serum amine oxidase = diamine oxidase
PLA_2 phospholipase A_2
PMA phorbol 12-myristate 13-acetate
PMN polymorphonuclear nucleocytes (neutrophils)
PNGase F peptide:N-glycosidase F (EC 3.5.1.52)
POPOP 1,4-di(2-(5-phenoxazolyl))-benzene
PPO 2,5-diphenoxazole
PQQ pyrroloquinoline quinone
PSG penicillin, streptomycin and glutamine medium supplement (*q.v.* §2.8.1.1)
PTH phenylthiohydantoin
PVA polyvinyl alcohol
PVDF polyvinylidene fluoride
RA PBMNC rheumatoid arthritis peripheral blood mononuclear cells
RA SFMNC rheumatoid arthritis synovial fluid mononuclear cells
RPS retroplacental serum
RID radial immunodiffusion
RPMI 1640 Roswell Park Memorial Institute 1640 medium (*q.v.* §2.8.1.1)
SD standard deviation
SDS sodium dodecyl sulphate
SE-HPLC size-exclusion high pressure liquid chromatography
SOD superoxide dismutase
SSAO semicarbazide-sensitive amine oxidase; EC 1.4.3.6?
SWR standard working reagent
TAO tyramine oxidase (EC 1.4.3.4)
[^{3}H]TdR [^{3}H]thymidine deoxyribose
TCA trichloroacetic acid
TEMED *N,N,N′,N′*-tetramethylethylenediamine
t.l.c. thin layer chromatography
TNF tumour necrosis factor
TOPA 2,4,5-trihydroxyphenylalanine
TPQ 2,4,5-trihydroxyphenylalanine quinone
Tris tris(hydroxymethyl)aminomethane
TST Tris-saline-Tween 20
Tx thromboxane

Appendix A. Distribution and Localization of Amine Oxidases

Notes to the Appendix. To be complete and unbiased, some enzymes from a single source have multiple listings and references. nd = Not done or not reported. PAO = polyamine oxidase (EC 1.5.3.11, EC 1.4.3.6, or EC 1.4.3.4), Plasma AO = plasma amine oxidase = diamine oxidase (usually EC 1.4.3.6); DAO = diamine oxidase = histaminase (EC 1.4.3.6 although may have EC 1.5.3.- activity); TAO = Tyramine oxidase (EC 1.4.3.4); AO = amine oxidase (EC 1.4.3.- or EC 1.5.3.-); MAO = monoamine oxidase (EC 1.4.3.4 although may have an EC 1.4.3.6 phenotype), the localization of monoamine oxidases has been reviewed [497,498] and is not considered in detail here; MAOx = methylamine oxidase (EC 1.4.3.-); PEAO = 2-phenylethylamine oxidase (EC 1.4.3.-); BAO = benzylamine oxidase (semicarbazide sensitive amine oxidase = SSAO; EC 1.4.3.6?); PO = putrescine oxidase (EC 1.4.3.10).

Table A.1 Distribution and Localization of Amine Oxidases

Source	*Enzyme*	*Location*	*Reference*
Bacteria			
Anacystis nidulans	PAO	cytoplasm	[510]
Arthrobacter	TAO	cytoplasm	[511]
Arthrobacter P1	MAOx	cytoplasm	[512-516]
Arthrobacter globoformis	PEAO	cytoplasm	[517-519]
	DAO	cytoplasm	[520]
Bacillus ceres	DAO	cytoplasm	[521]
Chromobacterium violaceum	DAO	cytoplasm	[521]
Cornynebacterium sp. 2-4-1	AO	cytoplasm	[522]
Escherichia coli	PAO	cytoplasm	[523]
Escherichia coli K12	AO	cytoplasm	[524-531]
Escherichia coli W3550	MAO(AO)	cytoplasm	[532]
Haemophilus parainfluenzae	PAO	cytoplasm	[521,533]
Micrococcus rubens	PO	cytoplasm	[534-538]
Mycobacterium smegmatis	PAO	cytoplasm	[521,539,540]
Neisseria perflava	PAO	cytoplasm	[541]
Pasteurella tularensis	PAO	cytoplasm	[533]
Phytomonas fascians	DAO	cytoplasm	[521]
Pseudomonas sp.	PAO	cytoplasm	[542]
Pseudomonas aeruginosa	PAO	cytoplasm	[221,521,543]
Pseudomonas pycyanoeae	DAO	cytoplasm	[544,545]
Serratia marcescens	PAO	cytoplasm	[537,546,547]
Sarcina lutea	TAO	cytoplasm	[548]
Fungi and Yeast			
Aspergillus niger	DAO	mycelia	[549-558]
	MAO	mycelia	[557,559]
Aspergillus terrus	PAO	mycelia	[560]
Candida boidinii	MAOx	nd	[561,562]
	BAO	nd	[561,562]
	PAO	peroxisomes	[561,563]
Candida nagoyaensis	PAO	nd	[563]
Candida utilis	MAOx	nd	[564,565]
	BAO	nd	[564,565]
	DAO	nd	[561]
Candida steatolytica	DAO	nd	[561]
Hansenula polymorpha	MAOx	peroxisomes	[566-569]
	PAO	nd	[563]
Kluyveromyces fragalis	BAO	nd	[570]
Lyophyllum aggeratum	PEAO	cytoplasm	[571]

Table A.1 *(Continued)*

Source	*Enzyme*	*Location*	*Reference*
Penicillium chrysogenum	agmatine oxidase	mycelia	[471,572,573]
	PAO	extracellular	[574]
Penicillium sp.	AO	extracellular	[575-577]
Pishia pastoris	MAOx	nd	[562,564]
	BAO	nd	[562,564,578]
	DAO	nd	[561]
Sporopachydermia cereana	PAO	nd	[561,563]
Trichosporon cutaneum X_4	BAO	nd	[579]
Trichosporon melibiosaceum	PAO	nd	[563]
Miscellaneous fungi	PAO	mycelia	[580]
Plants			
Arabidopsis thaliana	AO		[581]
Arachis hypogea (groundnut)	DAO		[582]
Atropa belladona	DAO		[583]
Avena sativa (oats)	PAO	seedlings	[584-586]
Camellia sinensis (tea)	BAO	young leaves	[587]
Canavalia ensiformis (jack bean)	DAO		[588]
Cicer arietinum (chick pea)	DAO	seedlings	[589-591]
Cucumis sativus (cucumber)	DAO	7-day-old seedlings	[592]
Euphorbia characias	DAO	latex	[593]
Eichhornia crassipes (water hyacinth)	PAO	leaves	[594]
Glycine max (soybean)	DAO	seedlings	[585,595-601]
Helianthus tuberousus	DAO	tubers	[602,603]
Hordeum vulgare (barley)	DAO	8-day-old seedlings	[604]
	PAO	seedlings	[584,605-607]
Hyoscyamus niger	DAO	cultured roots	[608,609]
Lathyrus cicera	DAO	8-day-old seedlings	[610]
Lathyrus sativus (chick pea)	DAO	5-day-old seedlings	[590,599,611-613]
Lens culinaris (lentil)	DAO	8-day-old seedlings	[614-617]
Lens esculenta (lentil)	DAO	seedlings	[590,614,618-621]
Lupinus luteus (lupine)	DAO	13-day-old seedlings	[622]
Nicotiana tabacum (tobacco)	DAO	roots	[623-626]
Nicotiana rustica	PO	roots	[627]
Onobrychis viciifolia (sainfoin)	DAO	7-day-old shoots/roots	[628]
Oryza sativa (rice)	PAO	seedlings	[629]
Phaseolus vulgaris (kidney bean)	DAO	8-day-old seedlings	[610]
Pisum sativum (pea)	DAO	seedlings	[185,515,590,599,607,613,630-650]
Secale cereale (rye)	PAO	seedlings	[584]
Setaria italica (millet)	PAO	seedlings	[584,651]
Triticum aestivum (wheat)	PAO	seedlings	[584]
Trifolium subterraneum (clover)	DAO	young leaves	[652]
Vicia faba (fava bean)	DAO	14-day-old leaves	[590,653]
Zea mays (corn/maize)	PAO	seedlings	[654-657]

Table A.1 *(Continued)*

Source	*Enzyme*	*Location*	*Reference*
Mammalian			
	MAO A & B	outer mitochondrial membrane, almost all mammalian tissues	[476,497,498,658]
Human	Plasma AO (DAO)	adult plasma/serum	[488,659-670]
	DAO	lymph	[671]
	BAO	adult plasma/serum	[672]
	DAO	post-heparin serum	[414,673-682]
	DAO	pregnancy plasma/serum	[421,488,505,660,663,664,671,678,683-721]
	PAO (DAO)	pregnancy serum	[169,420,718,719,722-725]
	PAO (DAO)	retroplacental serum	[420,442,726-729]
	DAO	placentae	[47,48,481,487,490,684,686,712,716,721,730-759]
	PAO	placentae (decidua)	[420]
	PAO	placental membranes	[684,760]
	DAO	amniotic fluid	[678,684,686,711,761-765]
	PAO	amniotic fluid	[760]
	PAO	milk	[766]
	(BAO) SSAO	vascular smooth muscle (umbilical artery)	[505,767,768]
	DAO	semen, spermatozoa	[751,769-776]
	PAO	testis	[410]
	DAO	kidney	[480,753,777-781]
	PAO	kidney	[410,725]
	DAO	intestine	[453,779,782-796]
	PAO	intestine	[410,797]
	PAO (DAO)	synovial fluid	[798]
	DAO	neutrophils, eosinophils	[755,799-806]
	PAO	macrophages	[807]
	DAO	macrophages	[807]
	PAO	liver	[410,725]
	DAO	liver	[761,783]
	PAO	spleen	[410]
	DAO	spleen	[783,785]
	DAO	submaxillary gland	[635]
	DAO	platelets	[808,809]
	AO	skin	[810]
	DAO, PAO	fibroblasts, vascular endothelial cells	[397,811-814]
	BAO (SSAO)	neonatal pharyngeal aspirate, amniotic fluid, placenta, umbilical vessels, placental vessels	[815,816]

Table A.1 *(Continued)*

Source	*Enzyme*	*Location*	*Reference*
Bovine	Plasma AO	plasma/serum	[49,214,222,223,411,458, 493,500,515,599,648,673, 718,721,756,817-855]
	PAO	serum	[856]
	DAO	liver	[411,778,857]
	PAO	liver	[858]
	DAO	intestine	[859]
	BAO	dental pulp	[828,860,861]
	BAO	aorta	[862]
	DAO	aorta	[855,863]
	lysyl Oxidase	aorta	[864]
	BAO	retina, optic nerve	[865]
	BAO (SSAO)	lung	[866,867]
	DAO	lung	[478]
	DAO	kidney	[478,635,753,778,857]
	PAO	milk	[766]
	DAO	submaxillary gland	[635]
	DAO	parotid gland	[635]
Deer	Plasma AO	serum	[458,500]
Giraffe	Plasma AO	serum	[458,500]
Porcine	DAO	kidney	[411,467,480,485-487,515,531,599,630,635, 642,730,731,745,753,756, 778,857,868-911]
	Plasma AO (DAO)	plasma	[500,613,648,912-932]
	PAO	liver	[409]
	DAO	liver	[857]
	BAO	aorta smooth muscle	[933]
	DAO	aorta	[934,935]
	BAO (SSAO)	dental pulp	[936]
	DAO	parotid gland	[635]
	DAO	intestine	[859,937]
Camel	Plasma AO	serum	[458,500]
Llama	Plasma AO	serum	[458,500]
Equine	DAO	lung, liver, kidney, intestine	[477,480,938]
	Plasma AO	serum	[222,500,648,721,939]
	BAO	serum	[940]
Elephant	BAO	serum	[500]
Ovine	Plasma AO	serum	[458,500,508,599,613,648 ,941,942]
	PAO	serum	[856]
	DAO	kidney	[480]
	SSAO	vascular smooth muscle	[942]
Goat	Plasma AO	serum	[458,500,721]
Rabbit	Plasma AO	plasma/serum	[599,648,943,944]
	PAO	serum	[432]
	DAO	post heparin plasma/lymph	[945,946]
	SSAO	lung and heart	[857,947-949]
	DAO	liver	[406,857,950-952]
	DAO	spleen	[857]
	DAO	placenta	[686]

Table A.1 *(Continued)*

Source	*Enzyme*	*Location*	*Reference*
Rabbit	DAO	kidney	[480,857,953]
	DAO	intestine	[782,784,857,859,937,954]
	PAO	macrophages (alveolar)	[955]
Rat	BAO (SSAO)	brown adipose tissue	[502,956-959]
	BAO (SSAO)	white adipose tissue	[959,960]
	DAO	postheparin-plasma	[945,946,961-963]
	BAO (SSAO)	liver microsomes, vascular smooth muscle	[505,507,810,964-966]
	DAO	smooth muscle	[967]
	PAO	liver	[164,165,167,176,179,412,486,491,880,881,968-976]
	DAO	liver	[857,967,975,977-982]
	BAO (SSAO)	vascular, heart, lung, testis	[983,984]
	BAO (SSAO)	aorta	[504,983,985-987]
	BAO (SSAO)	bone	[988]
	DAO	heart	[857,979]
	PAO	brain	[969,974]
	PAO	testis, prostate	[969,974]
	PAO	red blood cell	[55]
	AO	skin	[810]
	DAO	pregnancy serum	[690,989]
	DAO	placenta	[48,206,686,982]
	DAO	lung	[857,947,982,990,991]
	PAO	lung	[969,974]
	BAO	lymph, blood, intestinal mucosa, uterus	[945,992,993]
	PAO	pancreas	[969]
	DAO	intestine	[48,193,479,778,784,857,859,967,982,991,994-1002]
	PAO	intestine	[969,974,976]
	PAO	kidney	[412,969,974,976,1003]
	DAO	kidney	[857,979,980,1003]
	DAO	spleen	[48,982]
	PAO	spleen	[969,974]
	PAO	thymus	[969,974]
	DAO	thymus	[48,982,997,998,1004,1005]
Ox	DAO	lung, kidney	[477,480]
Dog	DAO	kidney, intestine, various tissues	[193,478,480,1006]
	DAO	intestine	[782,784,859,937,1007]
	DAO	serum, lymph	[478,662,1008-1010]
	DAO	liver	[193,778]
	BAO	serum	[458,500]
	DAO	submaxillary gland	[635]
Ferret	BAO	serum	[458,500]
Hamster (*Cercopithecus aethiops*)	PAO	ovary (CHO cells)	[1011]

Table A.1 *(Continued)*

Source	*Enzyme*	*Location*	*Reference*
Gerbil	DAO	intestine, thymus, spleen, liver	[48]
Cat	DAO	kidney	[480,778,1012]
	DAO	liver	[778]
	DAO	lymph	[1009,1013]
	DAO	intestine	[859,1006,1012]
Tiger	BAO	serum	[458,500]
Monkey	DAO	kidney	[480]
	DAO	serum	[721]
Guinea-pig	DAO	pregnancy serum	[690,1014]
	DAO	placenta	
	DAO	lung, spleen, kidney, heart	[857]
	DAO	liver	[407,408,733,857,967,1015-1017]
	DAO	serum	[662]
	PAO	serum	[432]
	DAO	post-heparin serum	[414,945,946,1018,1019]
	DAO	intestine	[48,784,857,859,967,1006]
	DAO	smooth muscle, endothelium	[967]
	SSAO	skin (fibroblasts)	[1020]
	DAO	skin	[193]
Mouse	DAO	liver	[857,1021,1022]
	DAO	intestine, kidney,	[857,967,1022]
	DAO	lung, heart, spleen	[857]
	PAO	macrophages (peritoneal)	[955]
	PAO	leukemia cells	[1023]
	PAO	serum	[432]
Rhesus monkey	DAO	serum	[662]
Squirrel monkey	DAO	serum	[662]
Marmoset monkey	Plasma AO	plasma	[704]
Various species inc. cat, dog, monkey, mouse, horse, goat, guinea pig, exotic species	DAO	various tissues, serum	[458,500,850,992,993]
Various species	DAO	post-heparin plasma	[946,1024]
Bird			
Chathartes aura (turkey buzzard)	DAO	liver	[475]
Coturnix coturnix japonica (quail)	DAO	liver microsomes	[965,1025,1026]
Chicken	AO	bone	[1027]
	DAO	intestine, liver, spleen	[778,1028]
	DAO	aorta	[1029]
	Lysyl Oxidase	aorta	[1030]
Fish			
Parasiluris asotus (catfish)	BAO	intestine, liver, etc.	[1031]
	PAO	intestine, liver, kidney	[1032]
Mugil cephalus (mullet)	Plasma AO	serum	[1033]
Salmo gairdneri	BAO	liver microsome	[965]
Teleostean sp.	DAO	intestine	[1034]

Table A.1 *(Continued)*

Source	*Enzyme*	*Location*	*Reference*
Helminths			
Setaria cervi	PAO	nd	[1035]
Ancylostoma ceylanicum	PAO	nd	[1035,1036]
Hippostrongylus brasiliensis	PAO	nd	[1035,1037]
Parasites			[1035,1037]
Acanthamoeba culbertsoni	PAO	nd	
Ascaris suum	PAO	nd	[1038,1039]
Onchocerca volvulus	PAO	nd	[1040]
Dirofilaria immitis	PAO	nd	[1041,1042]
Insects			[1042,1043]
cockroach	DAO	nd	[1044]

Appendix B. Reagents and Materials

B.1 Amine Oxidase Substrates

Table B.1 Amine Oxidase Substrates

Trivial Name	*Chemical Name*	*Source*
putrescine dihydrochloride	1,4-butanediamine.2HCl	Sigma Chemical Co., St. Louis, MO
spermidine trihydrochloride	*N*-[3-aminopropyl]-1,4-butane diamine.3HCl	Sigma Chemical Co., St. Louis, MO; Calbiochem–Novabiochem, San Diego, CA
spermine tetrahydrochloride	*N,N′*-bis[3-aminopropyl]-1,4-butane diamine.4HCl	Sigma Chemical Co., St. Louis, MO; Calbiochem–Novabiochem, San Diego, CA
N^1-Acetylspermine.3HCl		Sigma Chemical Co., St. Louis, MO
N^1-acetylspermidine.2HCl		Sigma Chemical Co., St. Louis, MO
histamine.2HCl		Sigma Chemical Co., St. Louis, MO
[^{14}C]spermine tetrahydrochloride	*N,N′*-bis(3-aminopropyl)-[1,4-^{14}C]tetramethylene-1,4-diamine.4HCl	Amersham, Buckinghamshire, UK
[^{14}C]spermidine trihydrochloride	*N*-(3-aminopropyl)-[1,4-^{14}C]tetramethylene-1,4-diamine.3HCl	Amersham, Buckinghamshire, UK
[^{14}C]putrescine dihydrochloride	[1,4-^{14}C]tetramethylene-diamine.2HCl	Amersham, Buckinghamshire, UK
[^{3}H]-Spermidine trihydrochloride	*N*-3[3-aminopropyl]-1,4-tetramethylene-1,4-diamine.3HCl [^{3}HN-terminal methylenes]	du Pont de Nemours & Co., Boston, MA

B.2 Amine Oxidase Inhibitors

Table B.2 Amine Oxidase Inhibitors

Generic Name	*Chemical Name*	*Source*
Quinacrine	6-chloro-9-[(4-diethylamino)-1-methylbutyl]amino-2-methoxy-acridine. dihydrochloride	Sigma Chemical Co., St Louis, MO
Isoniazid	isonicotinic acid hydrazide	Sigma Chemical Co., St Louis, MO
Pargyline	*N*-methyl-*N*-2-propynyl-benzylamine.hydrochloride	Sigma Chemical Co., St Louis, MO
	semicarbazide hydrochloride	Sigma Chemical Co., St Louis, MO
	aminoguanidine bicarbonate	Sigma Chemical Co., St Louis, MO
Clorgyline	*N*-methyl-*N*-propargyl-3(2,4-dichlorophenoxy)propylamine hydrochloride	Sigma Chemical Co., St Louis, MO
MGBG	methylglyoxal-bis-(guanyl-hydrazone) dihydrochloride	Sigma Chemical Co., St Louis, MO

B.3 General Reagents and Materials

Table B.3 General Reagents and Materials

Reagent/Material	*Grade*	*Source*
acrylamide	electrophoresis	Bio-Rad, Richmond, CA
acrylamide	Ultrapure™	Boehringer–Mannheim, Mannheim, Germany
aminopterin		Sigma Chemical Co, St Louis, MO
ammonium sulphate	HPLC	Bio-Rad, Richmond, CA
AP25 prefilters		Millipore, Bedford, MA
AP15 prefilters		Millipore, Bedford, MA
AP10 support pad		Millipore, Bedford, MA
2,2[1]-azino-bis(3-ethylbenzthiazoline 6-sulfonate)		Boehringer–Mannheim, Mannheim, Germany
bicinchoninic acid, disodium salt	high purity, for protein determination	Pierce, Rockford, IL
N,N'-bis-methylene acrylamide (BIS)	electrophoresis	Bio-Rad, Richmond, CA
bovine serum albumin (BSA)		Commonwealth Serum Laboratories, Melbourne, Australia
catalase (bovine liver EC 1.11.1.6)		Sigma Chemical Co., St Louis, MO
Centricon®-30 concentrators		Amicon, Danvers, MA
Centriprep®-30 concentrators		Amicon, Danvers, MA
dansyl chloride	biochemistry grade; >98% by argentometry, self fluorescence < 1 ppb Chinin, m.p. 69–71 °C	Merck, Darmstadt, Germany
o-dianisidine		Sigma Chemical Co, St Louis, MO
1,4-di(2-(5-phenoxazolyl))-benzene (POPOP)		Koch–Light Laboratories, Suffolk, UK
2,5-diphenoxazole (PPO)		Koch–Light Laboratories, Suffolk, UK
dithiothreitol (DTT)	high purity	Calbiochem, San Diego, CA
Durapore® 0.45 µm PVDF membrane		Millipore, Bedford, MA
Earle's medium	cell culture	Flow Laboratories, Irvine, Scotland, UK
fetal bovine serum (FBS)	cell culture	Flow Laboratories, Irvine, Scotland, UK; Cytosystems, Castle Hill, Australia
Folin–Ciocalteu phenol reagent, 2.0 *N*		Sigma Chemical Co, St Louis, MO
Freund's complete adjuvant		Difco, Detroit, MI
Freund's incomplete adjuvant		Sigma Chemical Co, St Louis, MO
gelatine	(EIA [enzyme immuno-assay)	Bio-Rad, Richmond, CA
gelatine	Bacto™ certified for tissue culture	Difco, Detroit, MI
D-[6-^{14}C]glucose		Amersham, Buckinghamshire, UK
L-glutamine	cell culture	Flow Laboratories, Irvine, Scotland, UK
glycine	electrophoresis	Bio-Rad, Richmond, CA
HEPES		Flow Laboratories, Irvine, Scotland, UK
Hypaque–Ficoll		Flow Laboratories, Irvine, Scotland, UK
high and low molecular weight standards, SDS - electrophoresis		Bio-Rad, Richmond, CA

Table B.3 *(Continued)*

Reagent/Material	*Grade*	*Source*
homovanillic acid		Sigma Chemical Co, St Louis, MO
horseradish peroxidase (donor: H_2O_2–oxidoreductase; HRPO; EC 1.11.1.7; Type II)		Sigma Chemical Co, St Louis, MO
hypoxanthine		Sigma Chemical Co, St Louis, MO
IgG lyophilised bovine γ-globulin	Bio-Rad protein standard I	Bio-Rad, Richmond, CA
Immulon® IV microtitre plates	ELISA	Dynatech, Chantilly, VA
Linbro® microtitre plates	cell culture	Flow Laboratories, Irvine, Scotland, UK
medium 199	cell culture	Flow Laboratories, Irvine, Scotland, UK
1.2 µm, 0.8 µm, 0.22 µm membrane filters		Sartørius, Gottingen, Germany
2-mercaptoethanol	electrophoresis	Bio-Rad, Richmond, CA
MicroFLUOR® "W" microtitre plates	fluorometric assay	Dynatech, Chantilly, VA
Millex® filter units, 0.45 µm, 0.2 µm		Millipore, Bedford, MA
Millex® FG_{50} 0.2 µm filter units		Millipore, Bedford, MA
Millipak® 40/60 membrane filters		Millipore, Bedford, MA
Millipore HV 0.22 µm Durapore™ membranes		Millipore, Bedford, MA
o-nitrophenol-β-D-galactopyranoside		Sigma Chemical Co, St Louis, MO
PEG 4000 fusogen		from BDH was a gift from Dr D. Brooks, Department of Chemical Pathology, Women's & Children's Hospital, Adelaide, Australia
penicillin	cell culture	Flow Laboratories, Irvine, Scotland, UK
polypropylene scintillation vials		Packard Instrument Co., Meriden, CT
o-phthaladehyde		Sigma Chemical Co, St Louis, MO
OptiPhase 'HiSafe' 3 scintillation fluid		LKB–Wallac, Sweden
potassium sulphate		Sigma Chemical Co, St Louis, MO
L-proline	as free Hydroxy-L-Proline	Sigma Chemical Co., St Louis, MO
Rainbow™ Protein Markers		Amersham, Buckinghamshire, UK
Ready-Solv® EP scintillation fluid		Beckman Instruments, Fullerton, CA
Reversed phase test mixture RP-mix D	HPLC	Alltech Association, Deerfield, IL
riboflavin-5′-phosphate		Sigma, St. Louis, MO
Roswell Park Memorial Institute 1640 medium (RPMI 1640)	cell culture	Flow Laboratories, Irvine, Scotland, UK; Cytosystems, Castle Hill, Australia
Sep-Pak® C_{18} cartridges		Waters Associates, Milford, MA
Silver Stain™ and Silver Stain Plus™ kits		Bio-Rad, Richmond, CA
sodium deoxycholate	high purity	Aldrich, Milwaukee, WI
sodium dodecyl sulphate (SDS)	electrophoresis	Bio-Rad, Richmond, CA
sodium dodecyl sulphate (SDS)	Special quality for protein chemistry [C_{12} > 99.5% GC;protease free]	Boehringer, Mannheim, Germany
sterile saline		Travanol, Australia
streptomycin	tissue culture	Flow Laboratories, Irvine, Scotland, UK

Table B.3 *(Continued)*

Reagent/Material	*Grade*	*Source*
N,N,N′,N′-tetramethylethylenediamine (TEMED)	electrophoresis	Bio-Rad, Richmond, CA
[*methyl*-^{3}H]thymidine		Amersham, Buckinghamshire, UK
Tissue cultureware		Nunc, Roskilde, Denmark; Corning, Corning, NY
Tris base	reagent grade	Sigma Chemical Co, St Louis, MO
Tris–HCl	reagent grade	Sigma Chemical Co, St Louis, MO
High purity Tris base	AnalaR™	BDH, Poole, UK
High purity Tris base	Ultrapure™	Boehringer–Mannheim, Mannheim, Germany
Triton X-100®	gas chromatography	Merck, Darmstadt, Germany
thymidine		Sigma Chemical Co, St Louis, MO
Trypan Blue		Sigma Chemical Co, St Louis, MO
YM30 membranes		Amicon, Danvers, MA

Other reagents were either high purity, analytical reagent, spectroscopic or HPLC grade where appropriate, from commercial sources including Ajax Chemicals, Auburn, Australia; BDH Chemicals, Poole, UK and Merck, Darmstadt, Germany. Dyes and indicators were of certified quality. Other reagents included:

ammonium persulphate
ammonium chloride
arsenious oxide
Brij 35®
borax (sodium tetraborohydrate)
bromophenol blue
calcium chloride
ceric(IV) sulphate
citric acid
Coatasil®
copper sulphate pentahydrate
diethyl ether
dimethyl sulfoxide
disodium hydrogen ortho-phosphate
ferroin indicator
glycerol
hydrogen peroxide
hydrochloric acid
magnesium chloride
magnesium sulphate
methanol
methyl red
octane-1-sulphonic acid
osmium tetroxide
oxalic acid
phenolphthalein
phenol red
potassium chloride
potassium dihydrogen ortho-phosphate
potassium iodide
potassium permanganate
sodium bicarbonate
sodium carbonate (anhydrous)
sodium chloride
sodium dihydrogen ortho-phosphate
sodium hydroxide
sodium (+) tartrate
sucrose
sulfuric acid
trichloroacetic acid
toluene
Tween 20®

B.4 Chromatographic Materials

Table B.4 Chromatographic Materials

Media	*Source*
Sephadex® G-50	Pharmacia, Uppsala, Sweden
Blue Sepharose® Cl-6B	Pharmacia, Uppsala, Sweden
Sephadex® G-25F	Pharmacia, Uppsala, Sweden
Sephacryl® S-200	Pharmacia, Uppsala, Sweden
Sephacryl® S-300	Pharmacia, Uppsala, Sweden
DEAE-Sephacel®	Pharmacia, Uppsala, Sweden
DEAE-Trisacryl®	Reactifs IBF, Villeneuve-la-Garenne, France
Fractogel® TSK HW-55 S	Merck, Darmstadt, Germany
Affi-Gel® 10	Bio-Rad Laboratories, Richmond, CA
Affi-Prep® 10	Bio-Rad Laboratories, Richmond, CA
Bio-Sil® TSK-400 (4000SW)	Bio-Rad Laboratories, Richmond, CA
Bio-Sil® TSK-250 (3000SW)	Bio-Rad Laboratories, Richmond, CA
BioSil® SEC 400	Bio-Rad Laboratories, Richmond, CA
AG-1-X8	Bio-Rad Laboratories, Richmond, CA
Chelex® 100	Bio-Rad Laboratories, Richmond, CA
Dowex™ 50W X 2 (200–400 mesh)	Bio-Rad Laboratories, Richmond, CA
Ultrasphere® ODS reversed phase C_{18} HPLC column 5 µm packing	Beckman, San Ramon, CA
Spherisorb® ODS-2	Phase Separations, Deeside, UK
butyl-agarose	Sigma Chemical Co., St Louis, MO
pentyl-agarose	Sigma Chemical Co., St Louis, MO
ω-aminobutyl-agarose	Sigma Chemical Co., St Louis, MO
ω-aminohexyl-agarose (attached through amino groups to 4% beaded agarose)	Sigma Chemical Co., St Louis, MO
homologous ω-aminoalkyl-agarose (DAA) and alkyl-agarose (MAA) series chromatography test kits	Sigma Chemical Co., St Louis, MO

B.5 Commercial Immunoglobulins, Antibodies and Antibody Conjugates

Table B.5 Commercial Immunoglobulins, Antibodies and Antibody Conjugates

Reagent	*Source*
sheep anti-mouse IgG	Silenus, Hawthorn, Australia
bovine γ-globulin	Silenus, Hawthorn, Australia
affinity purified sheep anti-mouse immunoglobulin	Silenus, Hawthorn, Australia
affinity purified sheep anti-rabbit.horseradish peroxidase conjugate	Silenus, Hawthorn, Australia
affinity purified sheep anti-mouse.horseradish peroxidase conjugate	Silenus, Hawthorn, Australia
affinity purified donkey anti-sheep IgG	Silenus, Hawthorn, Australia
sheep IgG	Silenus, Hawthorn, Australia
affinity purified donkey anti-sheep.horseradish peroxidase conjugate	Silenus, Hawthorn, Australia

Table B.5 *(Continued)*

Reagent	*Source*
affinity purified sheep anti-bovine immunoglobulin	Silenus, Hawthorn, Australia
affinity purified sheep anti-bovine.horseradish peroxidase conjugate	Silenus, Hawthorn, Australia
bovine IgG	Silenus, Hawthorn, Australia
Species specific affinity purified goat anti-mouse.β-galactosidase conjugate	Amersham, Buckinghamshire, UK
rabbit anti-mouse isotyping subclass specific antibodies (Typerpanel™)	Bio-Rad, Richmond, CA
Mouse myeloma IgG_1	Meloy, Springfield, VA

B.6 Reagent Grade Water

Reagent water was purified using a Milli-Q® polishing system (Millipore-Waters, Bedford, MA) fitted with a Milligaurd® microporous filtration cartridge, Super-C® carbon cartridge, Ion-Ex® ion exchange cartridge, Organex-Q® organics cartridge and a Pyrogard UF® ultra-filtration cartridge in sequence producing ultrapure pyrogen free water. Water used to prepare reagents for cell culture was sterile and pyrogen free (Travenol, Australia or Baxter, UK).

B.7 Laboratory animals

Specific pathogen free female BALB/c mice (*Mus musculus domesticus/musculus*), 6–8 weeks old and C3H/He mice were obtained from the University of Adelaide central animal house. The animals were housed at the Women's & Children's Hospital animal house, Adelaide, Australia under stringent hygienic conditions. Sterilised cages and autoclaved bedding were used and the mice maintained on autoclaved commer-cially prepared mouse pellets and sterile water *ad libertum* with a 14 h light/10 h dark cycle.

Procedures with animals were carried out in accordance with the 'Code of Practice for the Care and Use of Animals for Scientific Purposes', NH&MRC/CSIRO/AAC guidelines and the prevention of Cruelty to Animals Act 1985 with procedures recommended by, and with approval of, the ethics committees of the Women's Children's Hospital and The University of Adelaide.

Appendix C. Steady-State Kinetics Data

Introduction

Data from untransformed sets of experimental initial rate velocity data at varying substrate concentrations are displayed in plots overlaid with theoretical Michaelis-Menton curves using Michaelis parameters (*q.v.* 6.2.4) calculated by fitting the data to the Michaelis-Menton equation, modified to account for the observed substrate inhibition as described in section 2.3.2.4. Initial rate data were determined as described in sections 2.3.1.5 and 2.3.2. The theoretical maximum rate (V_{max}) is illustrated as a dashed line parallel to the abscissa.

The data are also displayed as Lineweaver-Burk transformations (insets) for illustrative purposes and are overlaid with a transformation of the theoretical curve. For reasons mainly related to non-standard error distribution, as described in references to sections 6.1 and 7.2.3, these double reciprocal plots were not used to analyse the data directly.

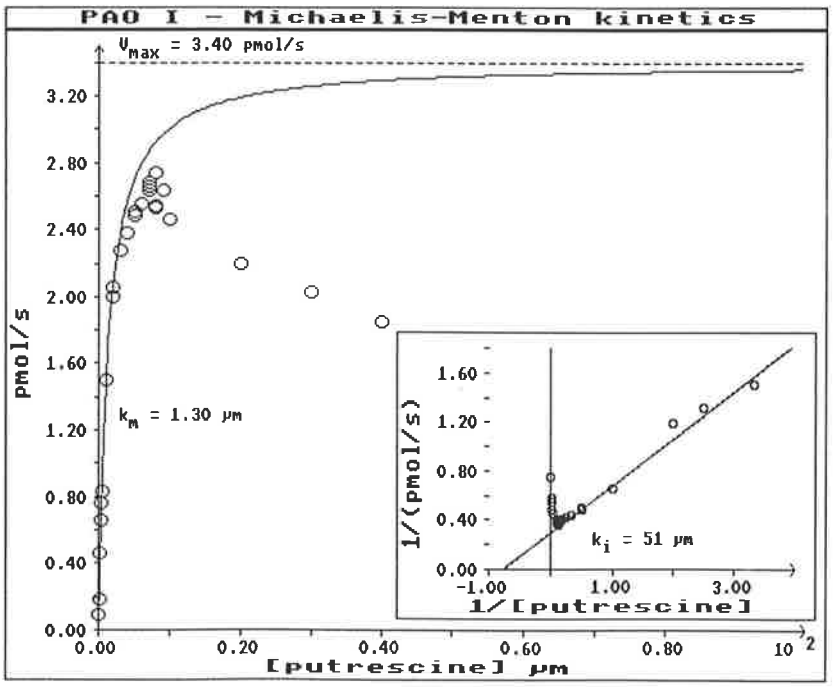


Figure C.1. Steady-State Kinetics of PAO I with Putrescine

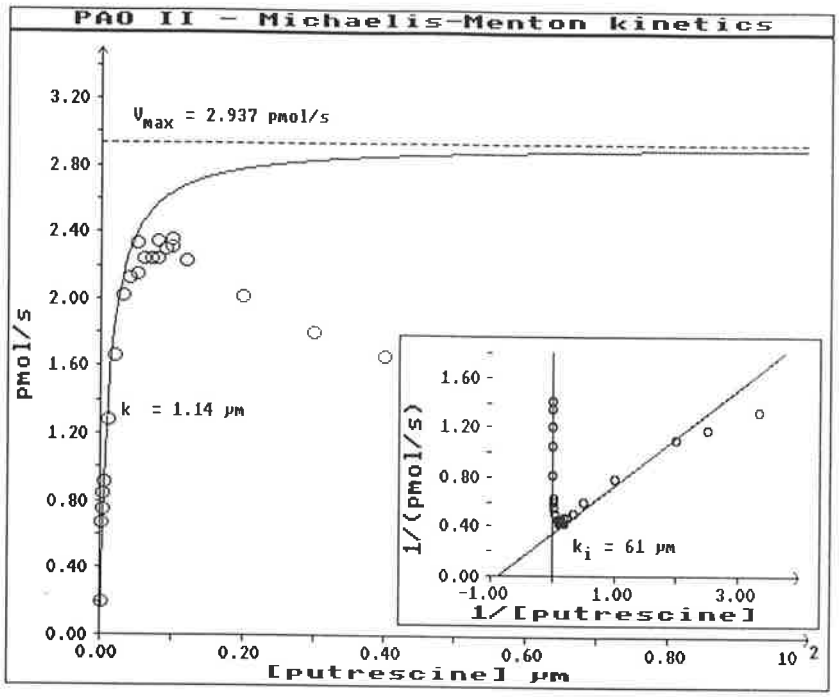


Figure C.2. Steady-State Kinetics of PAO II with Putrescine

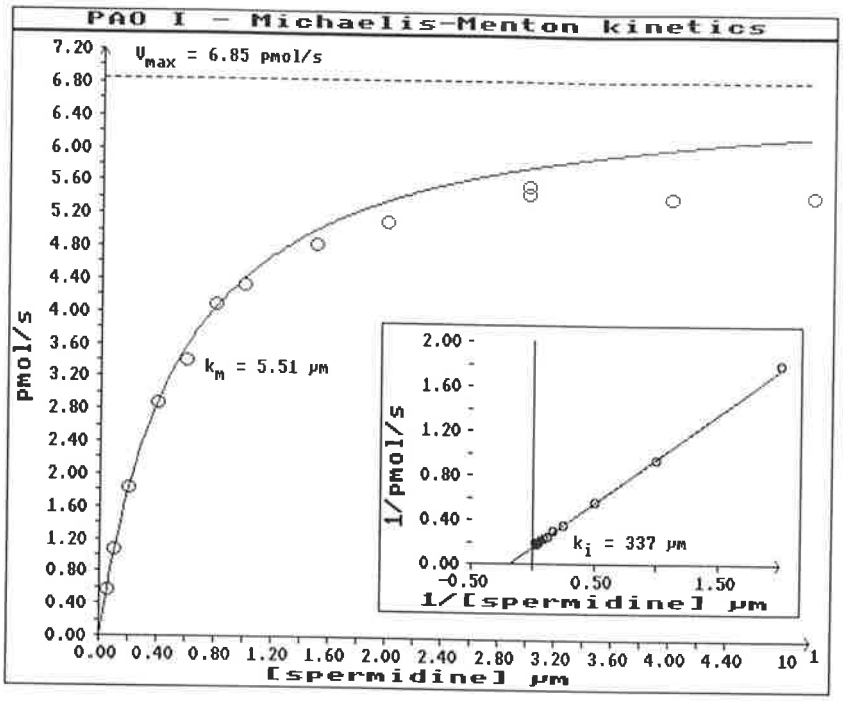


Figure C.3. Steady-State Kinetics of PAO I with Spermidine

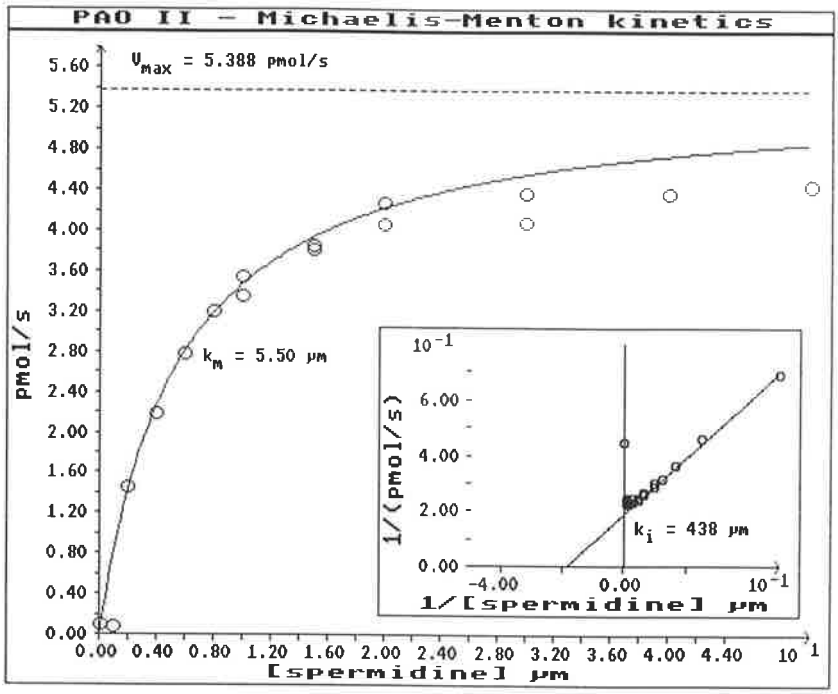


Figure C.4. Steady-State Kinetics of PAO II with Spermidine

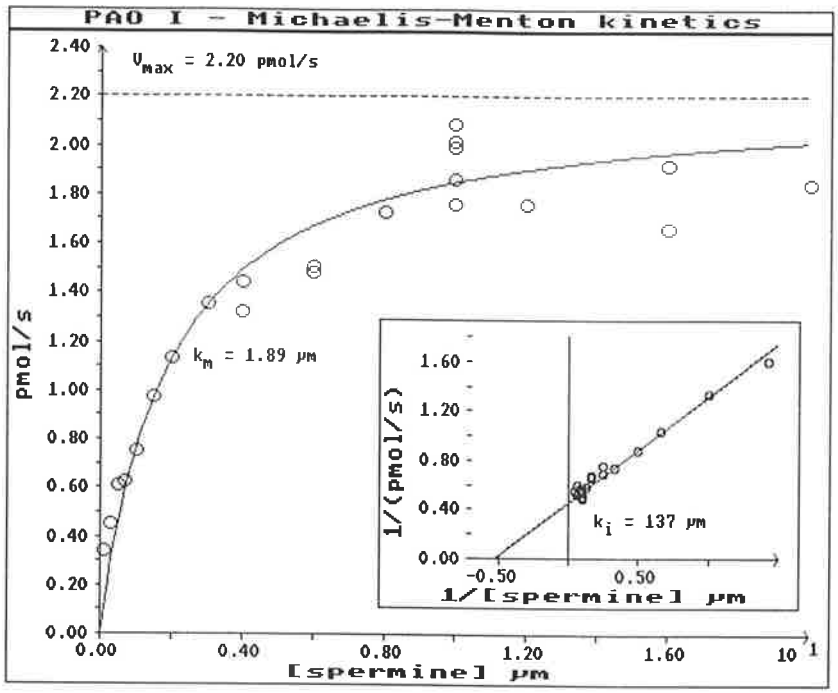


Figure C.5. Steady-State Kinetics of PAO I with Spermine

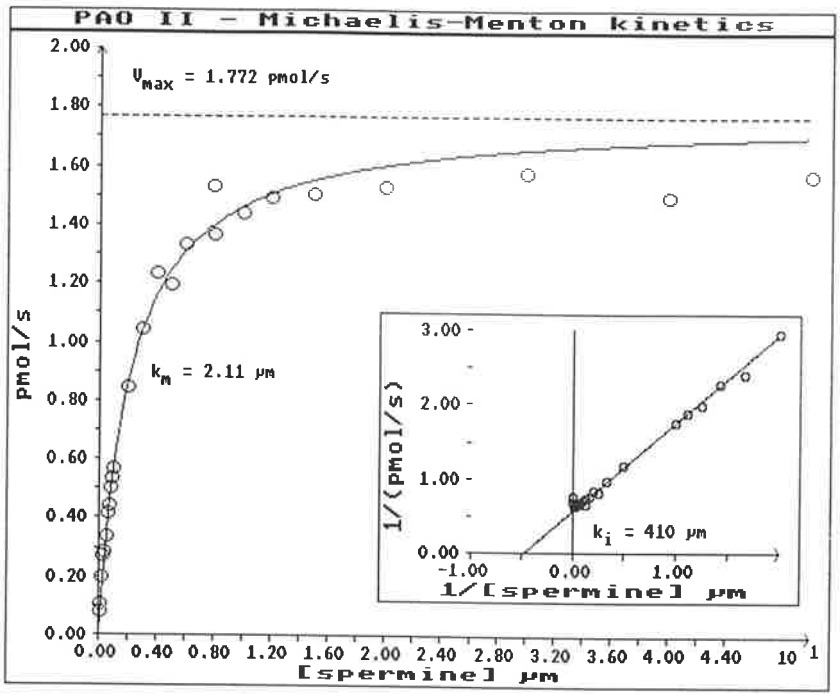


Figure C.6. Steady-State Kinetics of PAO II with Spermine

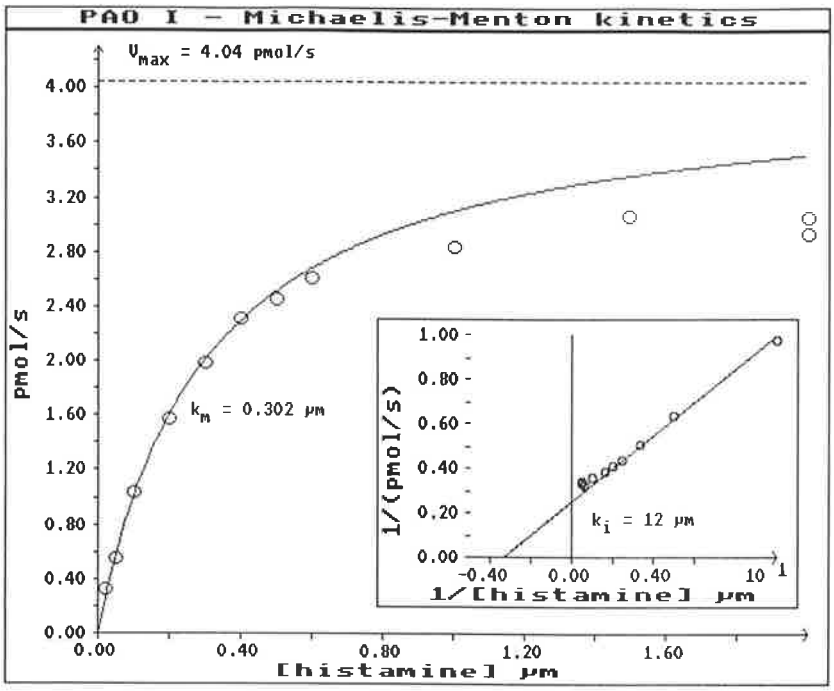
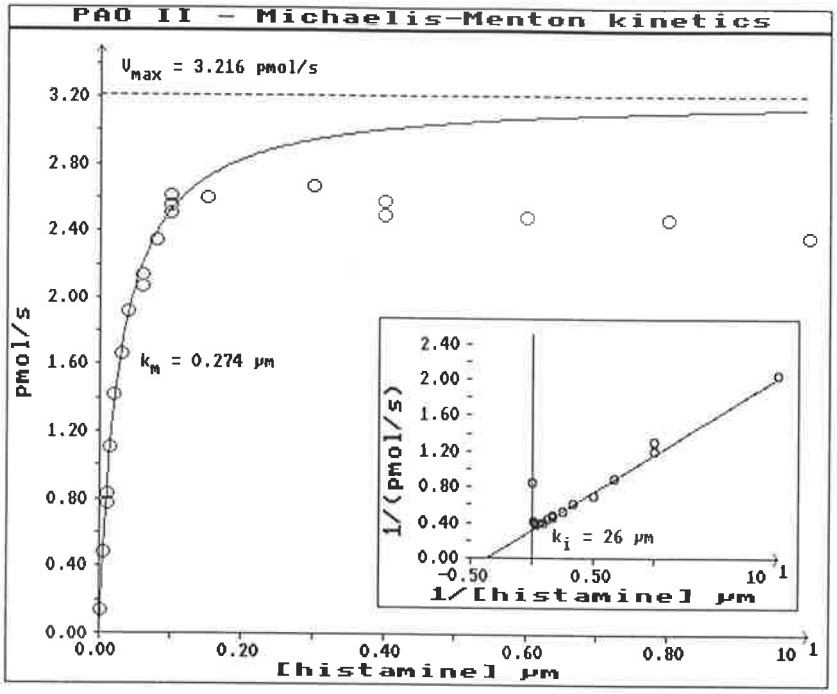


Figure C.7. Steady-State Kinetics of PAO I with Histamine



C.8. Steady-State Kinetics of PAO II with Histamine

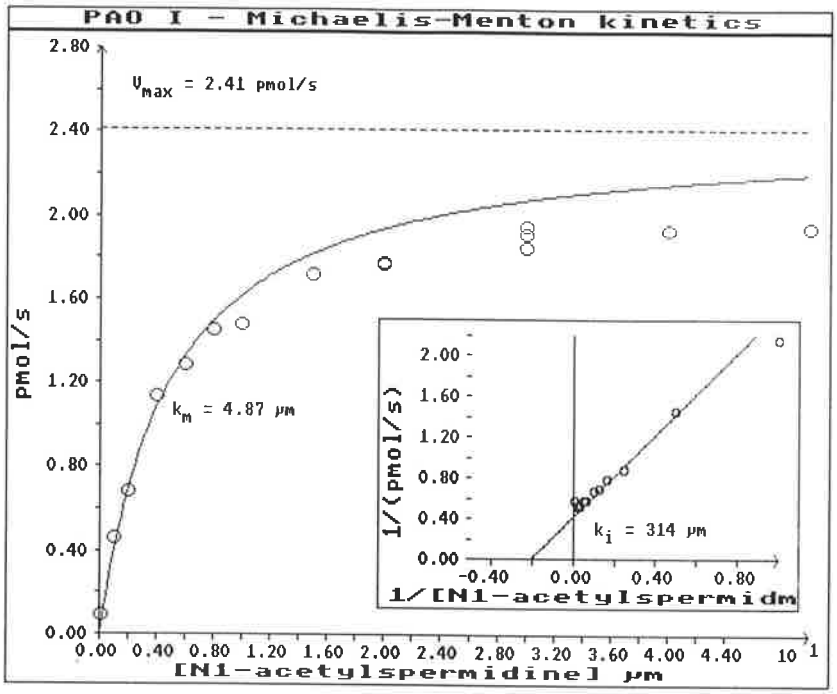


Figure C.9. Steady-State Kinetics of PAO I with N^1-Acetylspermidine

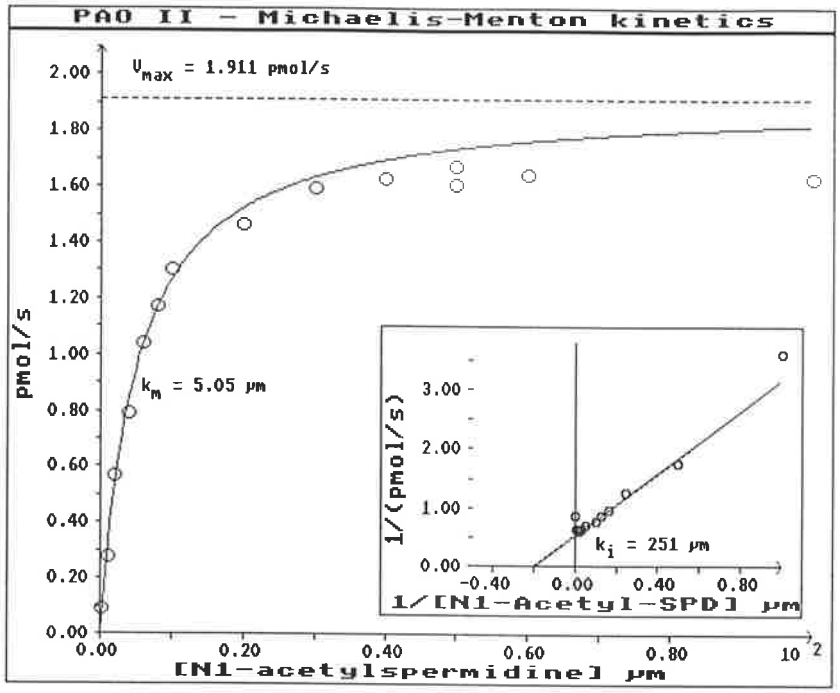


Figure C.10. Steady-State Kinetics of PAO II with N^1-Acetylspermidine

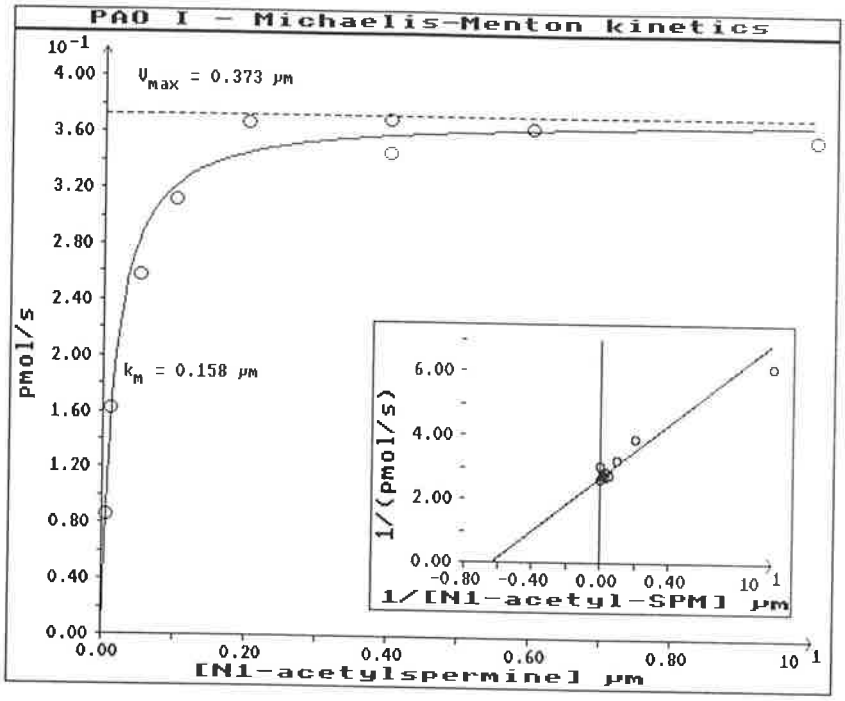


Figure C.11. Steady-State Kinetics of PAO I with N^1-Acetylspermine

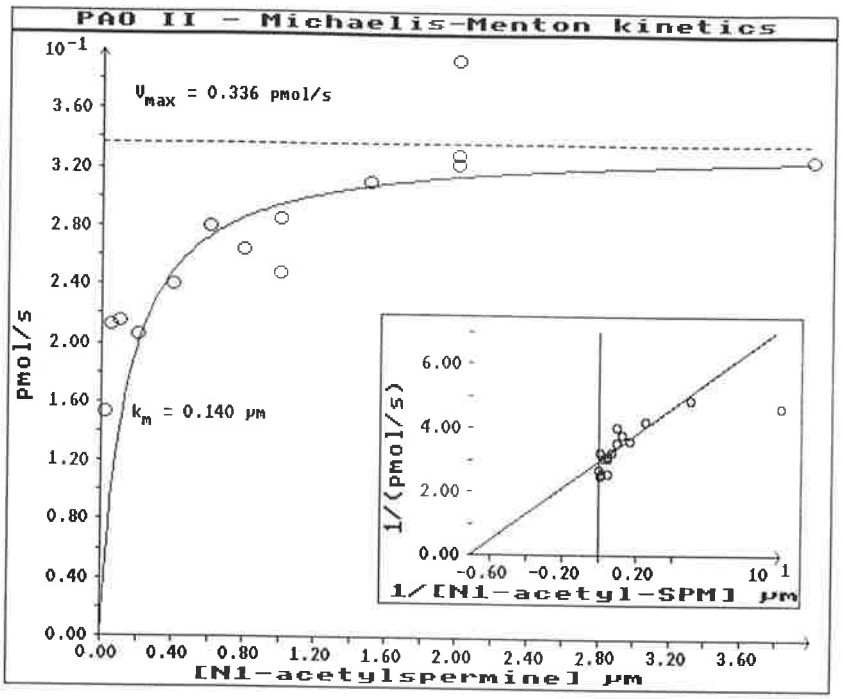


Figure C.12. Steady-State Kinetics of PAO II with N^1-Acetylspermine

Appendix D. Protein Sequencing Data

D.1 PAO I *Ref: PSE 265* *Date: 7/4/93*

Table D.1 ***N*-Terminal Amino Acid Sequence of PSE 265** [a]

aa no.	*1° Signal*	*2°*	*3°*	*4°*	*5°*	*6°*	*7°*	*8°*	*9°*
1	Glu				Arg				
2	Pro				Gly			Leu?	
3	Ser				Arg			Tyr/Asp/Phe	
4	Pro				Leu			Ala/Val	
5	Gly				Leu/Phe			Tyr/Val	
6	Thr				Tyr				
7	Leu				Phe			(Glu)	
8	Pro					Asp/Asn	Glu/Ala	Arg/Met/Val	
9	Arg								
10	-				Gly				
11	Ala				Asp				
12	Gly				Leu			Glu	
13	Val								
14	Phe				Arg				
15	Ser								
16	Asp								

Notes to the Tables

a. Interpretation of the chromatograms

The amino acid residue number is indicated in the first column on the left. The next column (headed '1° Signal') shows the amino acid responsible for the primary PTH(phenylthiohydantoin)–amino acid signal, amino acids from which other signals are derived are indicated across the table from left to right in decreasing order of PTH-amino acid signal strength.

The raw data from a sequencing run is a series of chromatograms of PTH-amino acids. PTH-Amino acids are identified by their retention times. The chromatogram also has peaks from the injection, and the derivatives diphenylthiourea (DPTU), dimethylphenylthiourea (DMPTU) and diphenylurea (DPU) formed by PTH-derivatization reactions involving 1,4-phenylenediisothiothiocyanate (PITC). These derivatives are useful for aligning successive chromatograms. Ideally there is one, large signal from an easily identified amino acid adduct. In impure samples and late in a run from even a pure sample, there are many 'amino acid' peaks. To identify the amino acid produced in a sequencing cycle, successive chromatograms are compared to identify which amino acid(s) increase in a cycle.

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

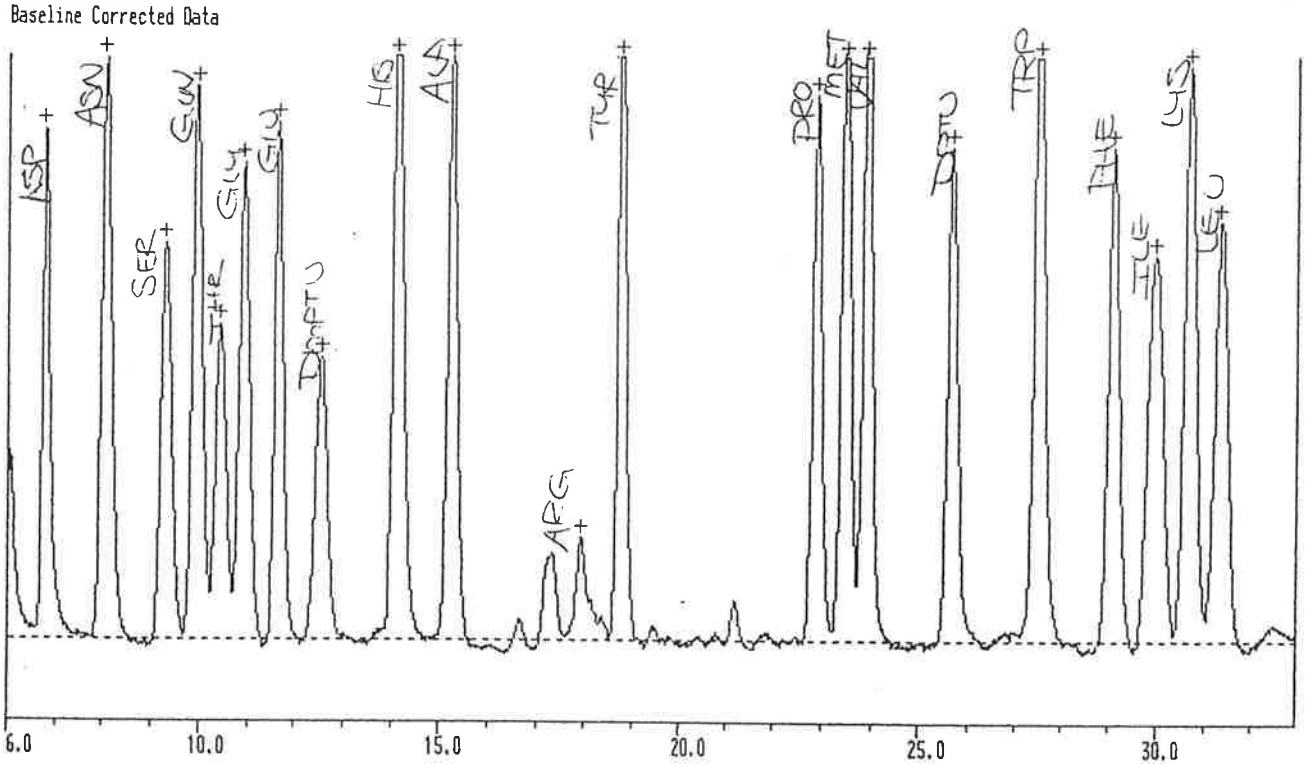
SAMPLE : JAMES STORER POA PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : BGN470-1
Conversion cycle : BGN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

CALIBRATION # 1 [7 Apr 1993 10:57am] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.07		6777			18.37		883	
ASP	6.80	6.80	18213	25.00	TYR	18.77	18.77	25932	25.00
ASN	8.07	8.07	20832	25.00		19.47		516	
SER	9.32	9.32	14184	25.00		21.20		1442	
GLN	9.97	9.97	19845	25.00	PRO	22.90	22.90	19584	25.00
THR	10.47	10.47	11318	25.00	MET	23.47	23.47	25862	25.00
GLY	10.93	10.93	17076	25.00	VAL	23.92	23.92	25106	25.00
GLU	11.67	11.67	18520	25.00	DPT	25.67	25.67	17714	25.00
DMP	12.57	12.57	10142	25.00	TRP	27.52	27.52	25320	25.00
HIS	14.13	14.13	24388	25.00	PHE	29.10	29.10	17704	25.00
ALA	15.25	15.25	21566	25.00	ILE	29.98	29.98	13814	25.00
	16.67		751		LYS	30.70	30.70	20623	25.00
	17.33		3122		LEU	31.35	31.35	15045	25.00
ARG	17.92	17.92	3710	25.00		32.45		578	
	18.15		1432			32.60		511	

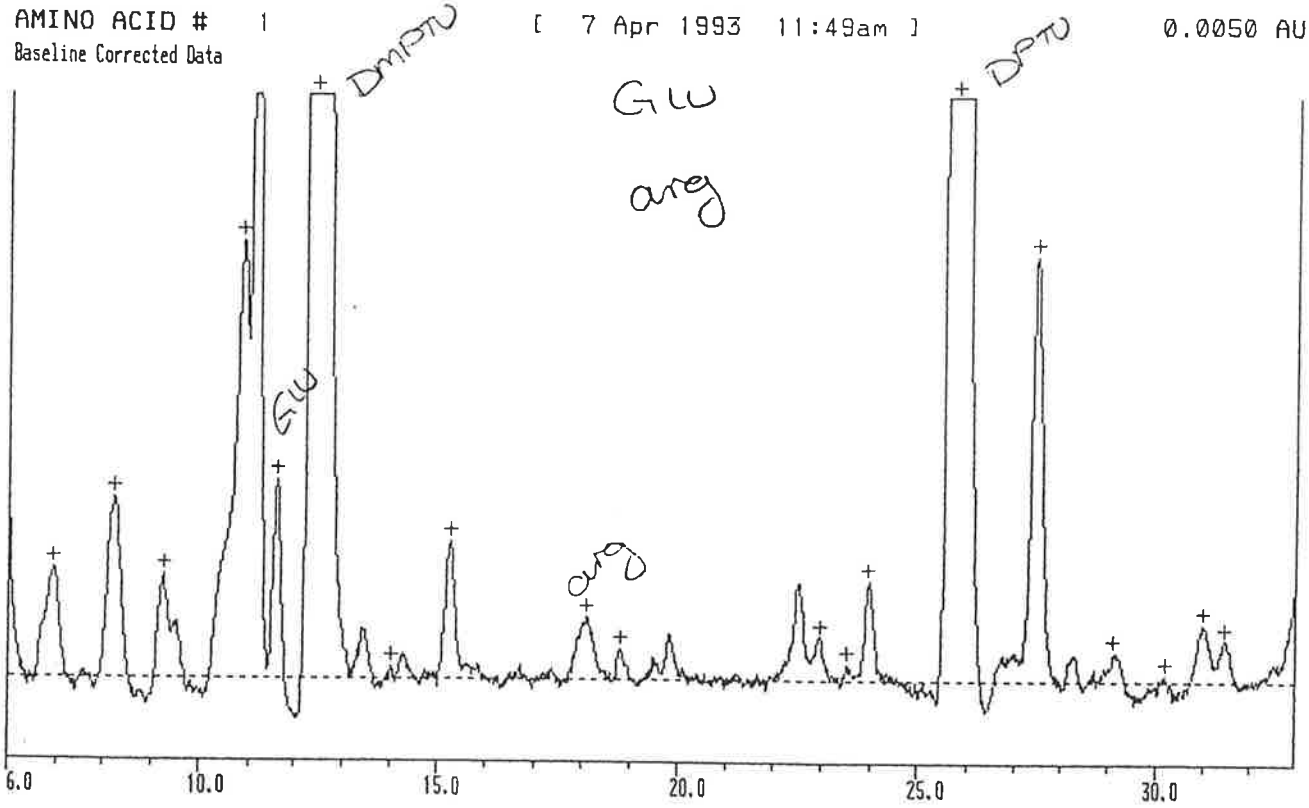
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 1 [7 Apr 1993 11:49am] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.00		3494			22.53		1788	
ASP	6.97	6.80	1994	2.74	PRO	22.98	22.90	770	0.98
ASN	8.22	8.07	3271	3.93	MET	23.53	23.47	283	0.27
SER	9.25	9.32	1874	3.30	VAL	23.97	23.92	1816	1.81
	9.52		1039		DPT	25.75	25.67	225127	317.72
GLY	10.88	10.93	7860	11.51		27.00		532	
	11.13		17961		TRP	27.43	27.52	7627	7.53
GLU	11.63	11.67	3583	4.84	PHE	29.12	29.10	530	0.75
DMP	12.43	12.57	183000	451.08	ILE	30.18	29.98	105	0.19
	13.47		890		LYS	31.00	30.70	1039	1.26
HIS	14.03	14.13	223	0.23	LEU	31.47	31.35	727	1.21
ALA	15.25	15.25	2498	2.90					
ARG	18.10	17.92	1132	7.63					
TYR	18.80	18.77	535	0.52					
	19.82		830						

Tabulation threshold : 500 uAU

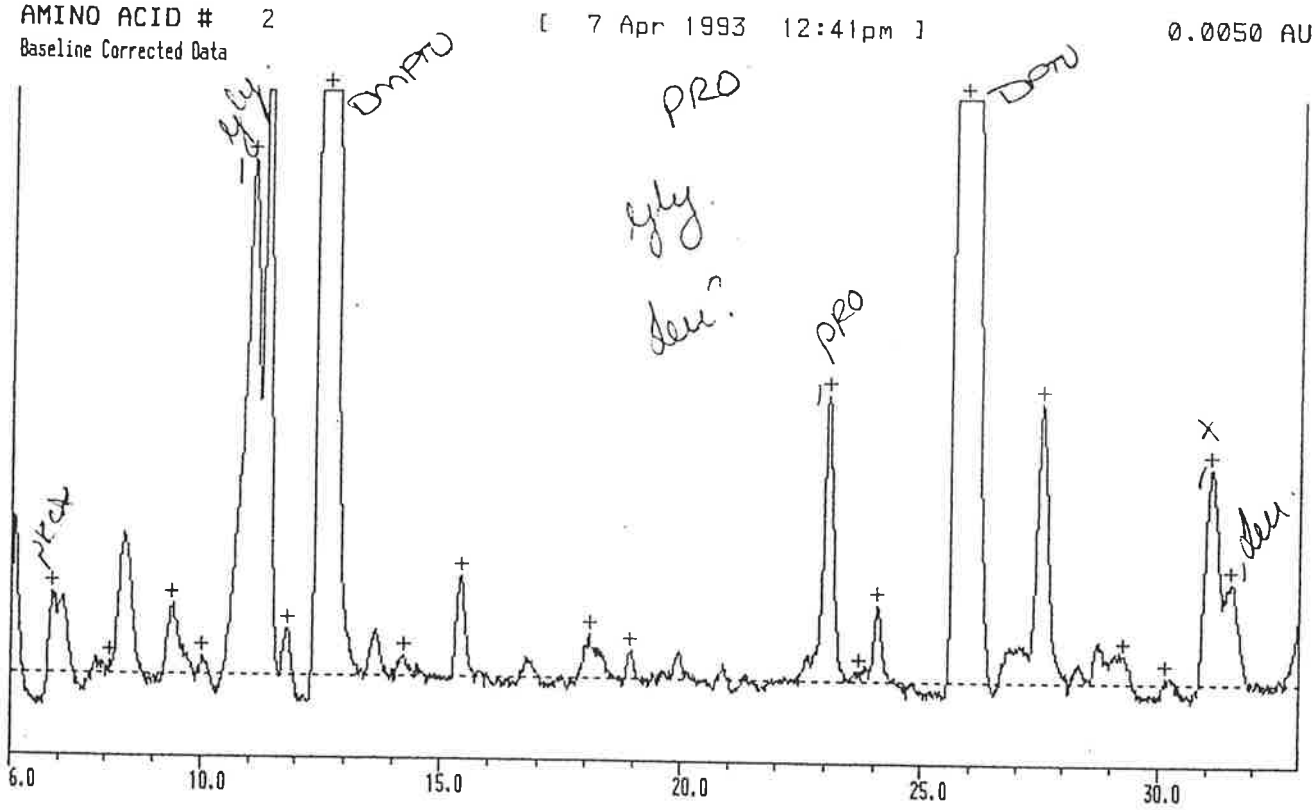
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 2 [7 Apr 1993 12:41pm] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		2784		ARG	18.07	17.92	796	5.37
ASP	6.88	6.80	1459	2.00		18.27		535	
	7.10		1401		TYR	18.95	18.77	513	0.50
ASN	8.10	8.07	240	0.29		19.97		523	
	8.40		2558		PRO	23.05	22.90	5150	6.57
SER	9.42	9.32	1291	2.28	MET	23.68	23.47	189	0.18
GLN	10.03	9.97	324	0.41	VAL	24.08	23.92	1392	1.39
GLY	11.05	10.93	9268	13.57	DPT	25.83	25.67	322898	455.70
	11.35		13677			26.85		679	
GLU	11.83	11.67	859	1.16		26.98		648	
DMP	12.58	12.57	75501	186.10		27.13		698	
	13.63		847		TRP	27.53	27.52	5049	4.99
HIS	14.23	14.13	384	0.39		28.73		756	
ALA	15.42	15.25	1807	2.09		29.08		537	
	17.98		607		PHE	29.28	29.10	520	0.74

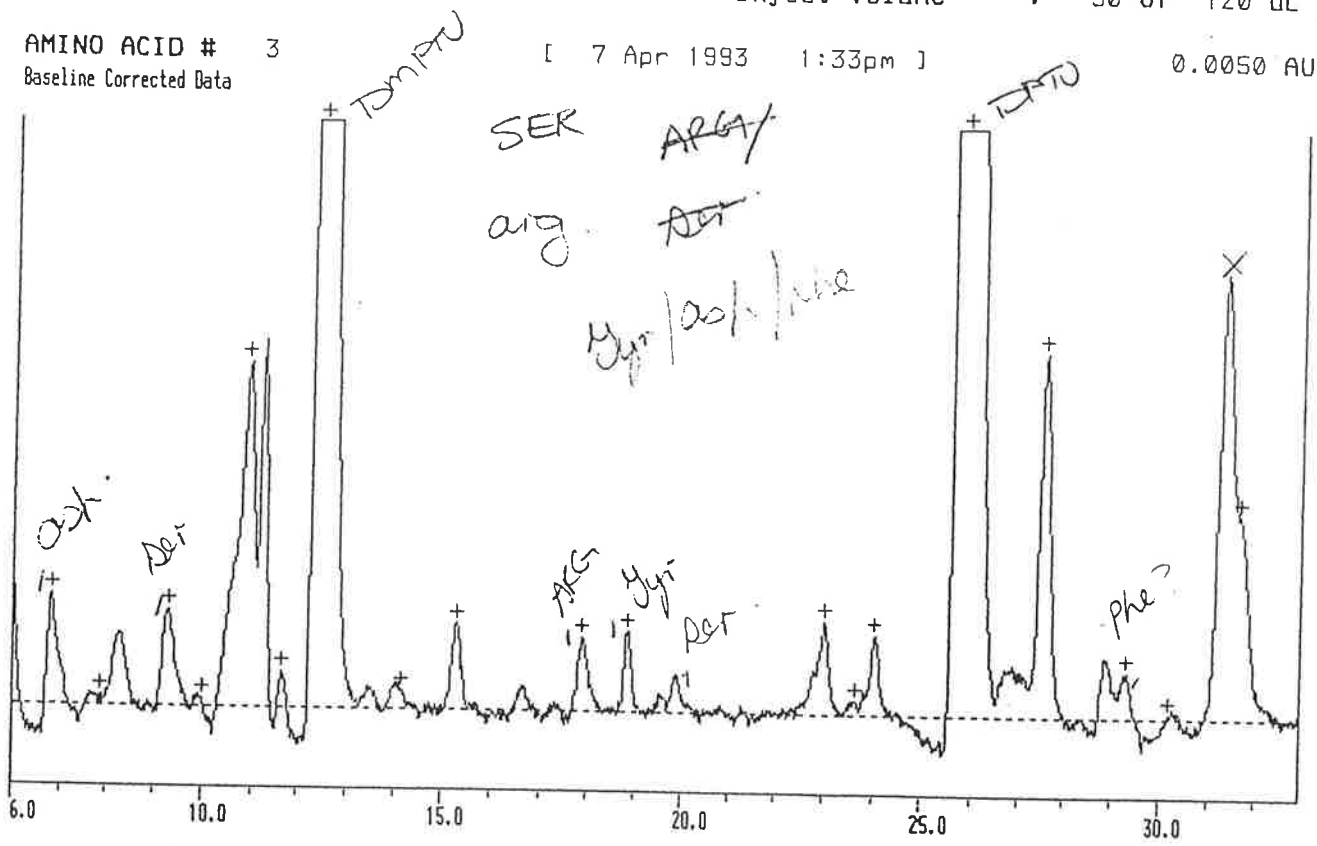
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 3
Baseline Corrected Data
[7 Apr 1993 1:33pm]
0.0050 AU



Retention Time: Minutes

PEAK TABULATION : (100% injection)
Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2119	
ASP	6.82	6.80	1989	2.73
ASN	7.88	8.07	192	0.23
	8.30		1317	
SER	9.32	9.32	1756	3.10
GLN	10.02	9.97	144	0.18
GLY	10.93	10.93	6194	9.07
	11.25		6573	
GLU	11.67	11.67	660	0.89
DMP	12.48	12.57	132631	326.92
HIS	14.18	14.13	345	0.35
ALA	15.28	15.25	1572	1.82
ARG	17.88	17.92	1339	9.02
TYR	18.85	18.77	1466	1.41
	19.93		676	
	22.80		808	
PRO	23.03	22.90	1699	2.17
MET	23.65	23.47	278	0.27
VAL	24.05	23.92	1454	1.45
DPT	25.83	25.67	532149	751.01
	26.90		936	
	27.03		856	
TRP	27.55	27.52	6528	6.45
	28.90		1060	
PHE	29.32	29.10	859	1.21
ILE	30.20	29.98	86	0.16
	31.33		7960	
LEU	31.65	31.35	3664	6.09
	32.03		537	

Tabulation threshold : 500 uAU

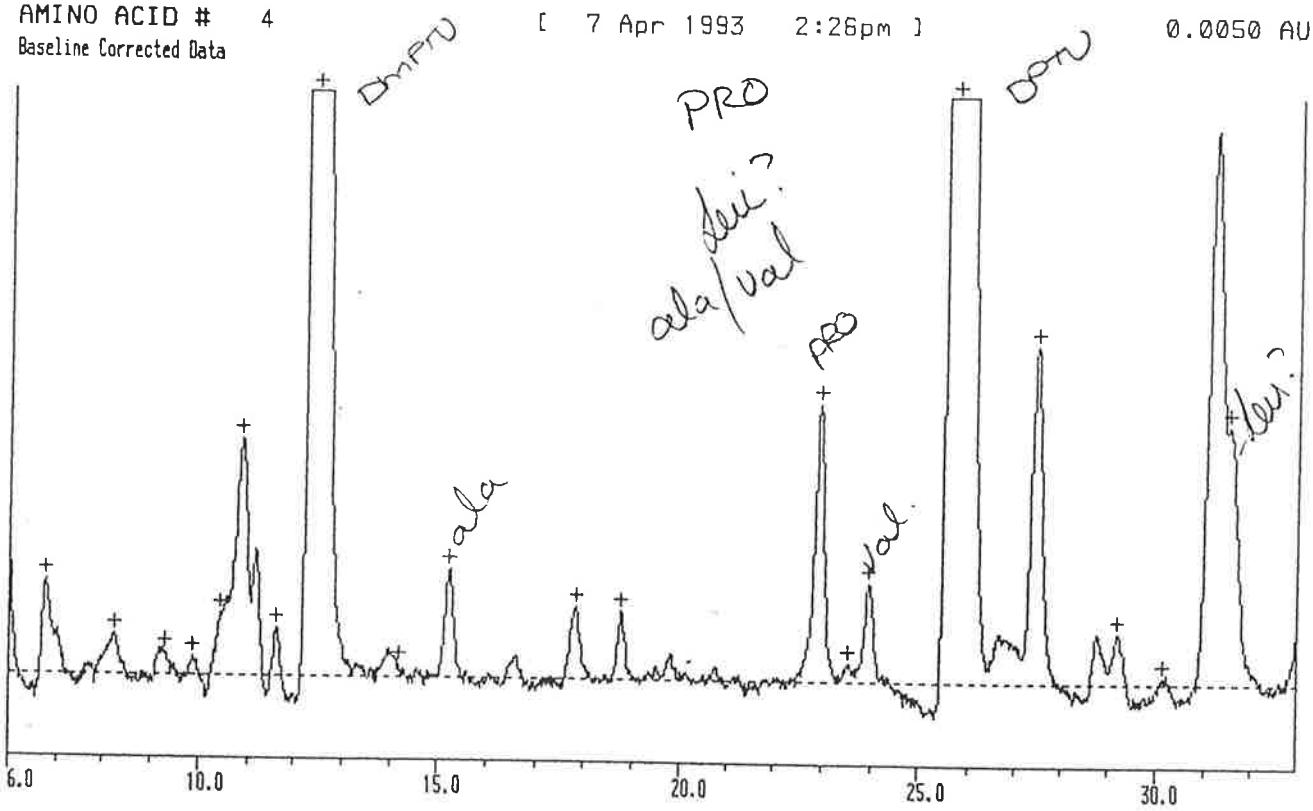
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 4 [7 Apr 1993 2:26pm] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.02		2325		ARG	17.82	17.92	1317	8.88
ASP	6.77	6.80	1718	2.36	TYR	18.77	18.77	1257	1.21
	7.02		796		PRO	22.93	22.90	4984	6.36
ASN	8.25	8.07	739	0.89	MET	23.53	23.47	340	0.33
SER	9.32	9.32	391	0.69	VAL	23.95	23.92	1790	1.78
GLN	9.90	9.97	338	0.43	DPT	25.73	25.67	476920	673.07
THR	10.45	10.47	1130	2.50		26.68		902	
	10.58		1430			26.82		799	
GLY	10.88	10.93	4260	6.24		27.00		741	
	11.18		2282		TRP	27.45	27.52	6055	5.98
GLU	11.63	11.67	864	1.17		28.73		921	
DMP	12.43	12.57	117530	289.70	PHE	29.20	29.10	916	1.29
	13.98		511		ILE	30.15	29.98	120	0.22
HIS	14.20	14.13	237	0.24		31.18		9919	
ALA	15.23	15.25	1958	2.27	LEU	31.50	31.35	4639	7.71

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

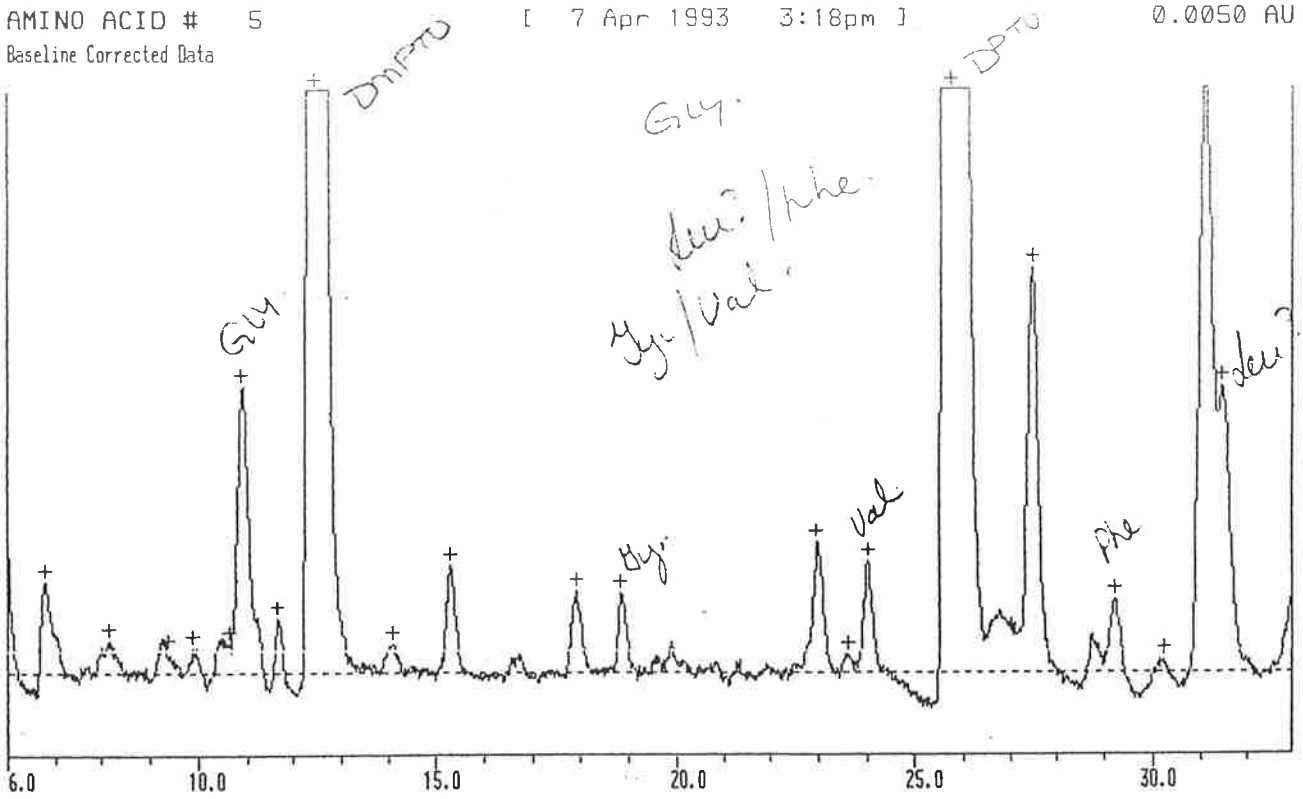
SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 5 [7 Apr 1993 3:18pm] 0.0050 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.02		2347			19.90		511	
ASP	6.82	6.80	1632	2.24		22.78		516	
ASN	8.17	8.07	552	0.66	PRO	22.98	22.90	2361	3.01
	9.28		597		MET	23.63	23.47	290	0.28
SER	9.42	9.32	343	0.60	VAL	24.02	23.92	1999	1.99
GLN	9.92	9.97	400	0.50	DPT	25.78	25.67	583632	823.67
THR	10.65	10.47	496	1.10		26.67		981	
GLY	10.93	10.93	5186	7.59		26.78		1077	
	11.23		981			27.00		952	
GLU	11.68	11.67	940	1.27		27.08		871	
DMP	12.48	12.57	126535	311.90	TRP	27.48	27.52	7288	7.20
HIS	14.13	14.13	492	0.50		28.73		660	
ALA	15.32	15.25	1920	2.23	PHE	29.22	29.10	1322	1.87
ARG	17.90	17.92	1449	9.77	ILE	30.22	29.98	216	0.39
TYR	18.85	18.77	1425	1.37		31.12		10776	

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

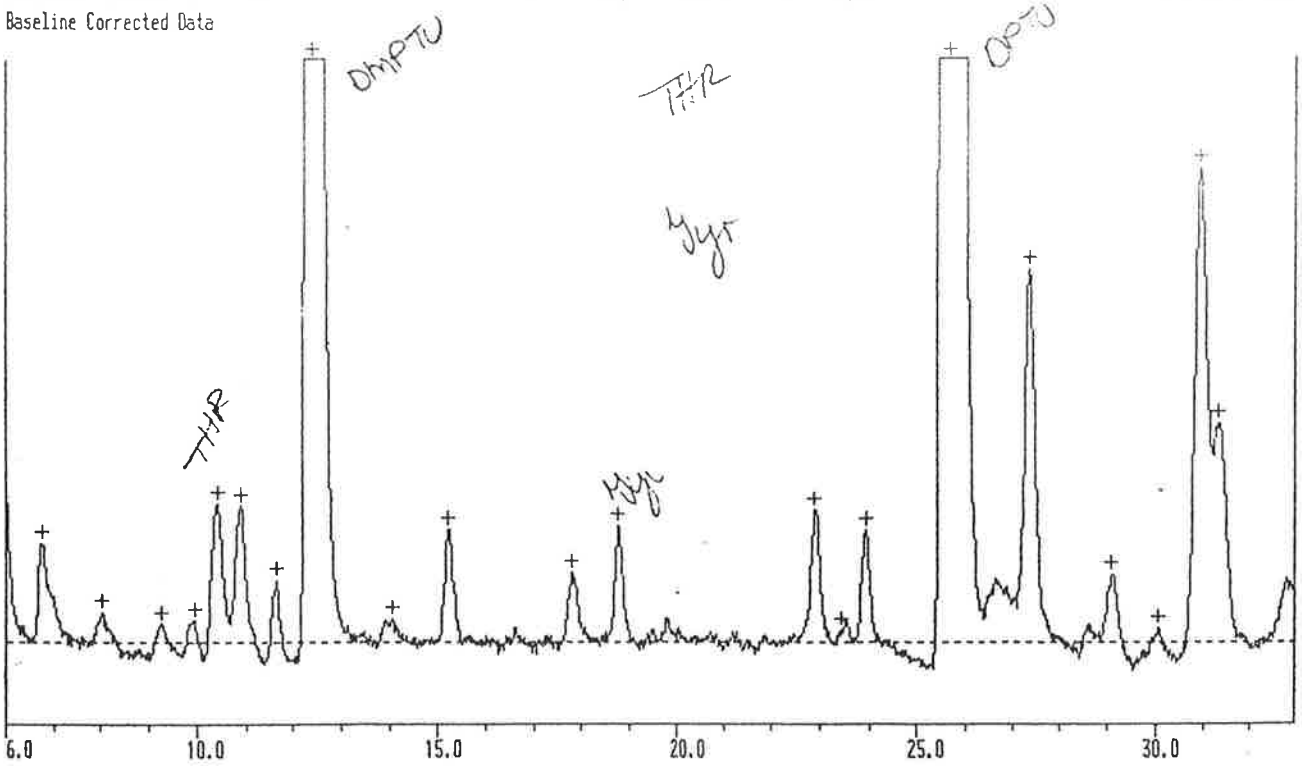
SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 6 [7 Apr 1993 4:11pm] 0.0050 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.02		2774		VAL	23.95	23.92	2025	2.02
ASP	6.80	6.80	1780	2.44	DPT	25.68	25.67	505994	714.10
ASN	8.05	8.07	566	0.68		26.37		612	
SER	9.28	9.32	336	0.59		26.55		962	
GLN	9.97	9.97	408	0.51		26.72		1080	
THR	10.43	10.47	2520	5.57		26.88		1020	
GLY	10.90	10.93	2455	3.59		27.05		813	
GLU	11.67	11.67	1113	1.50	TRP	27.37	27.52	6686	6.60
DMP	12.43	12.57	92954	229.12	PHE	29.12	29.10	1248	1.76
HIS	14.08	14.13	458	0.47	ILE	30.07	29.98	249	0.45
ALA	15.23	15.25	2032	2.36	LYS	30.95	30.70	8488	10.29
ARG	17.80	17.92	1276	8.60	LEU	31.35	31.35	3960	6.58
TYR	18.77	18.77	2112	2.04		32.80		1159	
PRO	22.92	22.90	2388	3.05		32.87		1092	
MET	23.43	23.47	237	0.23					

Tabulation threshold : 500 uAU

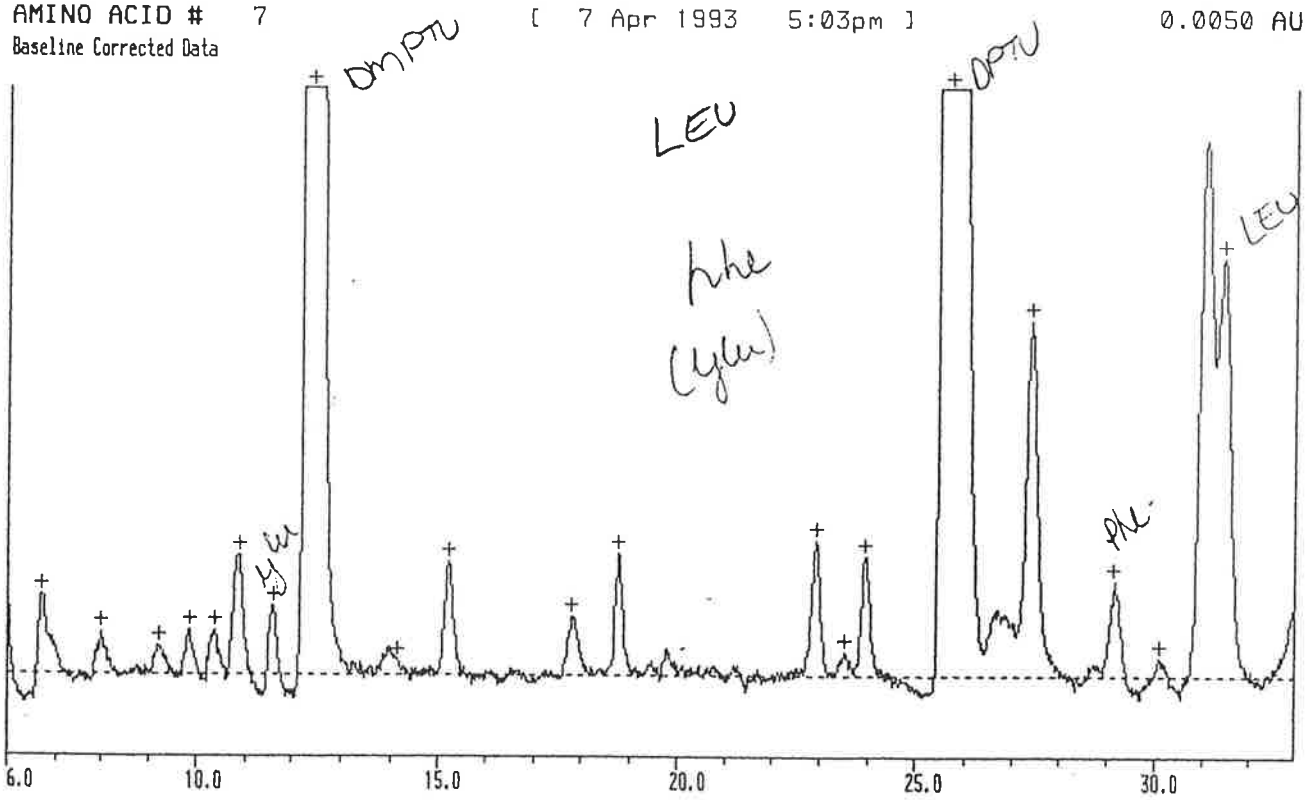
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 7 [7 Apr 1993 5:03pm] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
ASP	6.73	6.80	1437	1.97	VAL	23.93	23.92	2160	2.15
	6.88		715		DPT	25.72	25.67	462700	653.00
ASN	8.00	8.07	763	0.92		26.63		1166	
SER	9.23	9.32	518	0.91		26.70		1209	
GLN	9.88	9.97	794	1.00		27.03		988	
THR	10.40	10.47	794	1.75	TRP	27.40	27.52	6336	6.26
GLY	10.90	10.93	2145	3.14	PHE	29.15	29.10	1694	2.39
GLU	11.62	11.67	1243	1.68	ILE	30.10	29.98	352	0.64
DMP	12.42	12.57	79425	195.78		31.07		9554	
HIS	14.17	14.13	264	0.27	LEU	31.45	31.35	7459	12.39
ALA	15.22	15.25	2035	2.36		32.93		1188	
ARG	17.80	17.92	1058	7.13					
TYR	18.77	18.77	2176	2.10					
PRO	22.93	22.90	2421	3.09					
MET	23.52	23.47	436	0.42					

Tabulation threshold : 500 uAU

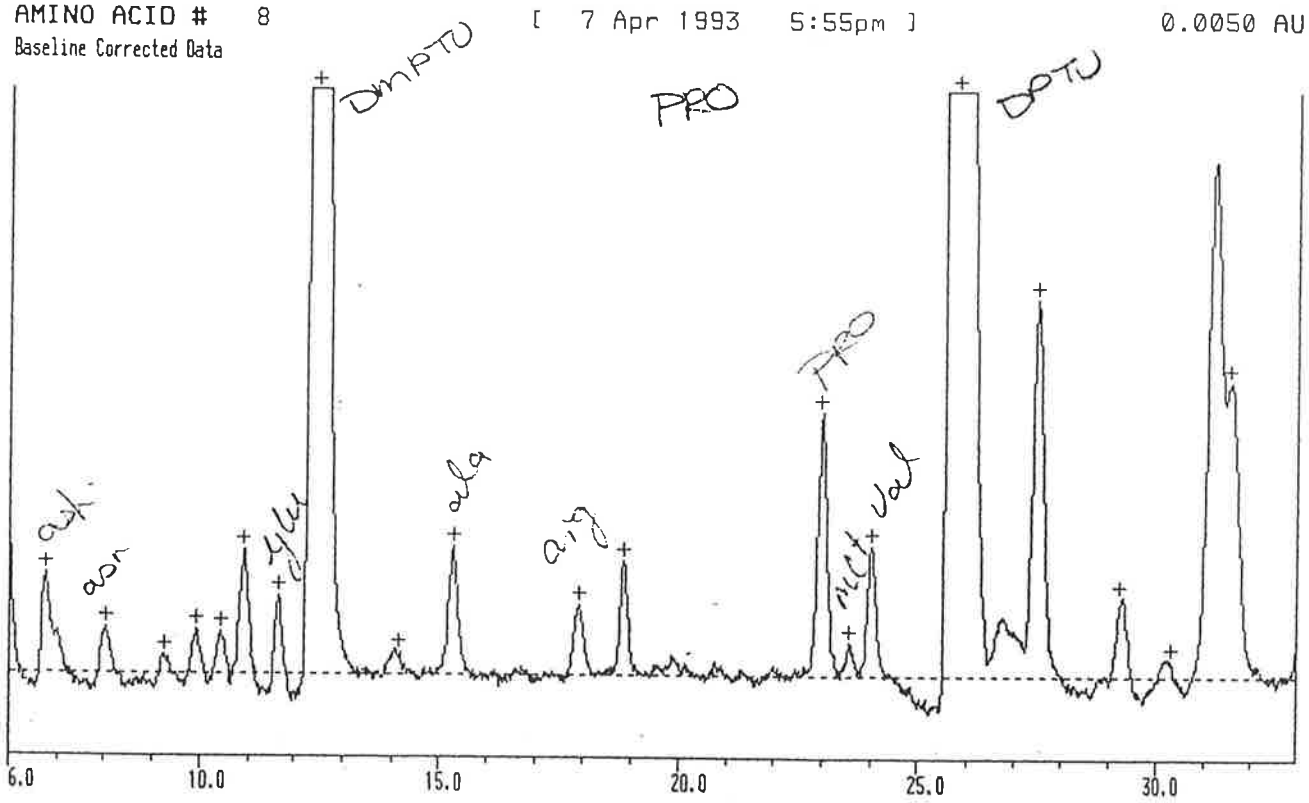
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 8 [7 Apr 1993 5:55pm] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2431		MET	23.57	23.47	609	0.59
ASP	6.77	6.80	1802	2.47	VAL	24.03	23.92	2352	2.34
ASN	8.03	8.07	835	1.00	DPT	25.80	25.67	530191	748.25
SER	9.27	9.32	321	0.57		26.77		1111	
	9.88		705			27.02		840	
GLN	9.95	9.97	818	1.03	TRP	27.48	27.52	6782	6.70
THR	10.45	10.47	777	1.72	PHE	29.23	29.10	1435	2.03
GLY	10.93	10.93	2234	3.27	ILE	30.30	29.98	312	0.56
GLU	11.68	11.67	1416	1.91		31.22		9242	
DMP	12.47	12.57	94579	233.13	LEU	31.55	31.35	5292	8.79
HIS	14.18	14.13	393	0.40					
ALA	15.28	15.25	2308	2.68					
ARG	17.90	17.92	1274	8.59					
TYR	18.85	18.77	2061	1.99					
PRO	23.00	22.90	4749	6.06					

Tabulation threshold : 500 uAU

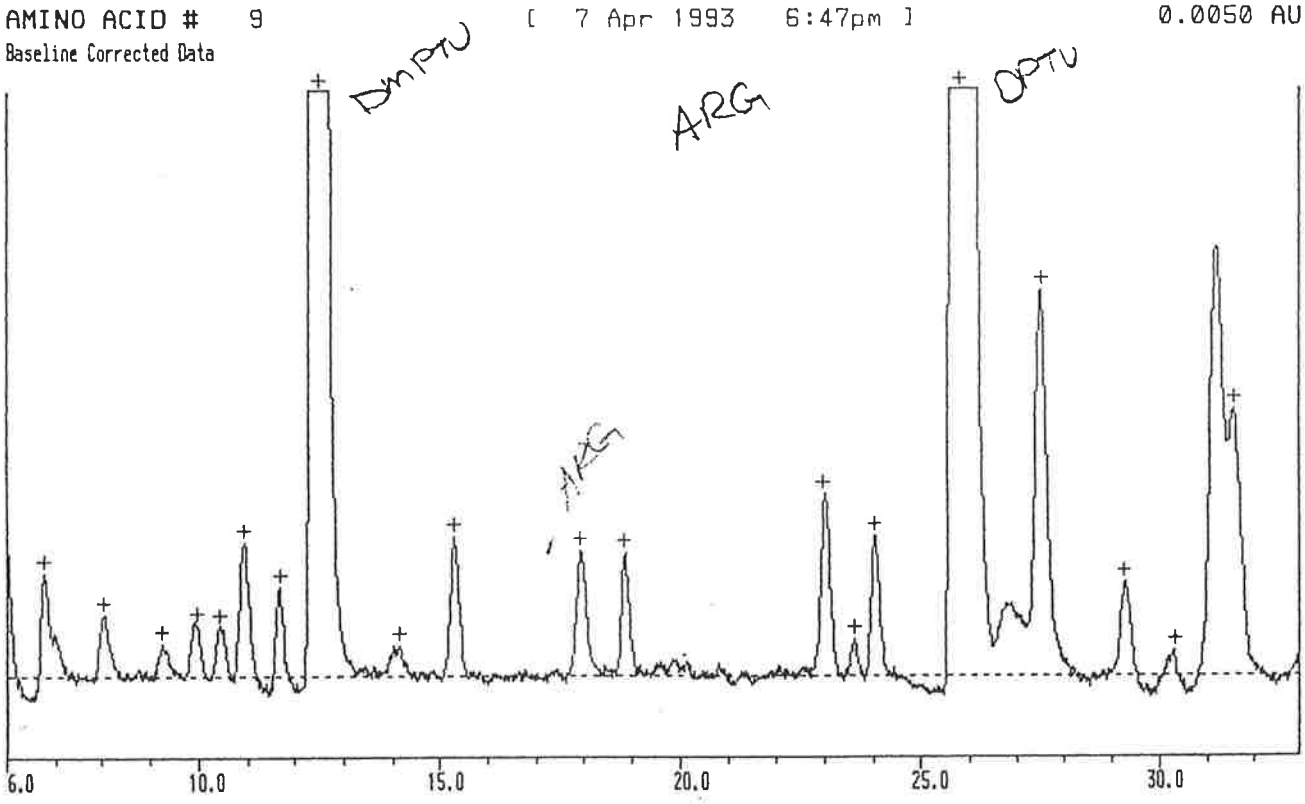
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1
Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 9 [7 Apr 1993 6:47pm] 0.0050 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R Time (min)	C Time (min)	Area (pAU)	pmol	Peak ID	R Time (min)	C Time (min)	Area (pAU)	pmol
	6.03		2229		TYR	18.87	18.77	2203	2.12
ASP	6.78	6.80	1852	2.54	PRO	23.00	22.90	3261	4.16
	7.02		751		MET	23.60	23.47	652	0.63
ASN	8.07	8.07	1096	1.32	VAL	24.03	23.92	2508	2.50
SER	9.27	9.32	576	1.02	DPT	25.82	25.67	515042	726.87
	9.93		1003			26.78		1284	
GLN	10.00	9.97	909	1.15	TRP	27.52	27.52	6876	6.79
THR	10.47	10.47	880	1.95	PHE	29.28	29.10	1656	2.34
GLY	10.97	10.93	2392	3.50	ILE	30.30	29.98	432	0.78
GLU	11.70	11.67	1600	2.16		31.22		7610	
DMP	12.50	12.57	83834	206.64	LEU	31.58	31.35	4735	7.87
	14.05		561						
HIS	14.15	14.13	542	0.56					
ALA	15.30	15.25	2493	2.89					
ARG	17.92	17.92	2251	15.17					

Tabulation threshold : 500 uAU

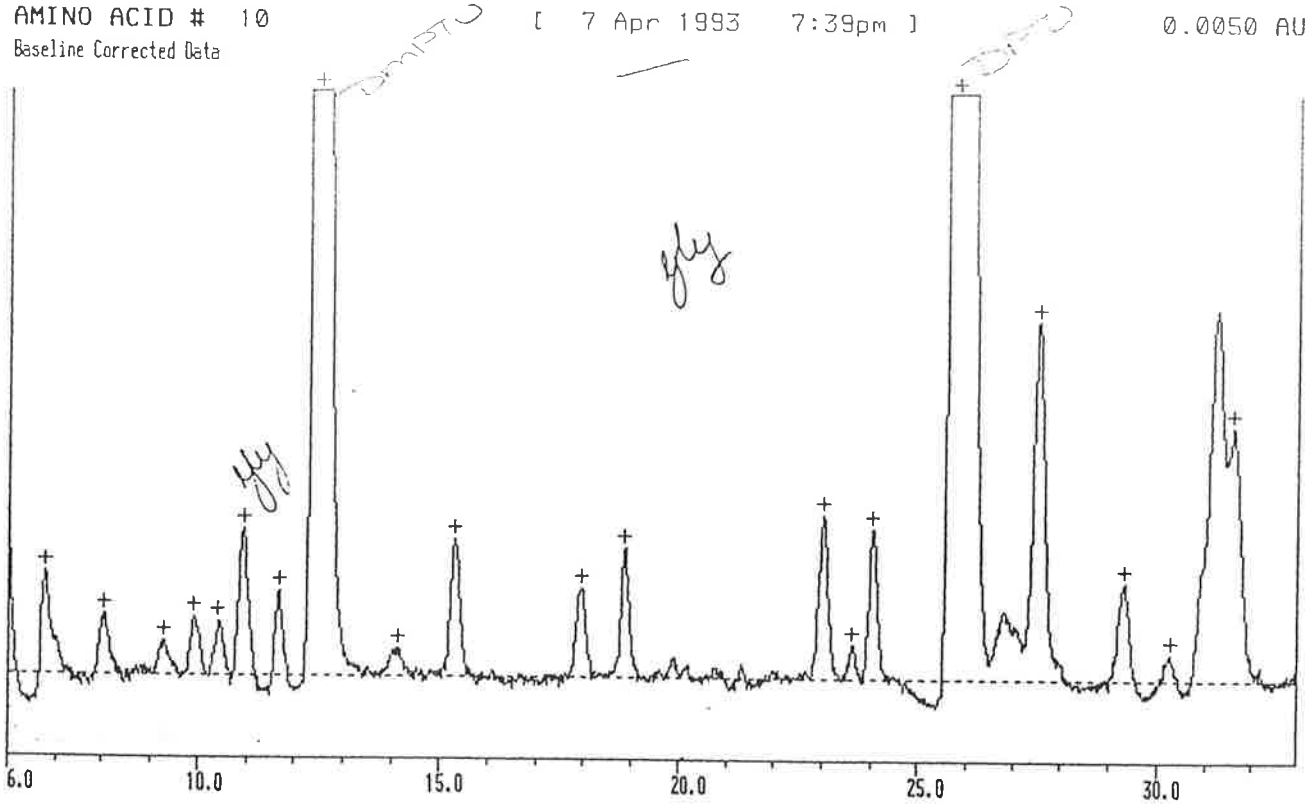
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 10 [7 Apr 1993 7:39pm] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2316		PRO	23.03	22.90	2928	3.74
ASP	6.80	6.80	1845	2.53	MET	23.62	23.47	638	0.62
ASN	8.07	8.07	1104	1.32	VAL	24.05	23.92	2678	2.67
	9.23		540		DPT	25.82	25.67	473796	668.66
SER	9.30	9.32	628	1.11		26.82		1224	
GLN	9.95	9.97	1068	1.35		26.90		1125	
THR	10.47	10.47	972	2.15		27.03		981	
GLY	10.95	10.93	2637	3.86	TRP	27.52	27.52	6403	6.32
GLU	11.70	11.67	1543	2.08	PHE	29.32	29.10	1730	2.44
DMP	12.50	12.57	81669	201.31	ILE	30.27	29.98	470	0.85
	14.10		530			31.23		6624	
HIS	14.17	14.13	508	0.52	LEU	31.58	31.35	4543	7.55
ALA	15.30	15.25	2455	2.85					
ARG	17.95	17.92	1591	10.72					
TYR	18.87	18.77	2335	2.25					

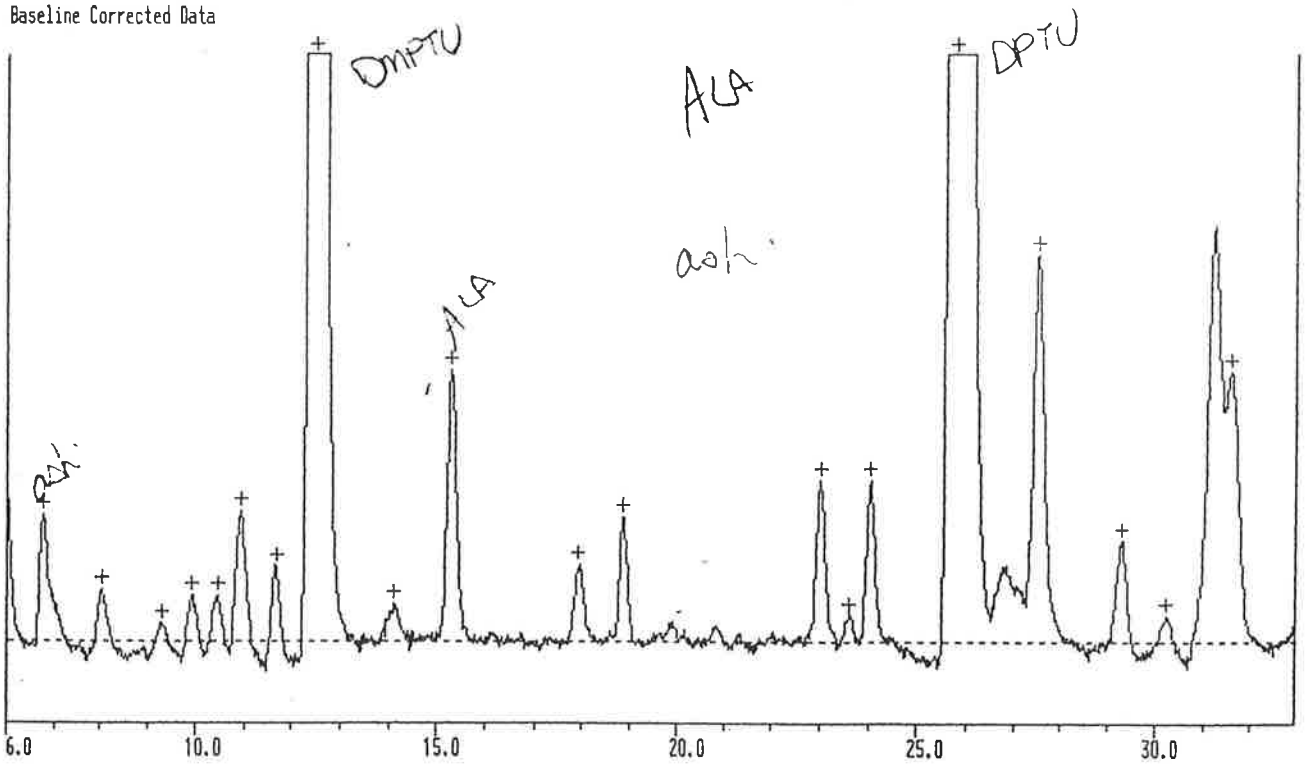
Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1
Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 11 [7 Apr 1993 8:32pm] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2616		VAL	24.05	23.92	2889	2.88
ASP	6.78	6.80	2301	3.16	DPT	25.82	25.67	517281	730.03
ASN	8.03	8.07	938	1.13		26.75		1334	
SER	9.30	9.32	352	0.62		26.83		1389	
GLN	9.95	9.97	856	1.08		27.05		1029	
THR	10.48	10.47	837	1.85	TRP	27.52	27.52	6921	6.83
GLY	10.95	10.93	2376	3.48	PHE	29.32	29.10	1812	2.56
GLU	11.70	11.67	1358	1.83	ILE	30.23	29.98	494	0.89
DMP	12.50	12.57	97624	240.64		31.22		7416	
HIS	14.13	14.13	688	0.71	LEU	31.58	31.35	4824	8.02
ALA	15.32	15.25	4855	5.63					
ARG	17.93	17.92	1375	9.27					
TYR	18.87	18.77	2253	2.17					
PRO	23.02	22.90	2892	3.69					
MET	23.63	23.47	470	0.45					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

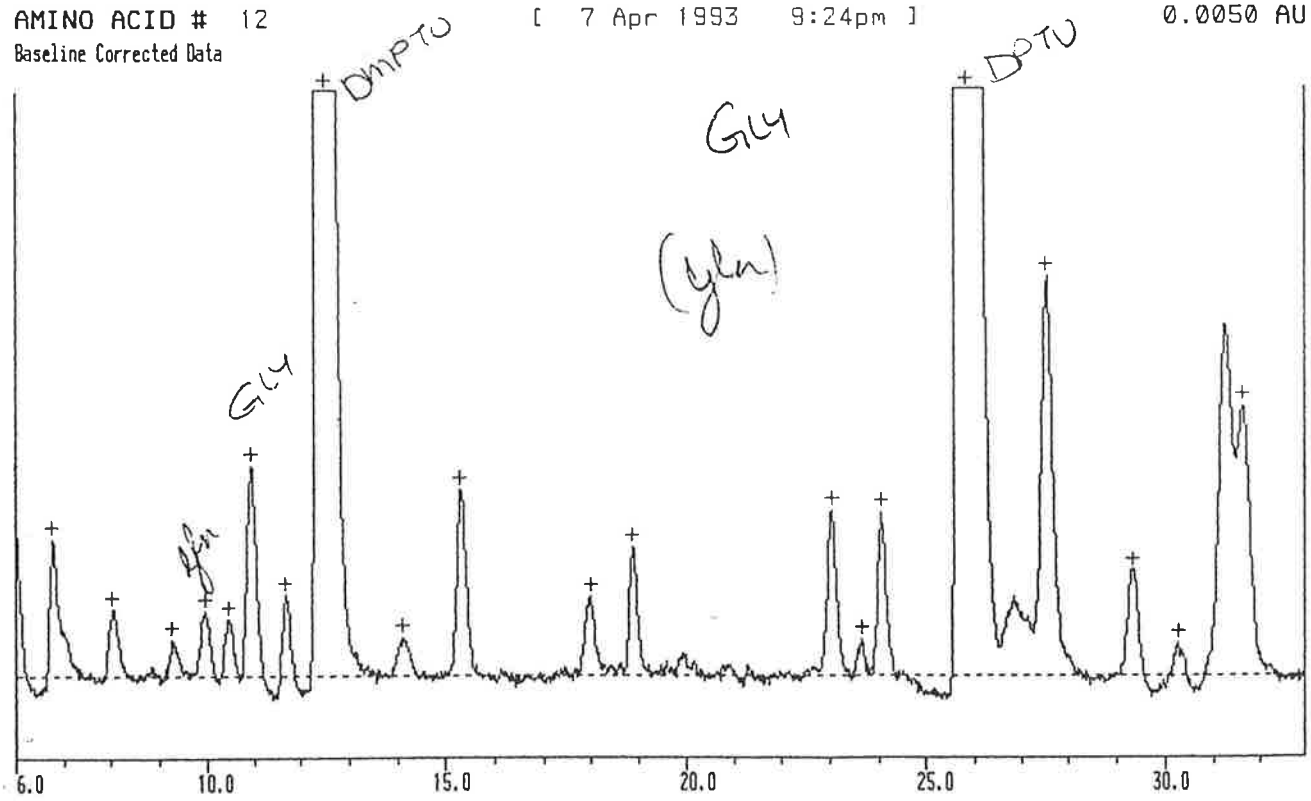
SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 12 [7 Apr 1993 9:24pm] 0.0050 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2539		VAL	24.07	23.92	2935	2.92
ASP	6.80	6.80	2457	3.37	DPT	25.85	25.67	534739	754.67
ASN	8.05	8.07	1202	1.44		26.85		1432	
SER	9.28	9.32	669	1.18		27.13		1063	
GLN	9.98	9.97	1161	1.46	TRP	27.55	27.52	7116	7.03
THR	10.48	10.47	1034	2.28	PHE	29.37	29.10	1905	2.69
GLY	10.97	10.93	3756	5.50	ILE	30.27	29.98	580	1.05
GLU	11.72	11.67	1468	1.98		31.25		6280	
DMP	12.52	12.57	110793	273.10	LEU	31.62	31.35	4831	8.03
HIS	14.13	14.13	693	0.71					
ALA	15.32	15.25	3343	3.88					
ARG	17.98	17.92	1411	9.51					
TYR	18.88	18.77	2332	2.25					
PRO	23.05	22.90	2980	3.81					
MET	23.65	23.47	652	0.63					

Tabulation threshold : 500 uAU

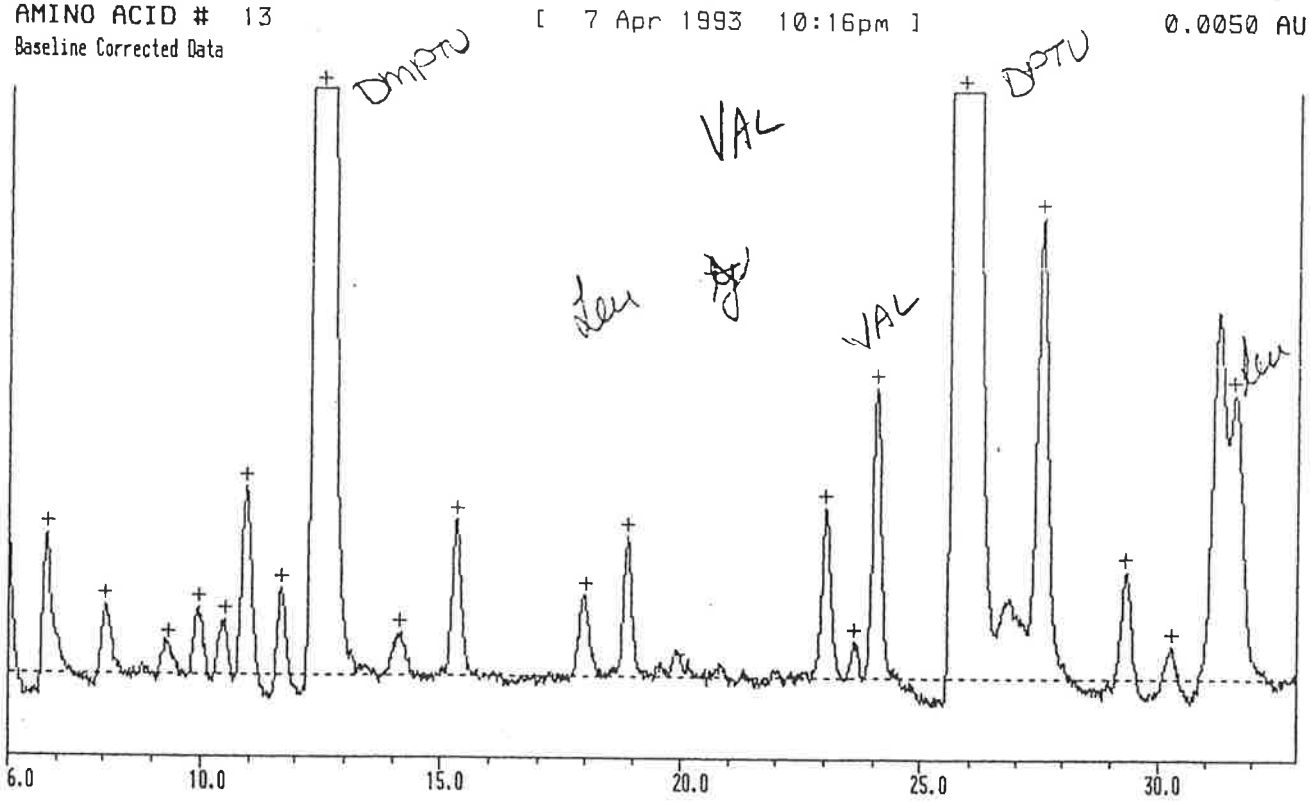
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 13 [7 Apr 1993 10:16pm] 0.0050 AU
Baseline Corrected Data



PEAK TABULATION : (100% injection) Retention Time: Minutes Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2301		PRO	23.03	22.90	3036	3.88
ASP	6.82	6.80	2491	3.42	MET	23.62	23.47	648	0.63
ASN	8.05	8.07	1245	1.49	VAL	24.08	23.92	5196	5.17
	9.28		619		DPT	25.83	25.67	625934	883.37
SER	9.37	9.32	568	1.00		26.85		1454	
GLN	9.97	9.97	1221	1.54		27.03		1156	
THR	10.52	10.47	1000	2.21		27.15		1032	
GLY	10.97	10.93	3369	4.93	TRP	27.55	27.52	8203	8.10
GLU	11.72	11.67	1581	2.13	PHE	29.32	29.10	1922	2.71
DMP	12.50	12.57	130960	322.81	ILE	30.28	29.98	571	1.03
	14.10		736			31.25		6554	
HIS	14.18	14.13	756	0.77	LEU	31.60	31.35	5064	8.41
ALA	15.33	15.25	2781	3.22					
ARG	17.98	17.92	1468	9.90					
TYR	18.88	18.77	2496	2.41					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

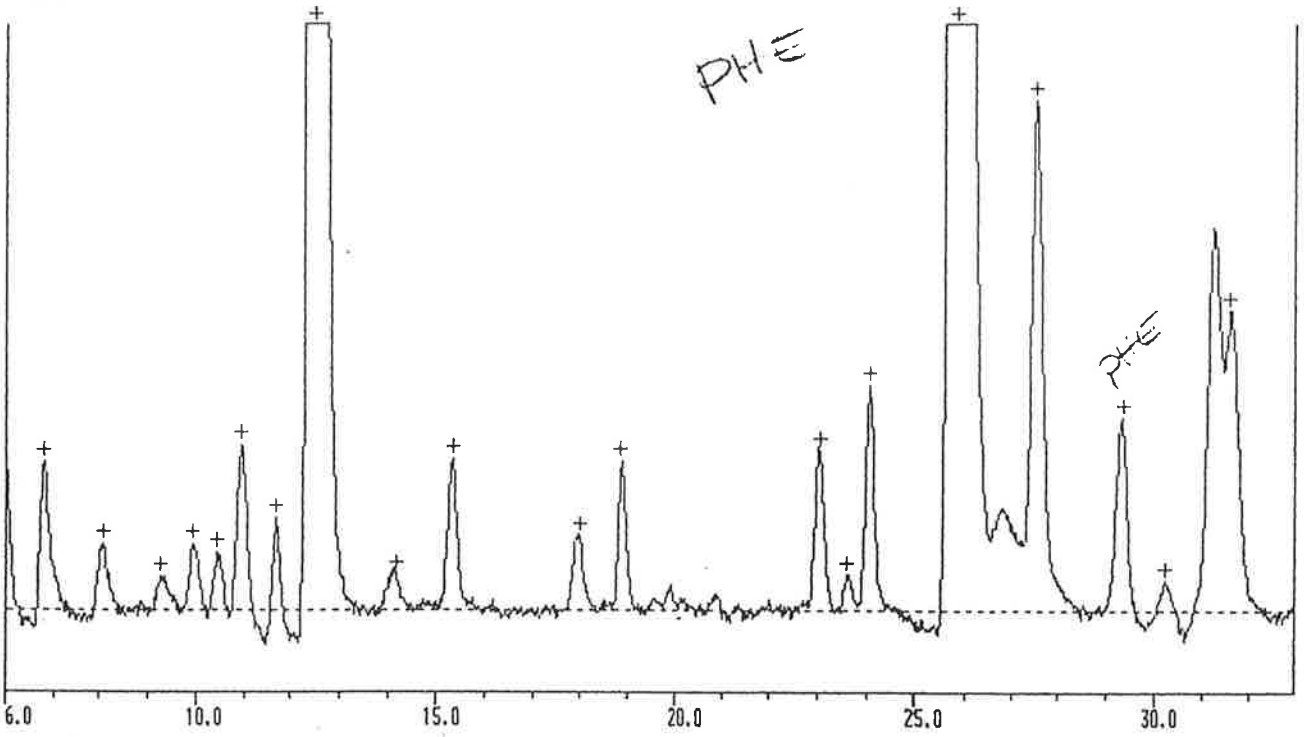
SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 14 [7 Apr 1993 11:08pm] 0.0050 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2515		MET	23.63	23.47	672	0.65
ASP	6.82	6.80	2695	3.70	VAL	24.07	23.92	4010	3.99
ASN	8.10	8.07	1185	1.42	DPT	25.83	25.67	689246	972.72
SER	9.28	9.32	614	1.08		26.85		1824	
GLN	9.93	9.97	1204	1.52		26.95		1692	
THR	10.47	10.47	1058	2.34		27.23		1269	
GLY	10.97	10.93	2968	4.35	TRP	27.53	27.52	9124	9.01
GLU	11.72	11.67	1665	2.25	PHE	29.33	29.10	3432	4.85
DMP	12.52	12.57	141792	349.50	ILE	30.22	29.98	556	1.01
	14.10		741			31.25		6844	
HIS	14.20	14.13	650	0.67	LEU	31.60	31.35	5356	8.90
ALA	15.33	15.25	2704	3.14					
ARG	17.98	17.92	1358	9.15					
TYR	18.87	18.77	2685	2.59					
PRO	23.05	22.90	2860	3.65					

Tabulation threshold : 500 uAU

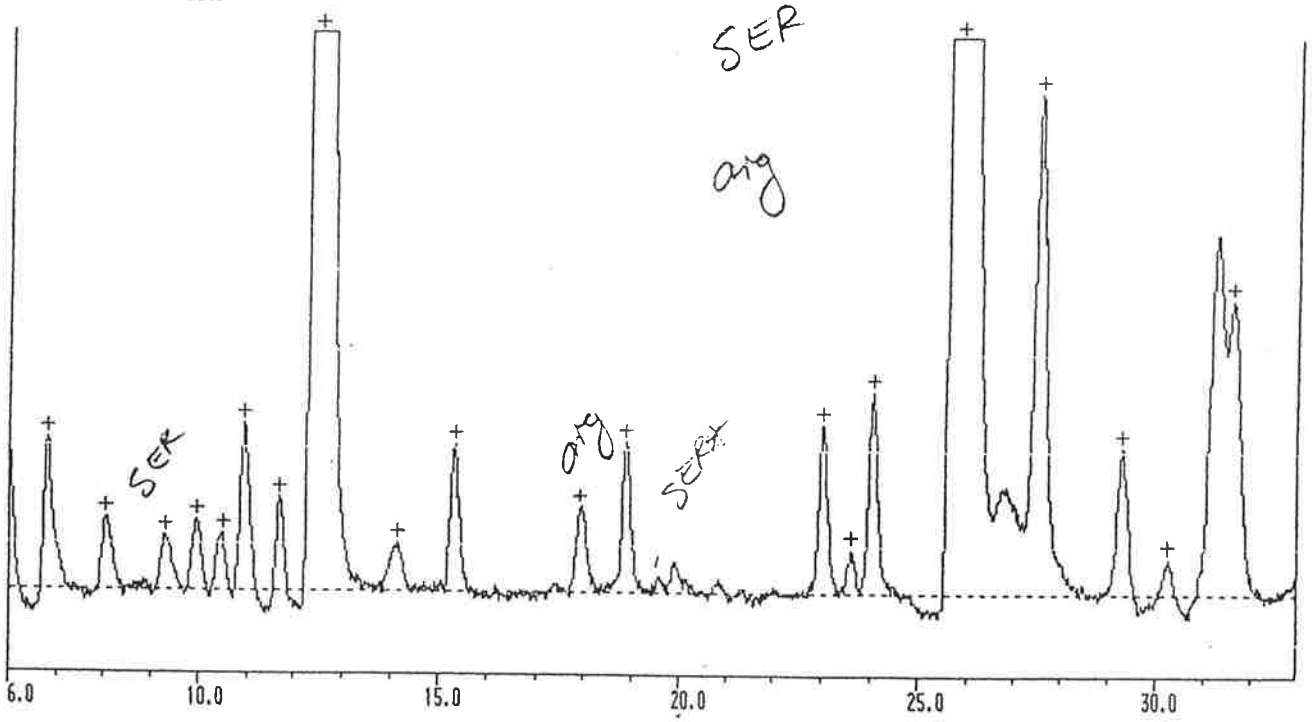
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 15 [8 Apr 1993 12:00am] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2428		MET	23.62	23.47	763	0.74
ASP	6.82	6.80	2688	3.69	VAL	24.07	23.92	3616	3.60
ASN	8.07	8.07	1293	1.55	DPT	25.83	25.67	717940	1013.22
SER	9.30	9.32	962	1.70		26.87		1958	
GLN	9.98	9.97	1250	1.58		27.02		1622	
THR	10.52	10.47	993	2.19		27.10		1468	
GLY	10.97	10.93	2961	4.34		27.18		1324	
GLU	11.73	11.67	1668	2.25	TRP	27.53	27.52	9492	9.37
DMP	12.52	12.57	160629	395.94	PHE	29.30	29.10	2630	3.71
HIS	14.15	14.13	837	0.86	ILE	30.25	29.98	609	1.10
ALA	15.33	15.25	2628	3.05		31.25		6468	
ARG	17.93	17.92	1536	10.35	LEU	31.57	31.35	5284	8.78
TYR	18.90	18.77	2697	2.60					
	19.93		566						
PRO	23.03	22.90	3009	3.84					

Tabulation threshold : 500 uAU

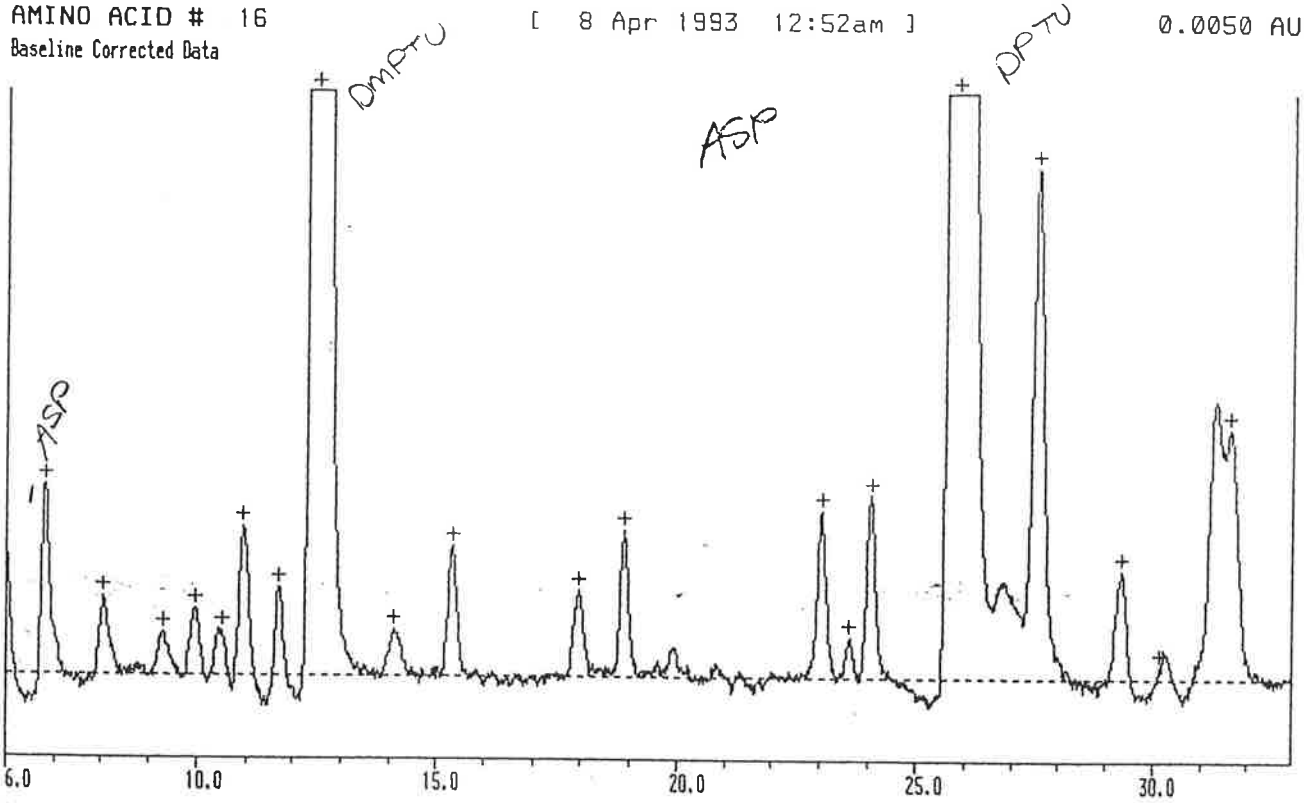
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 16 [8 Apr 1993 12:52am] 0.0050 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2150		MET	23.63	23.47	722	0.70
ASP	6.82	6.80	3412	4.68	VAL	24.07	23.92	3302	3.29
ASN	8.05	8.07	1411	1.69	DPT	25.83	25.67	706512	997.09
SER	9.30	9.32	777	1.37		26.82		1778	
GLN	10.00	9.97	1204	1.52		26.88		1749	
THR	10.53	10.47	808	1.79		27.02		1521	
GLY	10.97	10.93	2700	3.95	TRP	27.53	27.52	9144	9.03
GLU	11.72	11.67	1591	2.15	PHE	29.35	29.10	1956	2.76
DMP	12.52	12.57	140136	345.42	ILE	30.15	29.98	237	0.43
HIS	14.10	14.13	835	0.86		31.33		4984	
ALA	15.33	15.25	2359	2.73	LEU	31.63	31.35	4476	7.44
ARG	17.95	17.92	1579	10.64					
TYR	18.88	18.77	2644	2.55					
	19.92		542						
PRO	23.05	22.90	3012	3.84					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

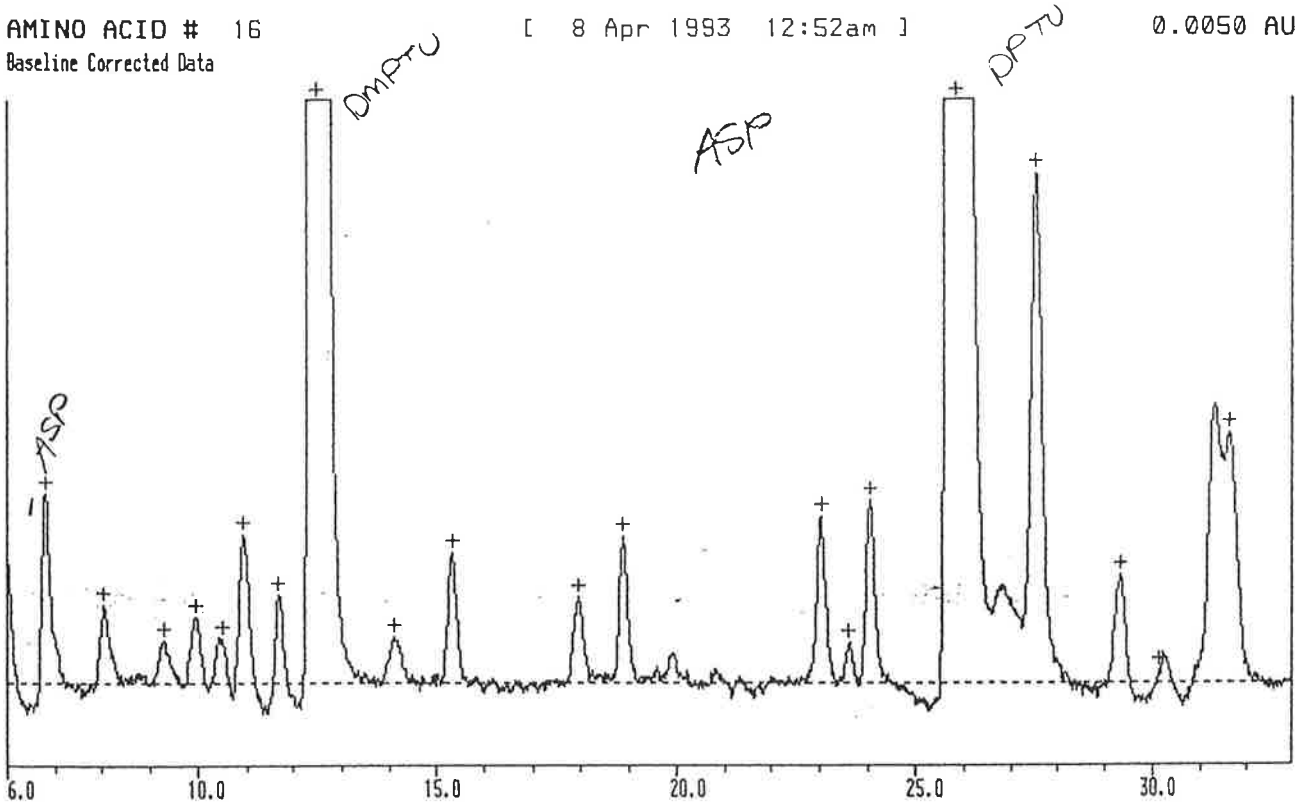
SAMPLE : JAMES STORER POA 1 PSE 265
[Initiated 7 Apr 1993 9:38am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 16 [8 Apr 1993 12:52am] 0.0050 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2150		MET	23.63	23.47	722	0.70
ASP	6.82	6.80	3412	4.68	VAL	24.07	23.92	3302	3.29
ASN	8.05	8.07	1411	1.69	DPT	25.83	25.67	706512	997.09
SER	9.30	9.32	777	1.37		26.82		1778	
GLN	10.00	9.97	1204	1.52		26.88		1749	
THR	10.53	10.47	808	1.79		27.02		1521	
GLY	10.97	10.93	2700	3.95	TRP	27.53	27.52	9144	9.03
GLU	11.72	11.67	1591	2.15	PHE	29.35	29.10	1956	2.76
DMP	12.52	12.57	140136	345.42	ILE	30.15	29.98	237	0.43
HIS	14.10	14.13	835	0.86		31.33		4984	
ALA	15.33	15.25	2359	2.73	LEU	31.63	31.35	4476	7.44
ARG	17.95	17.92	1579	10.64					
TYR	18.88	18.77	2644	2.55					
	19.92		542						
PRO	23.05	22.90	3012	3.84					

Tabulation threshold : 500 uAU

D.2 PAO II Ref: PSE 267 *Date: 13/4/93*

Table D.2 *N*-Terminal Amino Acid Sequence of PSE 267 [a.]

aa no.	*1° Signal*	*2°*	*3°*	*4°*	*5°*	*6°*	*7°*	*8°*	*9°*
1	Glu					Pro			
2	Pro								
3	Ser					Tyr/Val/Phe			
4	Pro					Thr/Glu/Tyr/Ala			Ile
5	Gly			Val/Glu		Tyr/Asp/Phe			
6	Thr					Phe			
7	Leu			Val		Ala/Gln		Ile/Asp/Glu/Asn	
8	Pro			Ile		Asn			Arg/Met
9	Arg			Phe		Gly			
10	Lys			Ile		His			Asp/Tyr
11	Ala			Leu		Thr/Met/Ile/Asp			
12	Gly								
13	Val			Leu		His			
14	Phe								
15	Ser								Glu
16	Asp					Lys			Ala/Arg
17	Leu					Asn			
18	Ser								
19	Asn								
20	Gln								
21	Glu								
22	Leu								
23	Lys								
24	Ala								
25	Val								
26	His								
27	Ser								
28	-								

a. Interpretation of the chromatograms

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

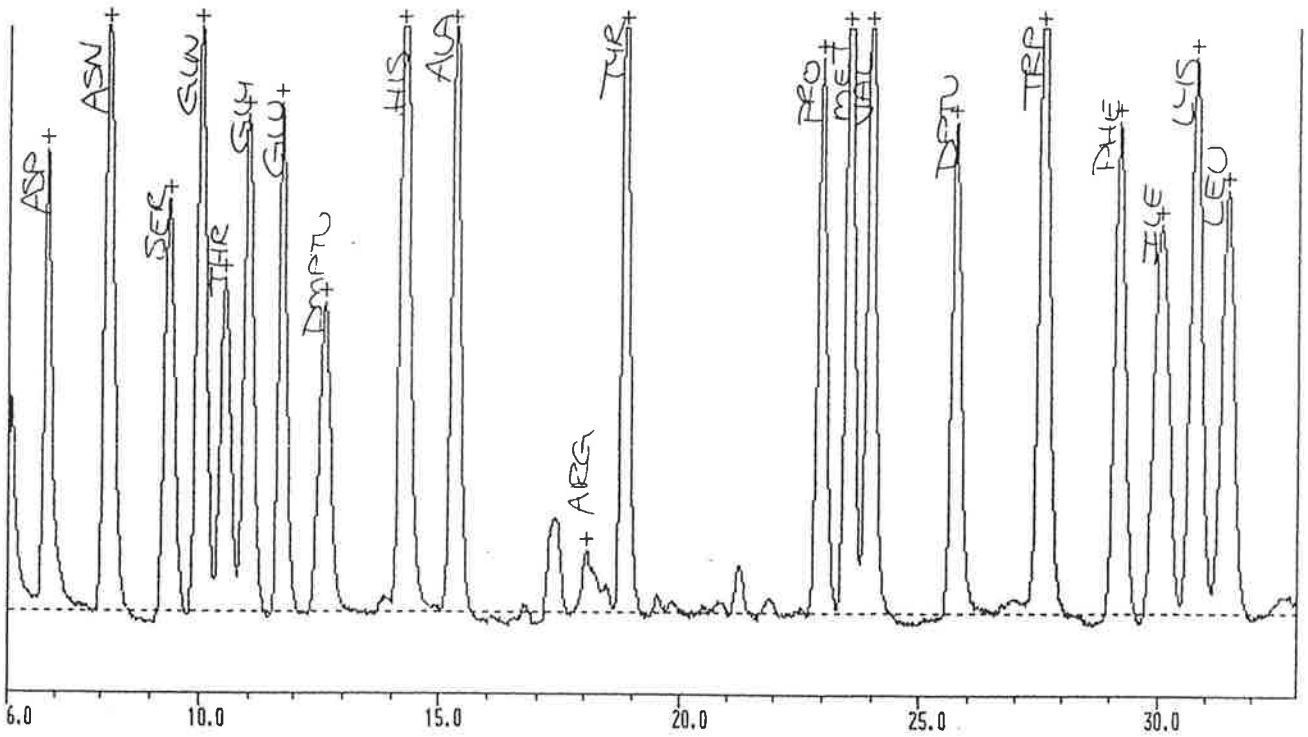
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: BGN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: BGN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

CALIBRATION # 1 [13 Apr 1993 10:39am] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		7692			17.37		3436	
	6.42		849		ARG	18.05	18.05	2280	25.00
	6.55		540			18.48		916	
	6.62		540		TYR	18.83	18.83	26414	25.00
ASP	6.83	6.83	16476	25.00		19.53		667	
ASN	8.13	8.13	21837	25.00		21.28		1732	
SER	9.38	9.38	14784	25.00		21.92		559	
GLN	10.03	10.03	21040	25.00	PRO	22.97	22.97	19908	25.00
THR	10.53	10.53	11971	25.00	MET	23.53	23.53	25759	25.00
GLY	11.00	11.00	17827	25.00	VAL	23.98	23.98	24972	25.00
GLU	11.73	11.73	18271	25.00	EMPT	25.73	25.73	17587	25.00
EMP	12.63	12.63	11095	25.00		26.93		544	
	13.88		583		TRP	27.58	27.58	25248	25.00
HIS	14.27	14.27	26318	25.00	PHE	29.18	29.18	17558	25.00
ALA	15.32	15.32	22322	25.00	ILE	30.05	30.05	13874	25.00

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

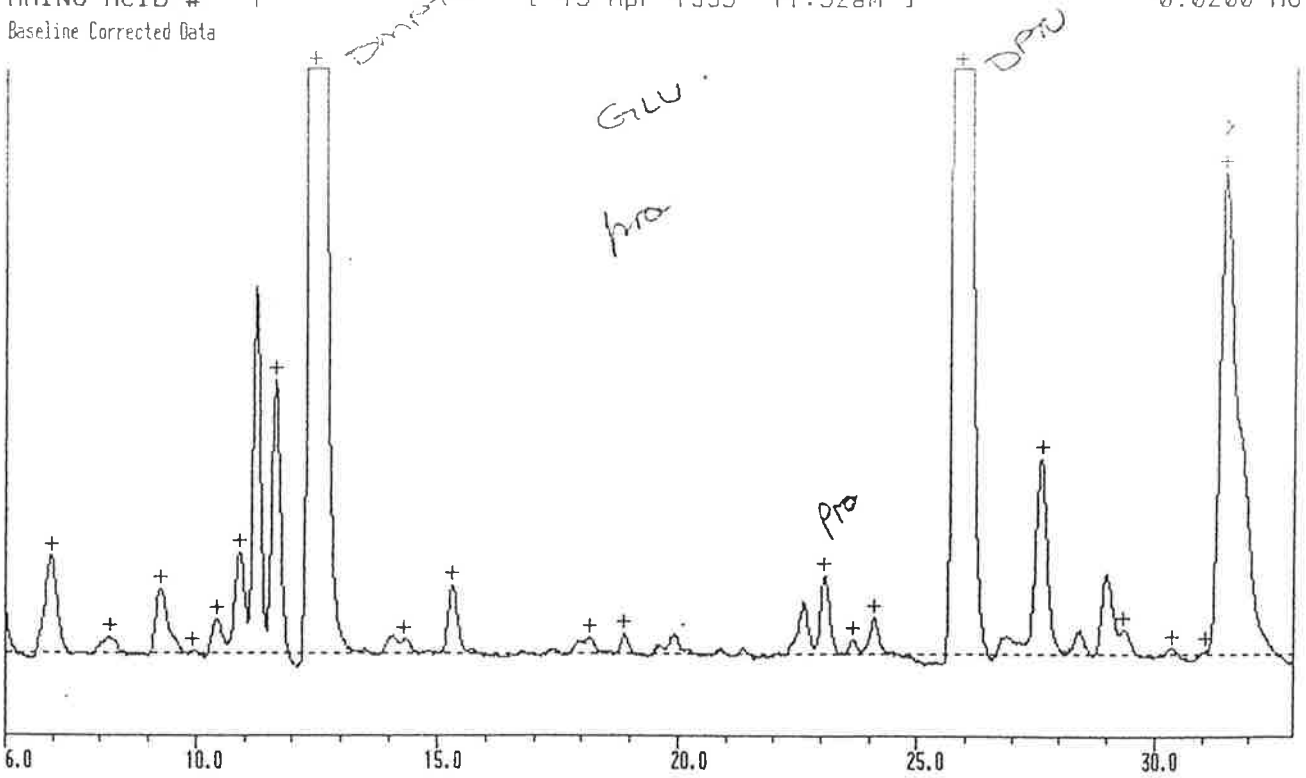
SAMPLE : JAMES STORER PA0 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 1 [13 Apr 1993 11:32am] 0.0200 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.02		2983		TYR	18.88	18.83	1423	1.35
ASP	6.98	6.83	6979	10.59		19.62		662	
ASN	8.20	8.13	1185	1.36		19.93		1555	
SER	9.27	9.38	4732	8.00		21.37		513	
GLN	9.95	10.03	256	0.31		22.65		3741	
THR	10.45	10.53	2476	5.17	PRO	23.08	22.97	5707	7.17
GLY	10.92	11.00	7236	10.15	MET	23.65	23.53	1118	1.09
	11.27		26380		VAL	24.12	23.98	2642	2.65
GLU	11.67	11.73	19706	26.96	DPT	25.88	25.73	437584	622.02
DMP	12.48	12.63	258619	582.73		26.90		1336	
	14.05		1269			26.97		1200	
HIS	14.35	14.27	1058	1.01		27.18		924	
ALA	15.30	15.32	5013	5.61	TRP	27.60	27.58	14037	13.90
	17.97		955			28.43		1783	
ARG	18.18	18.05	1152	12.63		28.98		5846	

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

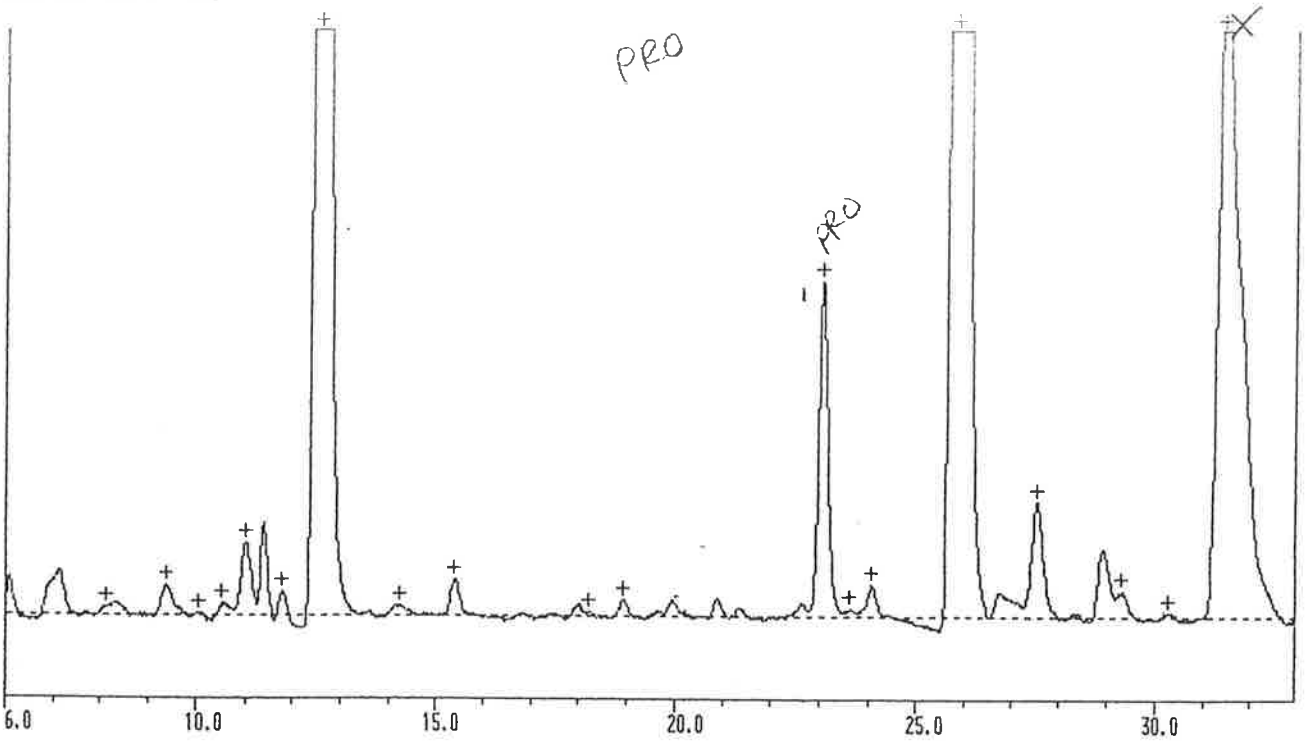
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 2 [13 Apr 1993 12:24pm] 0.0200 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.10		2757		ARG	18.23	18.05	376	4.13
	7.15		3252		TYR	18.95	18.83	1173	1.11
ASN	8.12	8.13	638	0.73		19.97		1135	
	8.33		945			20.90		1351	
SER	9.40	9.38	2145	3.63		21.40		679	
GLN	10.08	10.03	259	0.31		22.63		1020	
THR	10.55	10.53	878	1.83	PRO	23.07	22.97	23976	30.11
GLY	11.05	11.00	5277	7.40	MET	23.63	23.53	602	0.58
	11.40		6650		VAL	24.08	23.98	2289	2.29
GLU	11.80	11.73	1809	2.48	DPT	25.85	25.73	610036	867.16
DMP	12.58	12.63	220351	496.50		26.80		1831	
	14.20		832			26.95		1430	
HIS	14.27	14.27	787	0.75		27.13		1152	
ALA	15.40	15.32	2661	2.98	TRP	27.55	27.58	8265	8.18
	18.02		861			28.93		4972	

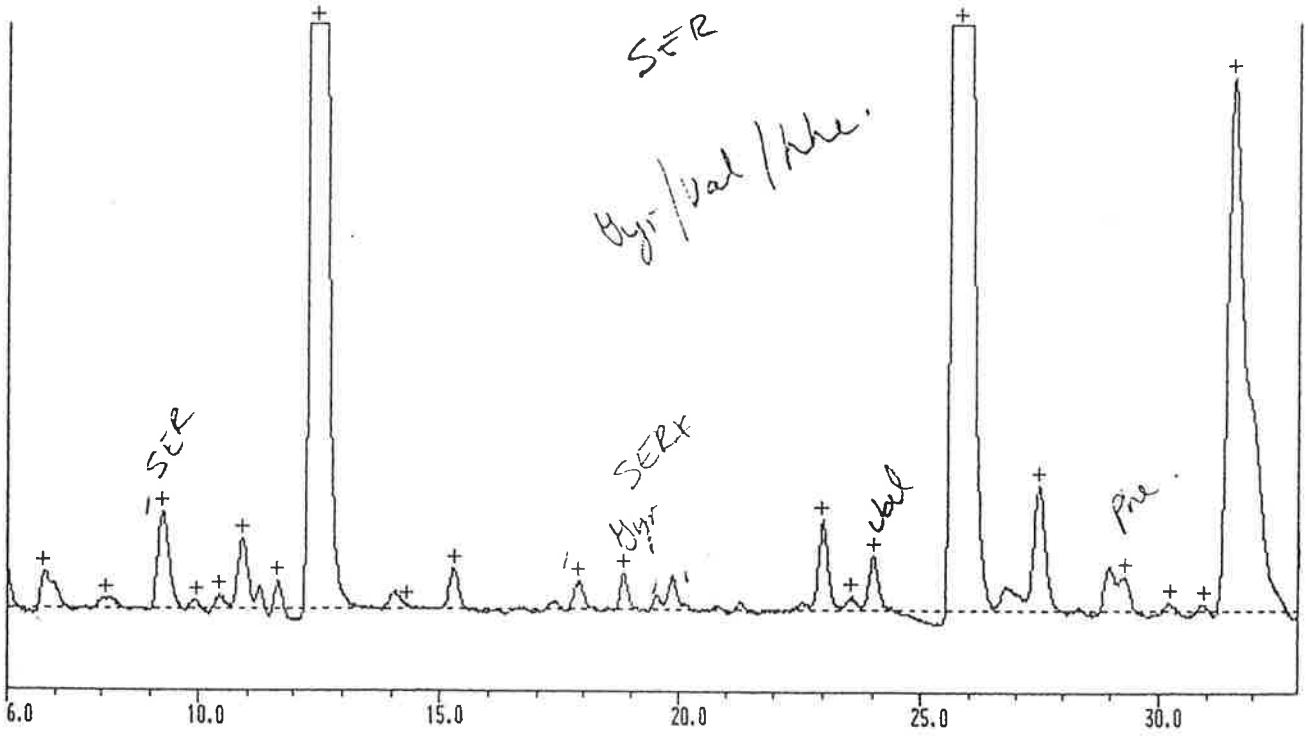
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 3 [13 Apr 1993 1:16pm] 0.0200 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.02		2971			17.40		559	
ASP	6.80	6.83	2702	4.10	ARG	17.88	18.05	2020	22.16
ASN	8.10	8.13	741	0.85	TYR	18.85	18.83	2654	2.51
	8.18		765			19.55		1027	
	8.27		638			19.88		2433	
SER	9.27	9.38	6940	11.74		21.32		648	
GLN	9.97	10.03	679	0.81		22.58		612	
THR	10.45	10.53	1024	2.14	PRO	23.00	22.97	6489	8.15
GLY	10.92	11.00	5037	7.06	MET	23.58	23.53	988	0.96
	11.27		1636		VAL	24.02	23.98	3916	3.92
GLU	11.67	11.73	2119	2.90	DPT	25.80	25.73	748334	1063.75
DMP	12.48	12.63	227690	513.04		26.80		1744	
	14.10		1291			27.18		890	
HIS	14.35	14.27	297	0.28	TRP	27.50	27.58	9076	8.99
ALA	15.28	15.32	2961	3.32		28.97		3237	

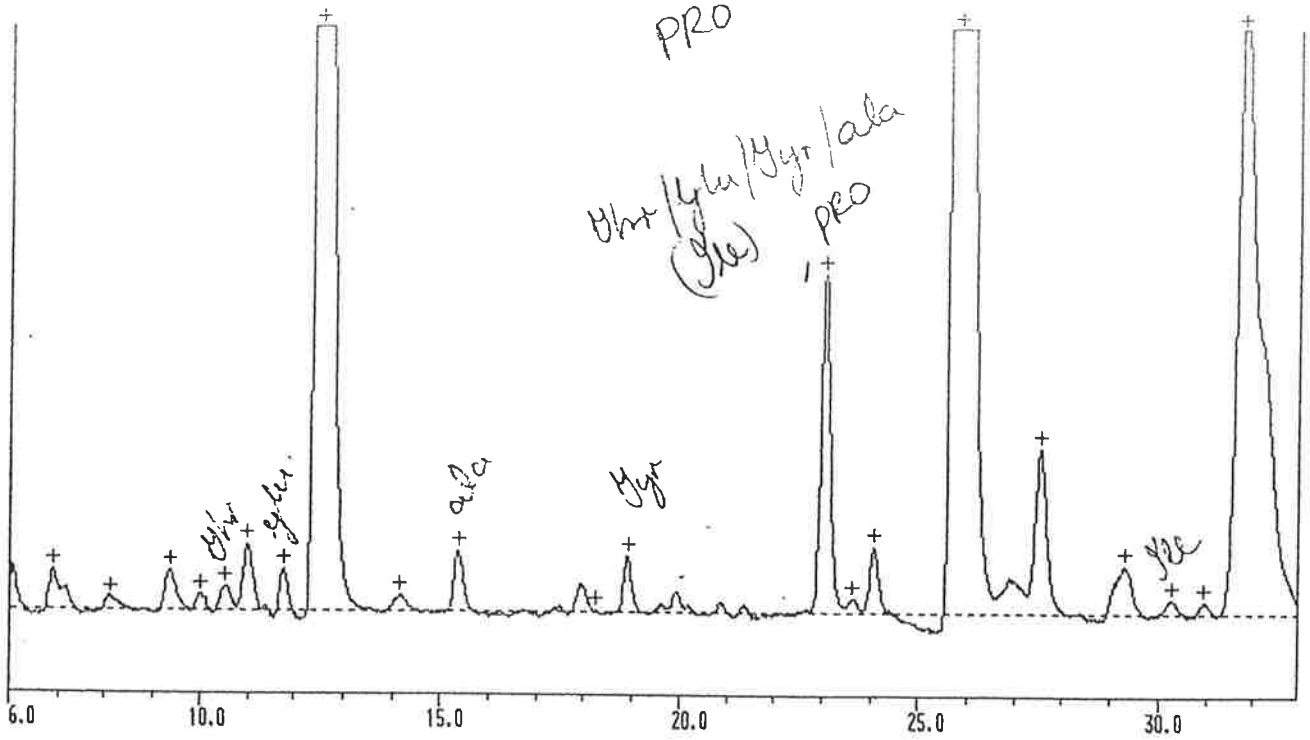
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 4 [13 Apr 1993 2:09pm] 0.0200 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		3165			19.67		528	
ASP	6.90	6.83	2868	4.35		19.93		1560	
	7.17		1670			20.20		549	
ASN	8.15	8.13	955	1.09		20.88		820	
SER	9.38	9.38	2899	4.90		21.37		600	
GLN	10.03	10.03	1197	1.42	PRO	23.05	22.97	24372	30.61
THR	10.53	10.53	1838	3.84	MET	23.62	23.53	1104	1.07
GLY	11.00	11.00	4821	6.76	VAL	24.07	23.98	4807	4.81
GLU	11.80	11.73	3033	4.15	DPT	25.85	25.73	988166	1404.67
DMP	12.57	12.63	232948	524.89		26.95		2553	
HIS	14.18	14.27	1200	1.14	TRP	27.53	27.58	11932	11.82
ALA	15.37	15.32	4435	4.97	PHE	29.30	29.18	3441	4.90
	17.92		2066		ILE	30.27	30.05	1106	1.99
ARG	18.25	18.05	225	2.47	LYS	30.95	30.78	861	1.08
TYR	18.92	18.83	4154	3.93	LEU	31.77	31.45	44364	73.32

Tabulation threshold : 500 uAU

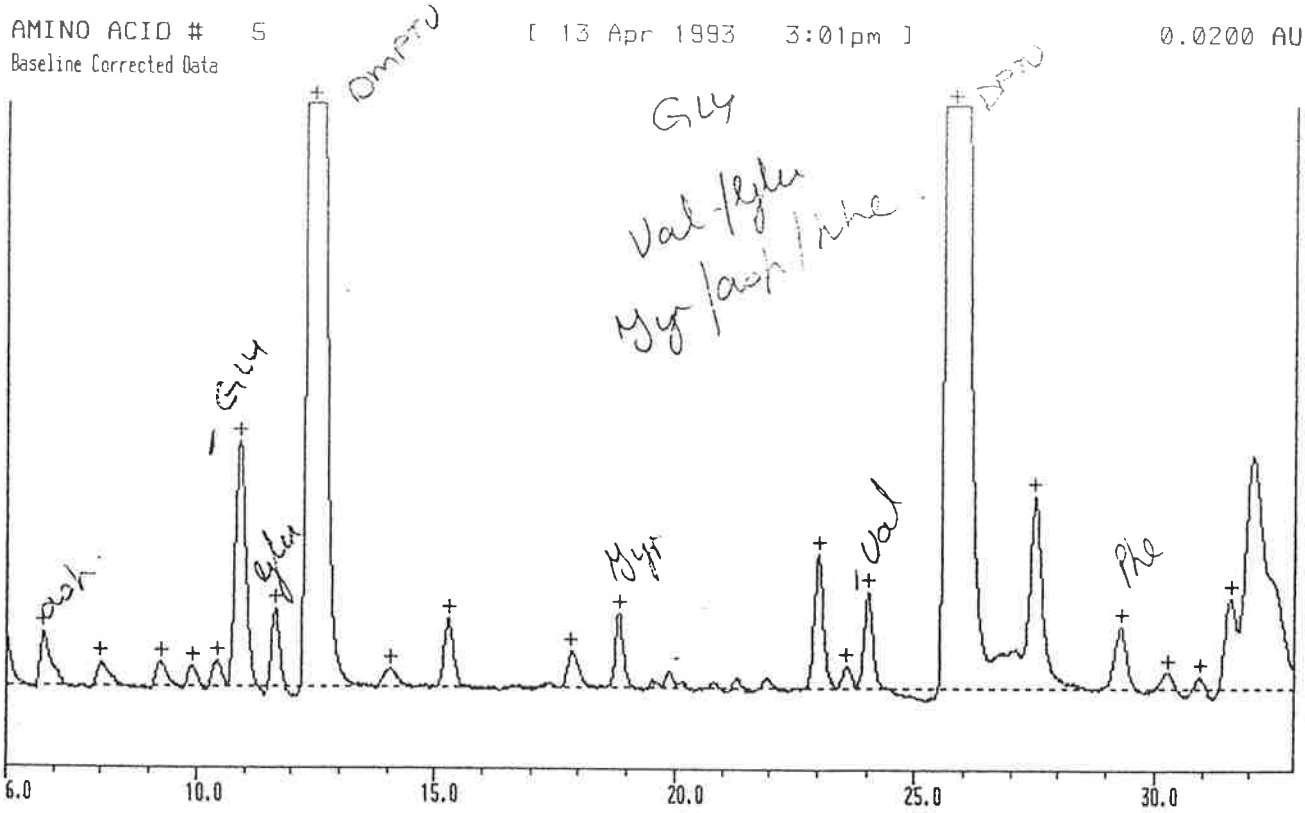
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 5 [13 Apr 1993 3:01pm] 0.0200 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3580			21.30		724	
ASP	6.80	6.83	3880	5.89		21.95		722	
ASN	8.02	8.13	1776	2.03	PRO	23.02	22.97	9775	12.28
SER	9.25	9.38	1776	3.00	MET	23.58	23.53	1653	1.60
GLN	9.93	10.03	1428	1.70	VAL	24.03	23.98	6955	6.96
THR	10.43	10.53	1876	3.92	DPT	25.80	25.73	1082937	1539.38
GLY	10.90	11.00	17635	24.73		26.78		2671	
GLU	11.67	11.73	5733	7.85		27.08		2865	
DMP	12.47	12.63	252487	568.91	TRP	27.50	27.58	13891	13.75
HIS	14.07	14.27	1308	1.24		28.03		696	
ALA	15.28	15.32	4980	5.58	PHE	29.30	29.18	4492	6.40
ARG	17.85	18.05	2656	29.13	ILE	30.27	30.05	1216	2.19
TYR	18.83	18.83	5397	5.11	LYS	30.93	30.78	878	1.10
	19.53	–	568		LEU	31.58	31.45	6616	10.94
	19.90		1180			32.05		16764	

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

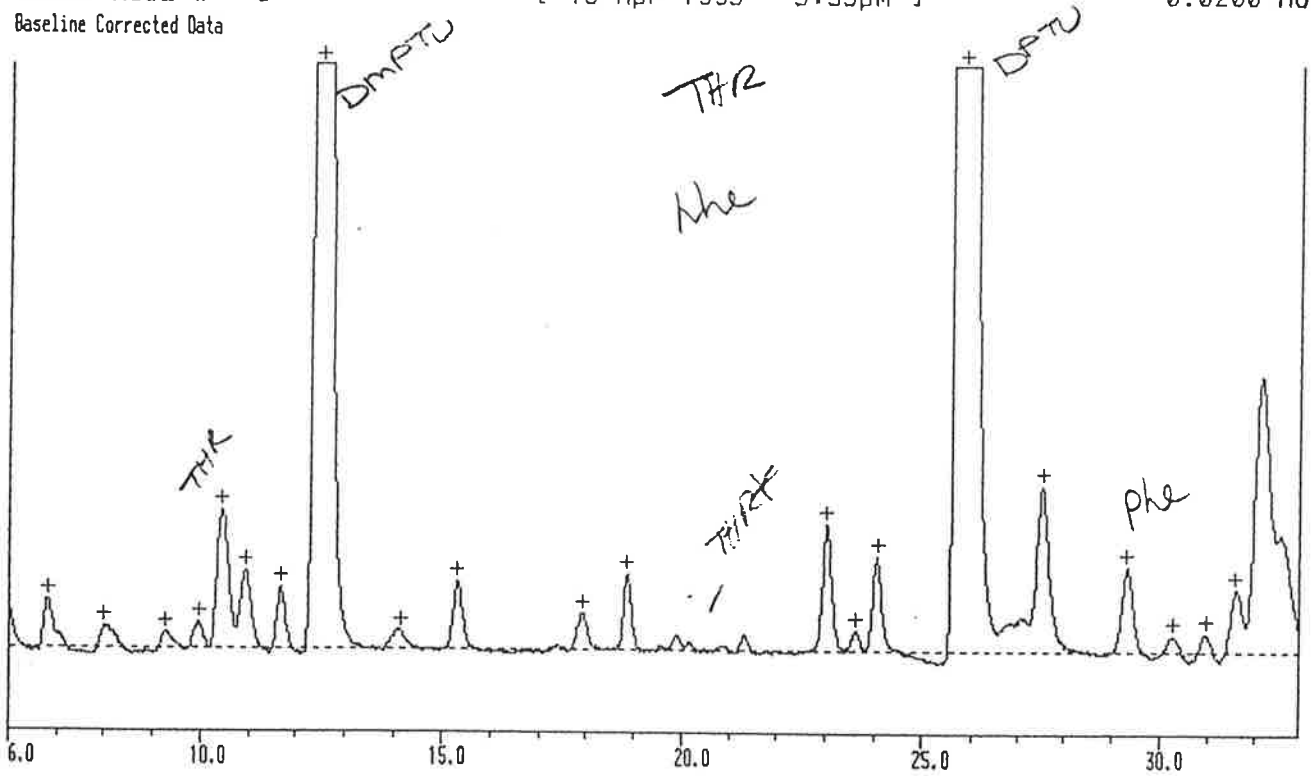
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 6 [13 Apr 1993 3:53pm] 0.0200 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2676			21.33		1161	
ASP	6.82	6.83	3561	5.40	PRO	23.03	22.97	9081	11.40
	7.08		993		MET	23.62	23.53	1512	1.47
ASN	8.02	8.13	1687	1.93	VAL	24.07	23.98	6885	6.89
SER	9.28	9.38	1192	2.02	DPT	25.83	25.73	939835	1335.96
GLN	9.97	10.03	1896	2.25		26.83		2169	
THR	10.47	10.53	10080	21.05		27.05		2546	
GLY	10.95	11.00	5685	7.97		27.12		2529	
GLU	11.70	11.73	4485	6.14	TRP	27.53	27.58	11872	11.76
DMP	12.52	12.63	226946	511.36		27.95		787	
HIS	14.15	14.27	1351	1.28		28.03		525	
ALA	15.32	15.32	4992	5.59	PHE	29.32	29.18	6062	8.63
ARG	17.92	18.05	2680	29.39	ILE	30.28	30.05	1183	2.13
TYR	18.87	18.83	5589	5.29	LYS	30.98	30.78	1348	1.69
	19.92		1089		LEU	31.58	31.45	4497	7.43

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

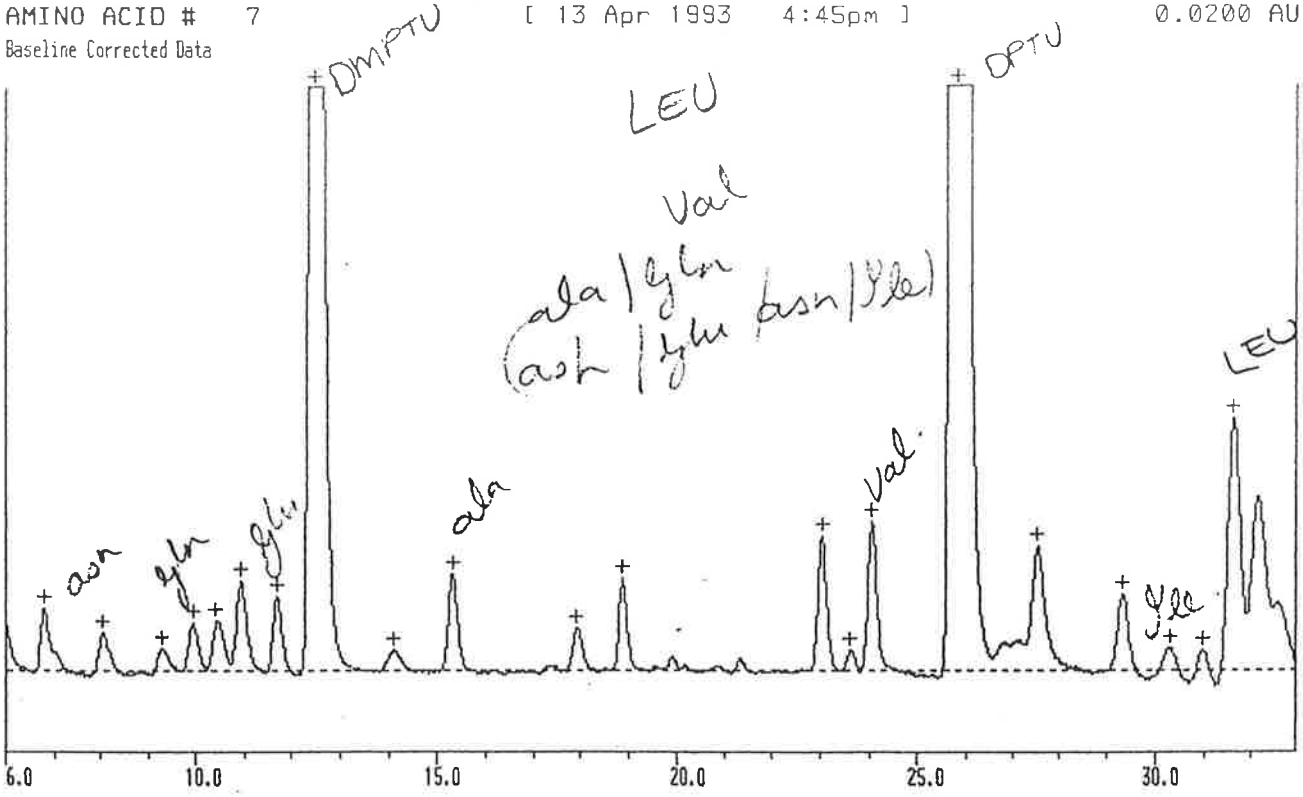
SAMPLE : JAMES STORER PA0 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 7 [13 Apr 1993 4:45pm] 0.0200 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3237		PRO	23.05	22.97	9720	12.21
ASP	6.82	6.83	4593	6.97	MET	23.63	23.53	1490	1.45
ASN	8.05	8.13	2853	3.27	VAL	24.07	23.98	10795	10.81
SER	9.30	9.38	1682	2.84	DPT	25.85	25.73	674688	959.06
GLN	9.98	10.03	3458	4.11		26.87		2133	
THR	10.47	10.53	3602	7.52		27.17		2152	
GLY	10.97	11.00	6535	9.16	TRP	27.55	27.58	9048	8.96
GLU	11.73	11.73	5419	7.41	PHE	29.35	29.18	5493	7.82
DMP	12.52	12.63	158143	356.33	ILE	30.33	30.05	1598	2.88
HIS	14.13	14.27	1492	1.42	LYS	30.98	30.78	1500	1.88
ALA	15.33	15.32	6938	7.77	LEU	31.63	31.45	18160	30.01
ARG	17.93	18.05	3117	34.18		32.15		12456	
TYR	18.88	18.83	6628	6.27					
	19.92		1044						
	21.35		938						

Tabulation threshold : 500 uAU

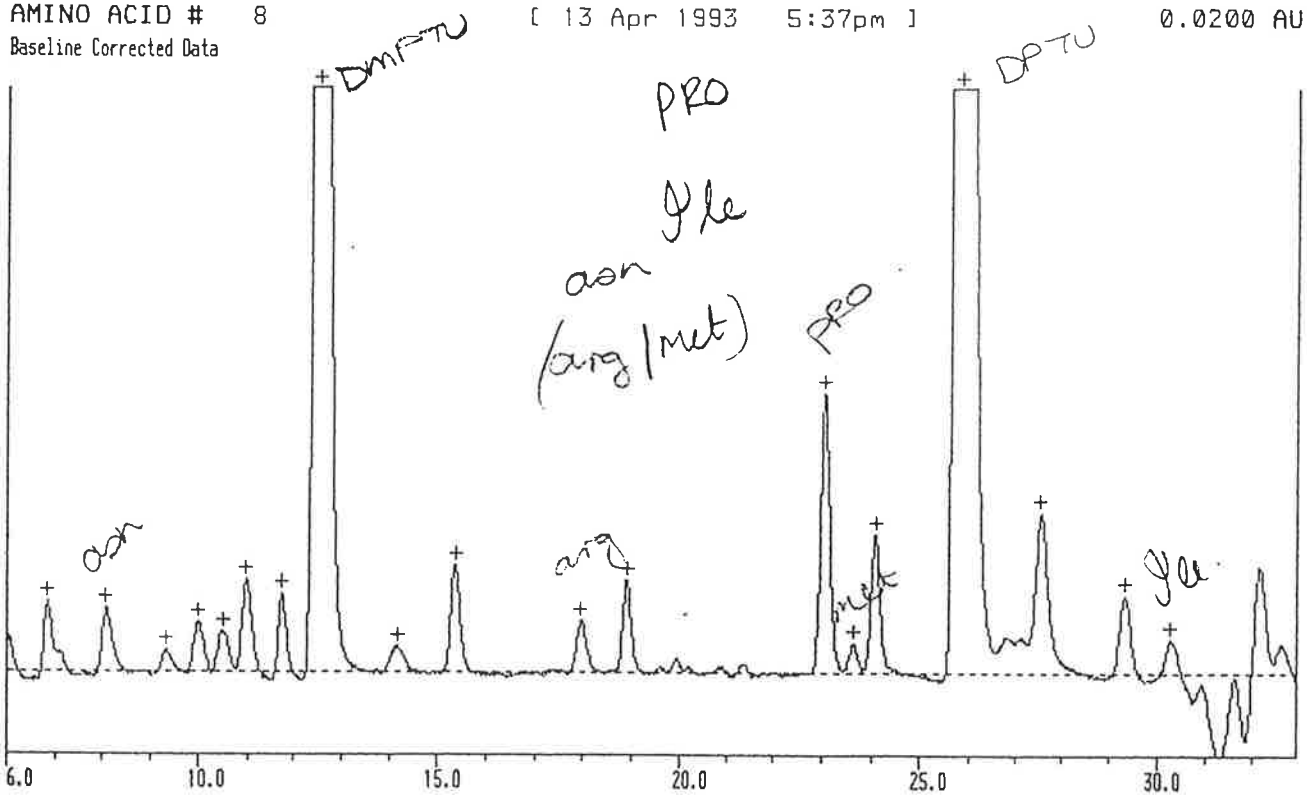
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PA0 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 8 [13 Apr 1993 5:37pm] 0.0200 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.07		2529			21.40		676	
ASP	6.87	6.83	5088	7.72	PRO	23.07	22.97	20088	25.23
	7.15		1392		MET	23.65	23.53	2174	2.11
ASN	8.10	8.13	4644	5.32	VAL	24.10	23.98	10034	10.05
SER	9.35	9.38	1581	2.67	DPT	25.85	25.73	913516	1298.55
GLN	10.03	10.03	3626	4.31		26.80		2714	
THR	10.53	10.53	2952	6.16		27.08		2611	
GLY	11.00	11.00	6638	9.31		27.15		2587	
GLU	11.77	11.73	5616	7.68	TRP	27.55	27.58	11666	11.55
DMP	12.57	12.63	235305	530.20	PHE	29.33	29.18	5656	8.05
HIS	14.20	14.27	1944	1.85	ILE	30.28	30.05	2467	4.45
ALA	15.37	15.32	7725	8.65		32.13		7644	
ARG	17.97	18.05	3775	41.39		32.62		2143	
TYR	18.92	18.83	6736	6.38					
	19.97		991						

Tabulation threshold : 500 uAU

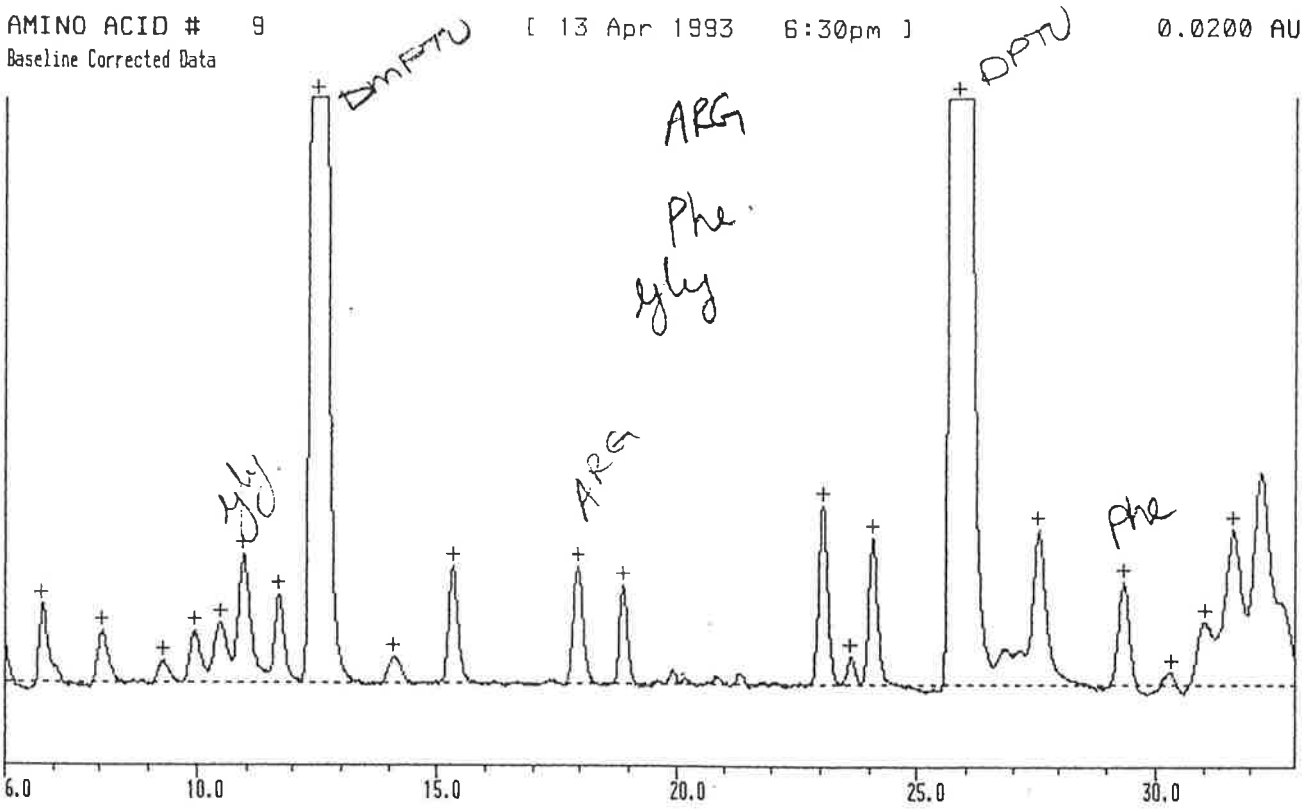
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 9 [13 Apr 1993 6:30pm] 0.0200 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		2512			19.92		988	
ASP	6.80	6.83	5707	8.66		20.85		585	
	7.07		1216			21.38		813	
ASN	8.07	8.13	3734	4.28	PRO	23.07	22.97	12902	16.20
SER	9.32	9.38	1581	2.67	MET	23.62	23.53	2047	1.99
GLN	9.97	10.03	3789	4.50	VAL	24.08	23.98	10584	10.60
THR	10.50	10.53	4420	9.23	DPT	25.85	25.73	847077	1204.11
GLY	10.97	11.00	9237	12.95		26.80		2623	
	11.43		904			26.87		2688	
GLU	11.72	11.73	6451	8.83		27.17		2515	
DMP	12.52	12.63	210398	474.08	TRP	27.55	27.58	11184	11.07
HIS	14.13	14.27	1970	1.87		28.05		823	
ALA	15.33	15.32	8440	9.45	PHE	29.33	29.18	7396	10.53
ARG	17.95	18.05	8378	91.87	ILE	30.32	30.05	856	1.54
TYR	18.88	18.83	7156	6.77	LYS	31.05	30.78	4581	5.75

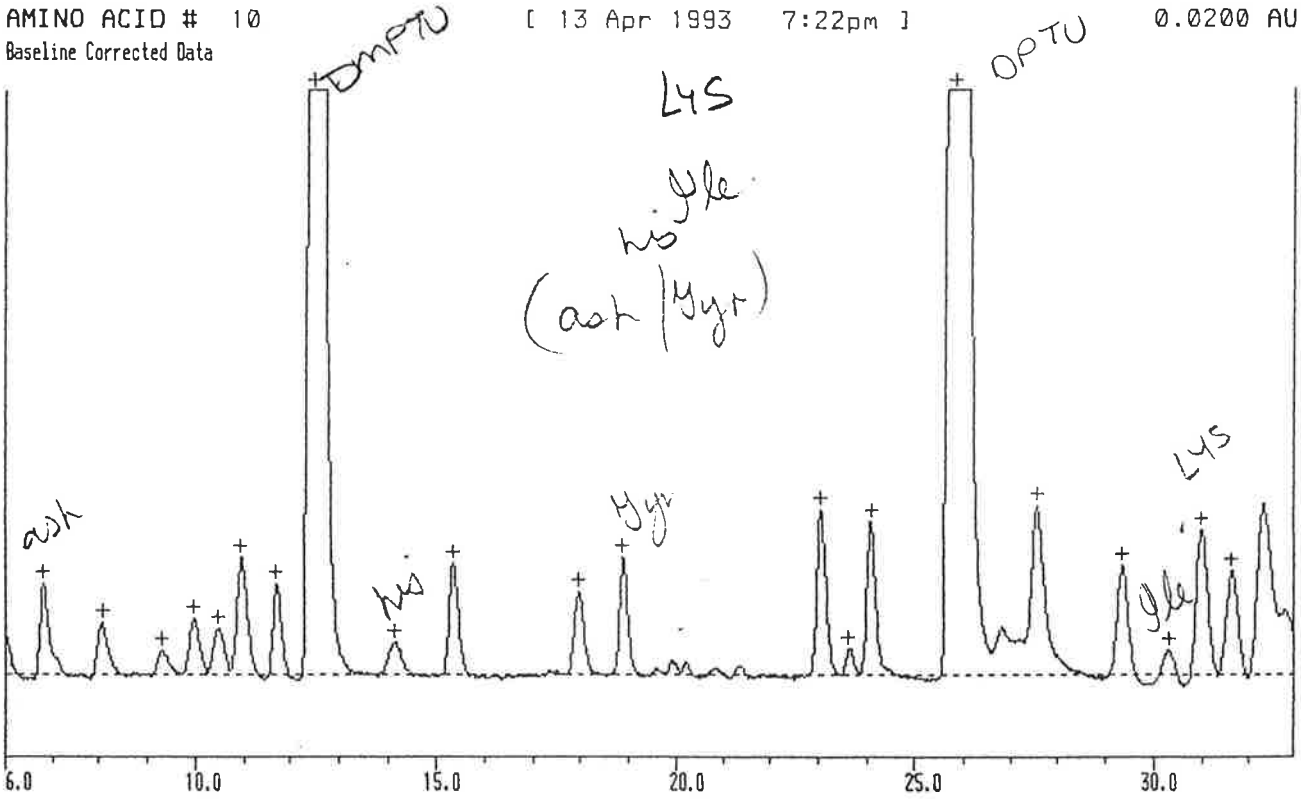
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 10 [13 Apr 1993 7:22pm] 0.0200 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2640			21.35		684	
ASP	6.82	6.83	6513	9.88	PRO	23.07	22.97	11846	14.88
ASN	8.08	8.13	3732	4.27	MET	23.63	23.53	1987	1.93
SER	9.32	9.38	1795	3.04	VAL	24.10	23.98	11044	11.06
GLN	9.98	10.03	4084	4.85	OPT	25.87	25.73	918076	1305.04
THR	10.50	10.53	3424	7.15		26.82		3472	
GLY	10.97	11.00	8455	11.86		27.20		2556	
GLU	11.72	11.73	6588	9.01	TRP	27.57	27.58	12160	12.04
DMP	12.53	12.63	229516	517.15	PHE	29.35	29.18	7802	11.11
HIS	14.17	14.27	2409	2.29	ILE	30.32	30.05	1742	3.14
ALA	15.33	15.32	7980	8.94	LYS	31.00	30.78	10442	13.10
ARG	17.95	18.05	6033	66.16	LEU	31.63	31.45	7382	12.20
TYR	18.90	18.83	8359	7.91		32.32		12328	
	19.92		1065						
	20.23		960						

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

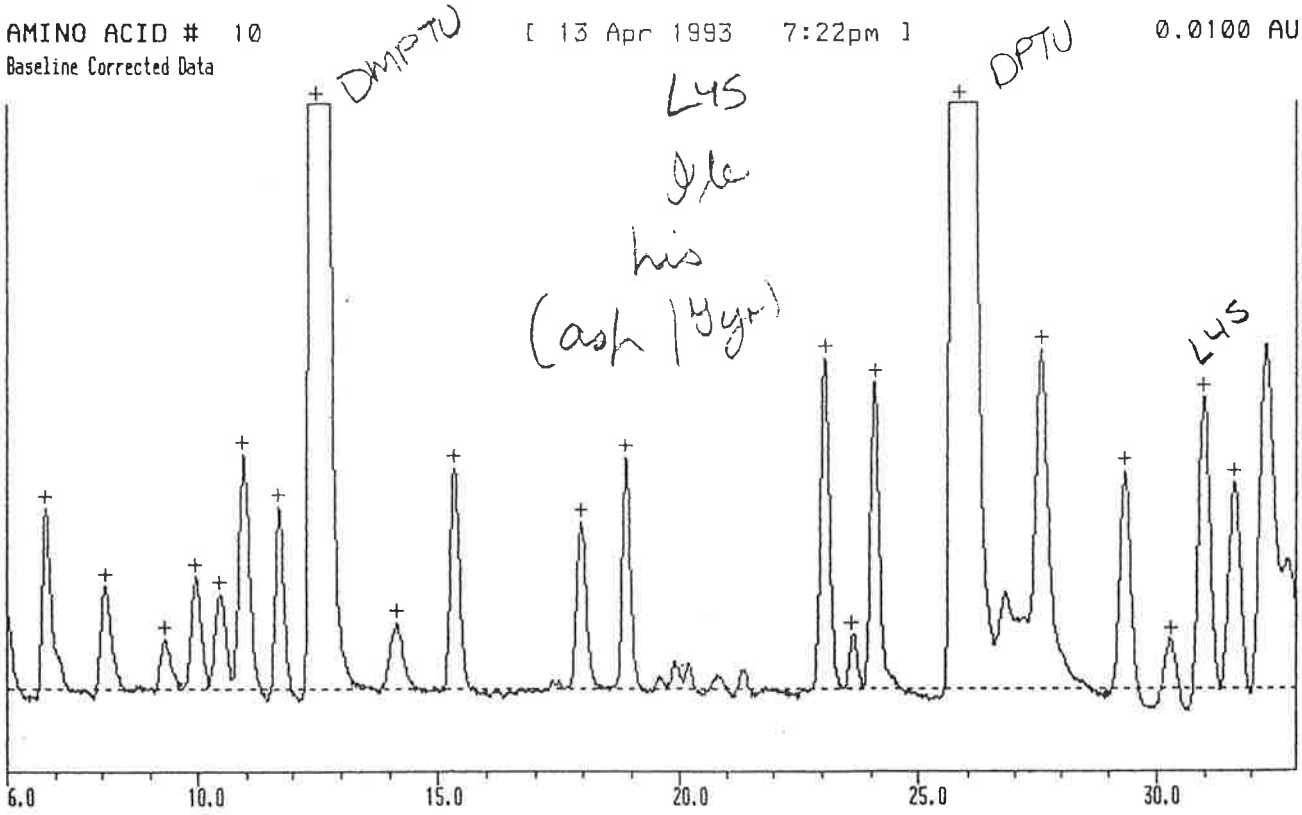
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 10 [13 Apr 1993 7:22pm] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2640			21.35		684	
ASP	6.82	6.83	6513	9.88	PRO	23.07	22.97	11846	14.88
ASN	8.08	8.13	3732	4.27	MET	23.63	23.53	1987	1.93
SER	9.32	9.38	1795	3.04	VAL	24.10	23.98	11044	11.06
GLN	9.98	10.03	4084	4.85	DPT	25.87	25.73	918076	1305.04
THR	10.50	10.53	3424	7.15		26.82		3472	
GLY	10.97	11.00	8455	11.86		27.20		2556	
GLU	11.72	11.73	6588	9.01	TRP	27.57	27.58	12160	12.04
DMP	12.53	12.63	229516	517.15	PHE	29.35	29.18	7802	11.11
HIS	14.17	14.27	2409	2.29	ILE	30.32	30.05	1742	3.14
ALA	15.33	15.32	7980	8.94	LYS	31.00	30.78	10442	13.10
ARG	17.95	18.05	6033	66.16	LEU	31.63	31.45	7382	12.20
TYR	18.90	18.83	8359	7.91		32.32		12328	
	19.92		1065						
	20.23		960						

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

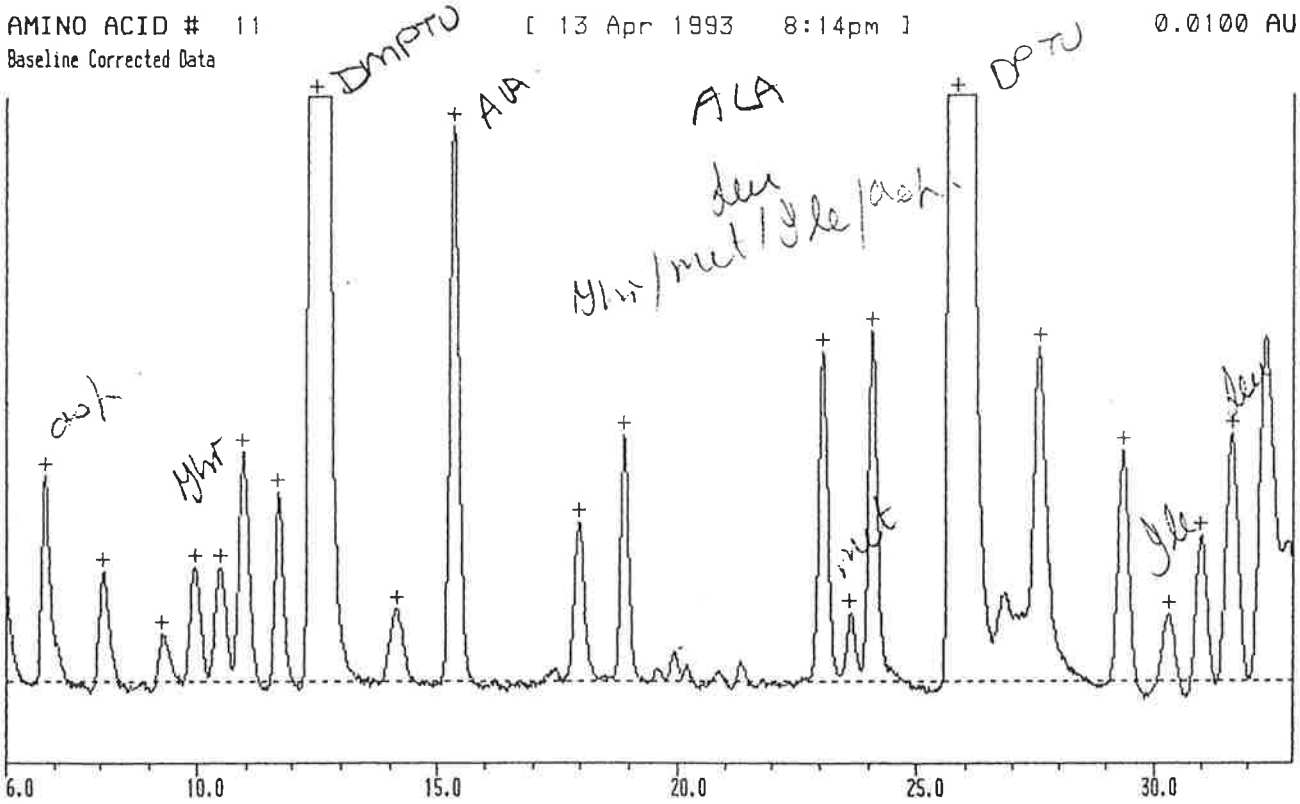
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 11 [13 Apr 1993 8:14pm] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		3074			21.37		703	
ASP	6.82	6.83	7380	11.20	PRO	23.07	22.97	11820	14.84
ASN	8.07	8.13	4005	4.59	MET	23.63	23.53	2455	2.38
SER	9.28	9.38	1730	2.93	VAL	24.08	23.98	12492	12.51
GLN	9.98	10.03	4171	4.96	DPT	25.87	25.73	894777	1271.92
THR	10.48	10.53	4120	8.61		26.87		3180	
GLY	10.97	11.00	8294	11.63		27.08		2400	
GLU	11.73	11.73	6787	9.29		27.20		2472	
DMP	12.53	12.63	218695	492.77	TRP	27.57	27.58	11956	11.84
HIS	14.15	14.27	2620	2.49	PHE	29.35	29.18	8275	11.78
ALA	15.35	15.32	19884	22.27	ILE	30.30	30.05	2428	4.38
ARG	17.97	18.05	5712	62.63	LYS	31.02	30.78	5200	6.53
TYR	18.90	18.83	8810	8.34	LEU	31.65	31.45	8808	14.56
	19.95		1111			32.37		12316	
	20.20		624						

Tabulation threshold : 500 uAU

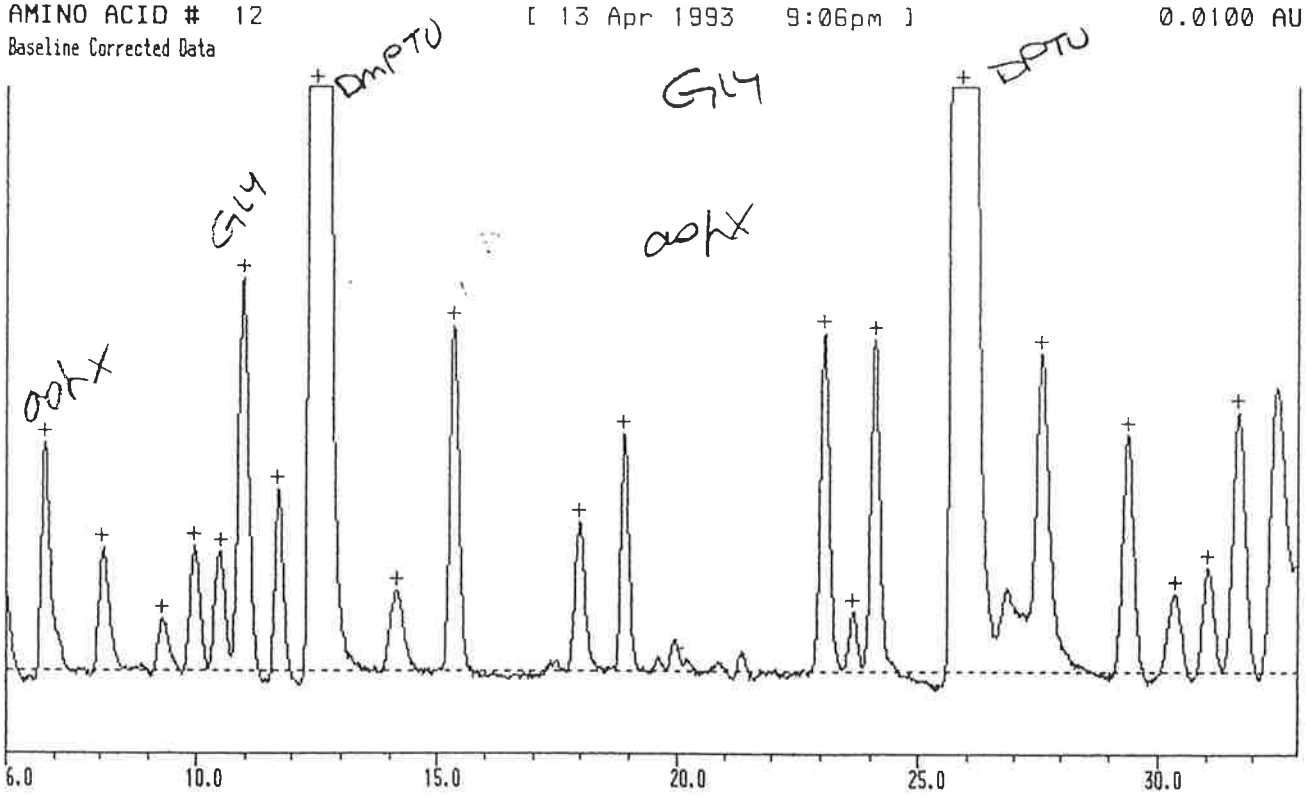
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PA0 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 12 [13 Apr 1993 9:06pm] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2656			19.97		1164	
ASP	6.83	6.83	8186	12.42		21.38		722	
ASN	8.07	8.13	4396	5.03	PRO	23.08	22.97	12132	15.24
SER	9.30	9.38	1884	3.19	MET	23.67	23.53	2176	2.11
GLN	9.98	10.03	4526	5.38	VAL	24.10	23.98	11930	11.94
THR	10.52	10.53	4305	8.99	DPT	25.88	25.73	891374	1267.08
GLY	10.98	11.00	14145	19.84		26.87		3036	
GLU	11.73	11.73	6508	8.91		27.22		2138	
DMP	12.53	12.63	218428	492.17	TRP	27.58	27.58	11426	11.31
	13.20		568			28.03		943	
HIS	14.15	14.27	2916	2.77	PHE	29.38	29.18	8520	12.13
ALA	15.35	15.32	12352	13.83	ILE	30.35	30.05	2851	5.14
ARG	17.97	18.05	5359	58.76	LYS	31.03	30.78	3792	4.76
TYR	18.90	18.83	8560	8.10	LEU	31.68	31.45	9314	15.39
	19.60		516			32.50		10248	

Tabulation threshold : 500 uAU

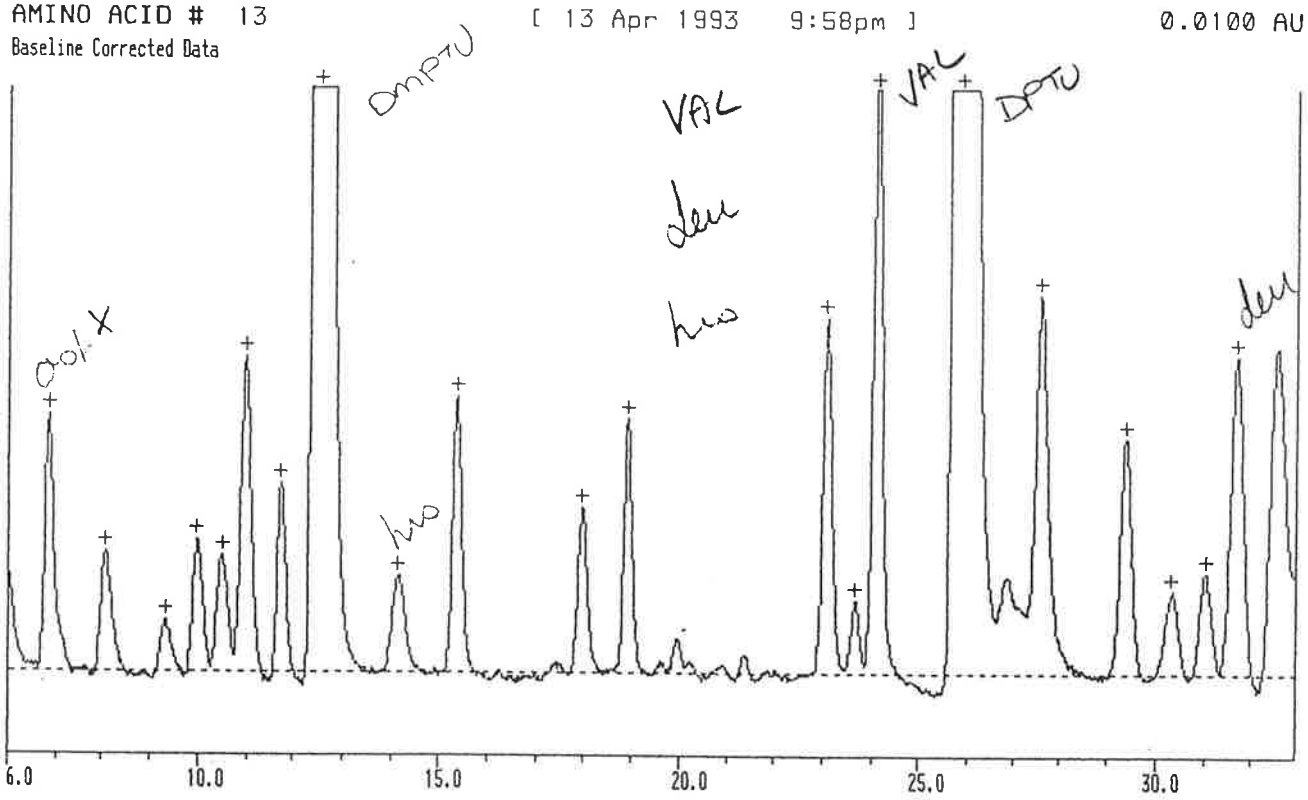
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 13 [13 Apr 1993 9:58pm] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		3482		PRO	23.08	22.97	12712	15.96
ASP	6.85	6.83	9295	14.10	MET	23.65	23.53	2618	2.54
ASN	8.08	8.13	4389	5.03	VAL	24.12	23.98	23385	23.41
SER	9.33	9.38	1932	3.27	DPT	25.88	25.73	1071816	1523.57
GLN	10.00	10.03	4764	5.66		26.62		2044	
THR	10.53	10.53	4216	8.81		26.87		3506	
GLY	11.00	11.00	11376	15.95	TRP	27.58	27.58	13552	13.42
GLU	11.75	11.73	6818	9.33		28.05		909	
DMP	12.55	12.63	249129	561.35	PHE	29.38	29.18	8431	12.00
HIS	14.18	14.27	3463	3.29	ILE	30.33	30.05	2959	5.33
ALA	15.37	15.32	9921	11.11	LYS	31.03	30.78	3636	4.56
ARG	17.97	18.05	5928	65.00	LEU	31.67	31.45	11371	18.79
TYR	18.92	18.83	9134	8.65		32.57		11683	
	19.97		1269						
	21.35		686						

Tabulation threshold : 500 uAU

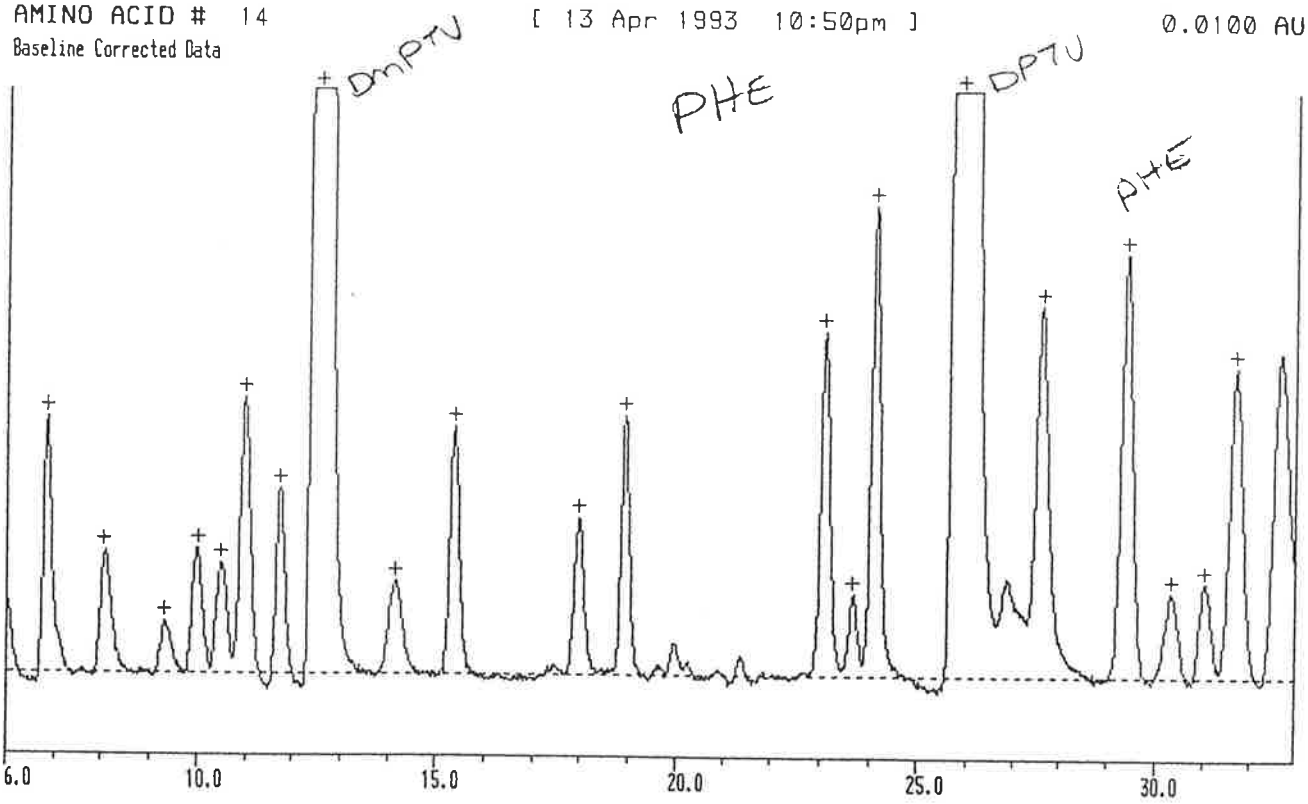
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 14 [13 Apr 1993 10:50pm] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.07		2541		PRO	23.08	22.97	12410	15.58
ASP	6.85	6.83	9230	14.01	MET	23.67	23.53	2954	2.87
ASN	8.07	8.13	4416	5.06	VAL	24.12	23.98	16869	16.89
SER	9.32	9.38	1891	3.20	DPT	25.90	25.73	1050897	1493.84
GLN	10.02	10.03	4514	5.36		26.87		3552	
THR	10.50	10.53	4000	8.36	TRP	27.60	27.58	13296	13.17
GLY	10.98	11.00	9955	13.96	PHE	29.38	29.18	15211	21.66
GLU	11.77	11.73	6679	9.14	ILE	30.32	30.05	3045	5.49
DMP	12.55	12.63	226521	510.40	LYS	31.03	30.78	3448	4.33
HIS	14.17	14.27	3355	3.19	LEU	31.68	31.45	11124	18.38
ALA	15.37	15.32	8913	9.98		32.63		11671	
ARG	17.97	18.05	5625	61.68					
TYR	18.93	18.83	9333	8.83					
	19.97		1171						
	21.38		710						

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

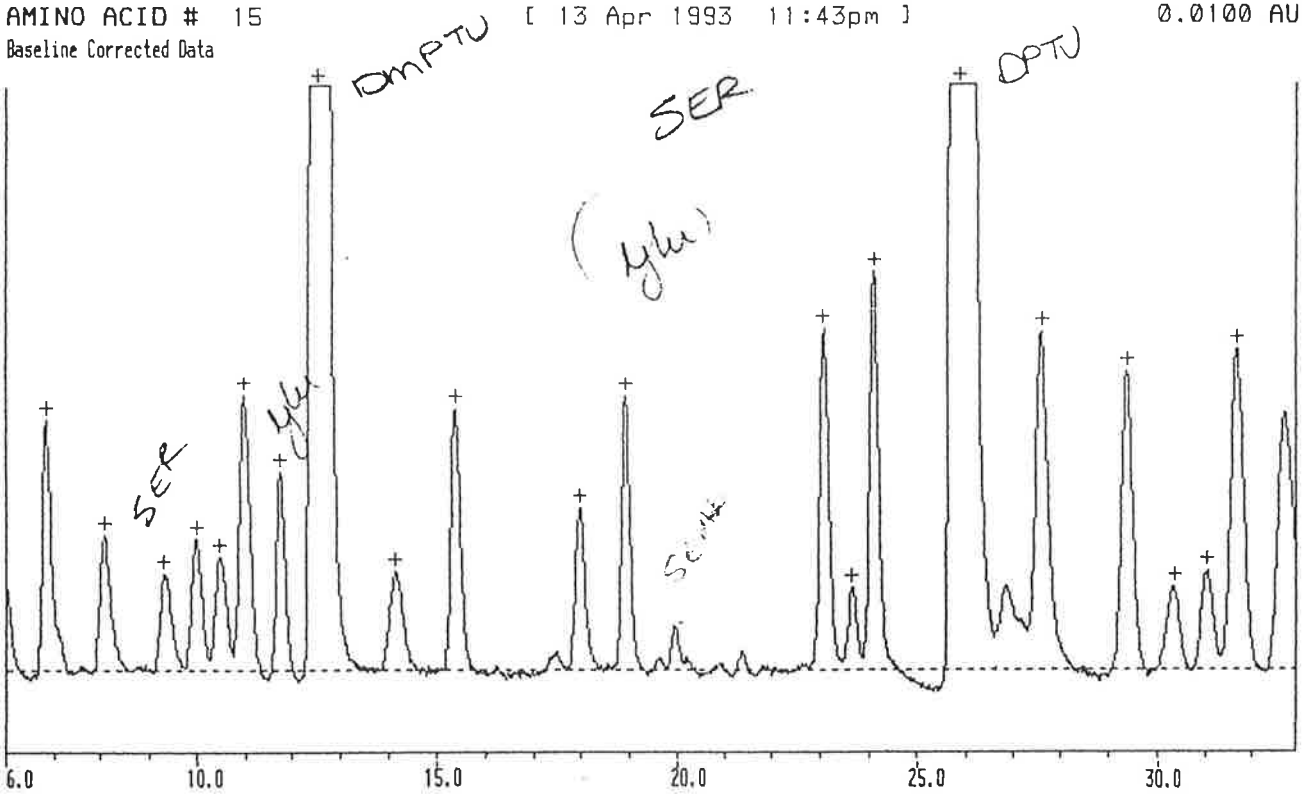
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 15 [13 Apr 1993 11:43pm] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2937			21.40		664	
ASP	6.85	6.83	8980	13.63	PRO	23.08	22.97	12256	15.39
ASN	8.10	8.13	4891	5.60	MET	23.65	23.53	3021	2.93
SER	9.32	9.38	3468	5.86	VAL	24.12	23.98	14282	14.30
GLN	10.02	10.03	4742	5.63	DPT	25.88	25.73	970132	1379.03
THR	10.50	10.53	4104	8.57		26.85		3031	
GLY	11.00	11.00	9852	13.82		27.17		1807	
GLU	11.77	11.73	7113	9.73	TRP	27.60	27.58	12060	11.94
DMP	12.55	12.63	220140	496.03	PHE	29.38	29.18	10660	15.18
HIS	14.17	14.27	3549	3.37	ILE	30.35	30.05	3004	5.41
ALA	15.38	15.32	9364	10.49	LYS	31.05	30.78	3588	4.50
	17.48		640		LEU	31.67	31.45	11457	18.94
ARG	17.97	18.05	5834	63.97					
TYR	18.92	18.83	9760	9.24					
	19.93		1588						

Tabulation threshold : 500 uAU

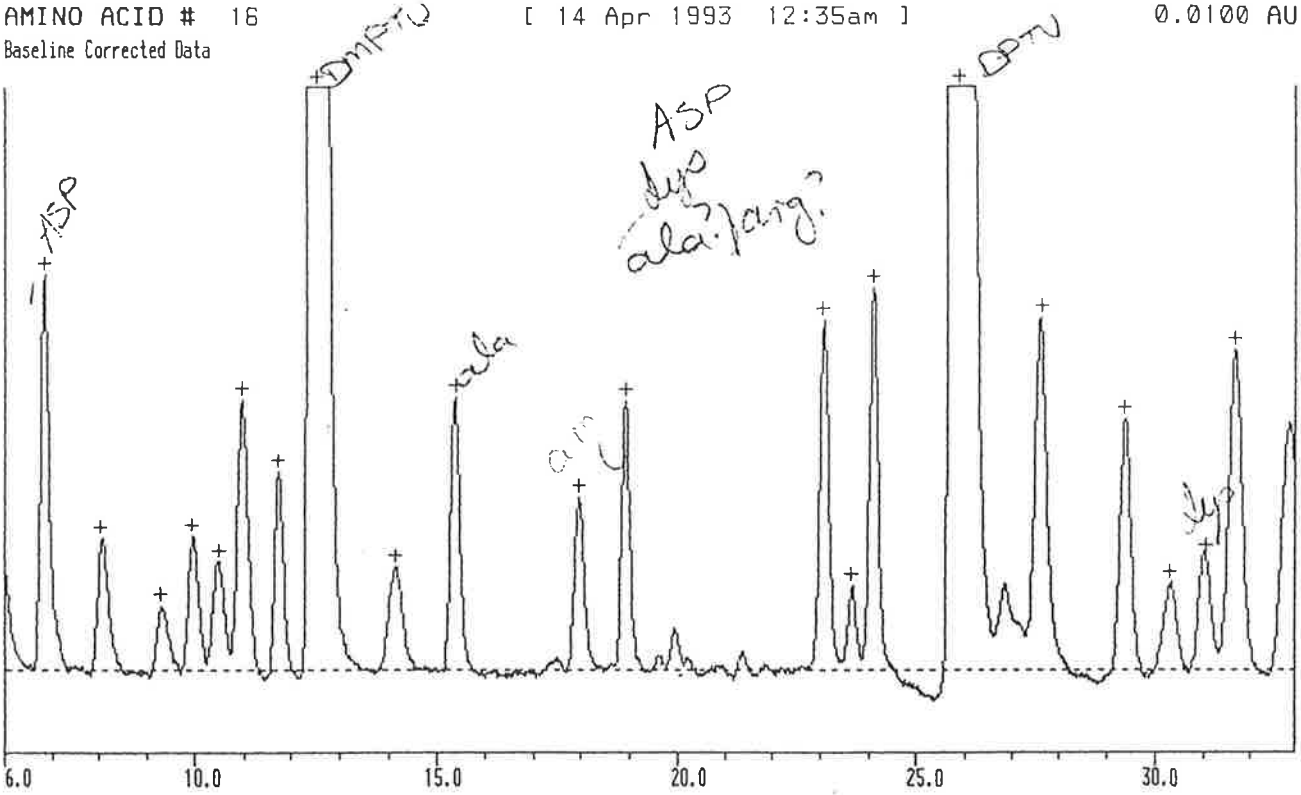
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 16 [14 Apr 1993 12:35am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		3434			21.40		688	
ASP	6.85	6.83	14251	21.62	PRO	23.08	22.97	12588	15.81
ASN	8.07	8.13	4812	5.51	MET	23.67	23.53	3048	2.96
SER	9.32	9.38	2299	3.89	VAL	24.12	23.98	13732	13.75
GLN	10.00	10.03	4831	5.74	DPT	25.88	25.73	1066555	1516.10
THR	10.52	10.53	3943	8.23		26.87		3108	
GLY	10.98	11.00	9777	13.71	TRP	27.60	27.58	12676	12.55
GLU	11.75	11.73	7149	9.78	PHE	29.37	29.18	9043	12.88
DMP	12.55	12.63	225460	508.01	ILE	30.33	30.05	3163	5.70
HIS	14.18	14.27	3739	3.55	LYS	31.07	30.78	4104	5.15
ALA	15.37	15.32	9854	11.04	LEU	31.67	31.45	11500	19.01
ARG	17.95	18.05	6218	68.18					
TYR	18.93	18.83	9744	9.22					
	19.67		506						
	19.95		1516						

Tabulation threshold : 500 uAU

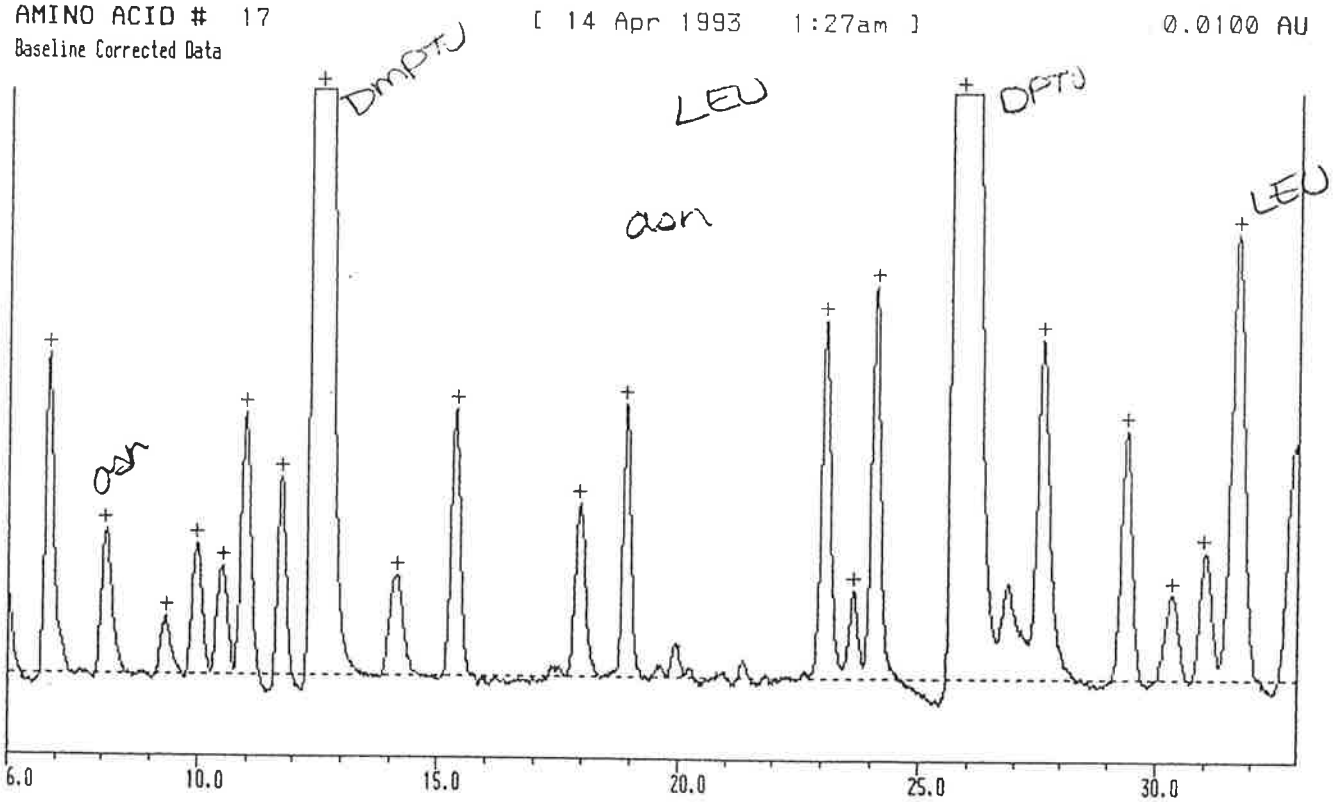
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 17 [14 Apr 1993 1:27am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2784		PRO	23.07	22.97	12823	16.10
ASP	6.85	6.83	11440	17.36	MET	23.65	23.53	3213	3.12
ASN	8.07	8.13	5215	5.97	VAL	24.10	23.98	14020	14.04
SER	9.33	9.38	2097	3.55	DPT	25.87	25.73	1039000	1476.93
GLN	10.00	10.03	4735	5.63		26.87		3470	
THR	10.52	10.53	3900	8.14		27.13		1800	
GLY	10.98	11.00	9396	13.18		27.25		1447	
GLU	11.75	11.73	7106	9.72	TRP	27.58	27.58	12158	12.04
DMP	12.55	12.63	213770	481.67	PHE	29.38	29.18	8930	12.72
HIS	14.17	14.27	3650	3.47	ILE	30.33	30.05	3048	5.49
ALA	15.37	15.32	9542	10.69	LYS	31.02	30.78	4569	5.73
ARG	17.93	18.05	6261	68.66	LEU	31.67	31.45	15955	26.37
TYR	18.90	18.83	9758	9.24					
	19.95		1267						
	21.37		708						

Tabulation threshold : 500 uAU

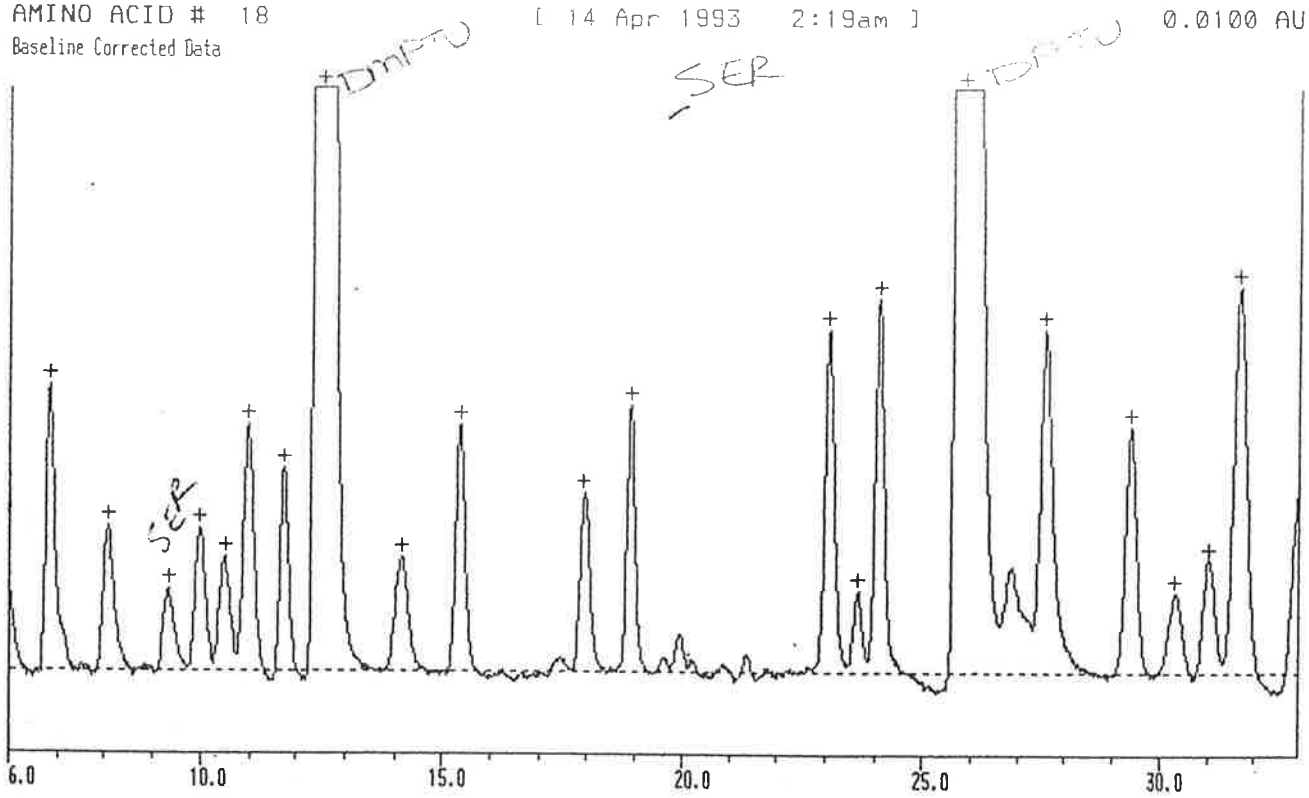
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 18 [14 Apr 1993 2:19am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2776			19.95		1411	
ASP	6.85	6.83	10300	15.63		21.35		648	
ASN	8.08	8.13	5217	5.97	PRO	23.08	22.97	12384	15.55
SER	9.33	9.38	2954	5.00	MET	23.67	23.53	2959	2.87
GLN	10.00	10.03	5186	6.16	VAL	24.10	23.98	13435	13.45
THR	10.52	10.53	4108	8.58	DPT	25.88	25.73	1037716	1475.10
GLY	10.98	11.00	8916	12.50		26.83		3789	
GLU	11.75	11.73	7293	9.98	TRP	27.58	27.58	12384	12.26
DMP	12.55	12.63	231801	522.30	PHE	29.38	29.18	8880	12.64
HIS	14.15	14.27	4137	3.93	ILE	30.32	30.05	2937	5.29
ALA	15.37	15.32	8920	9.99	LYS	31.05	30.78	4075	5.11
	17.47		501		LEU	31.68	31.45	13924	23.01
ARG	17.93	18.05	6434	70.55					
TYR	18.92	18.83	9657	9.14					
	19.60		554						

Tabulation threshold : 500 uAU

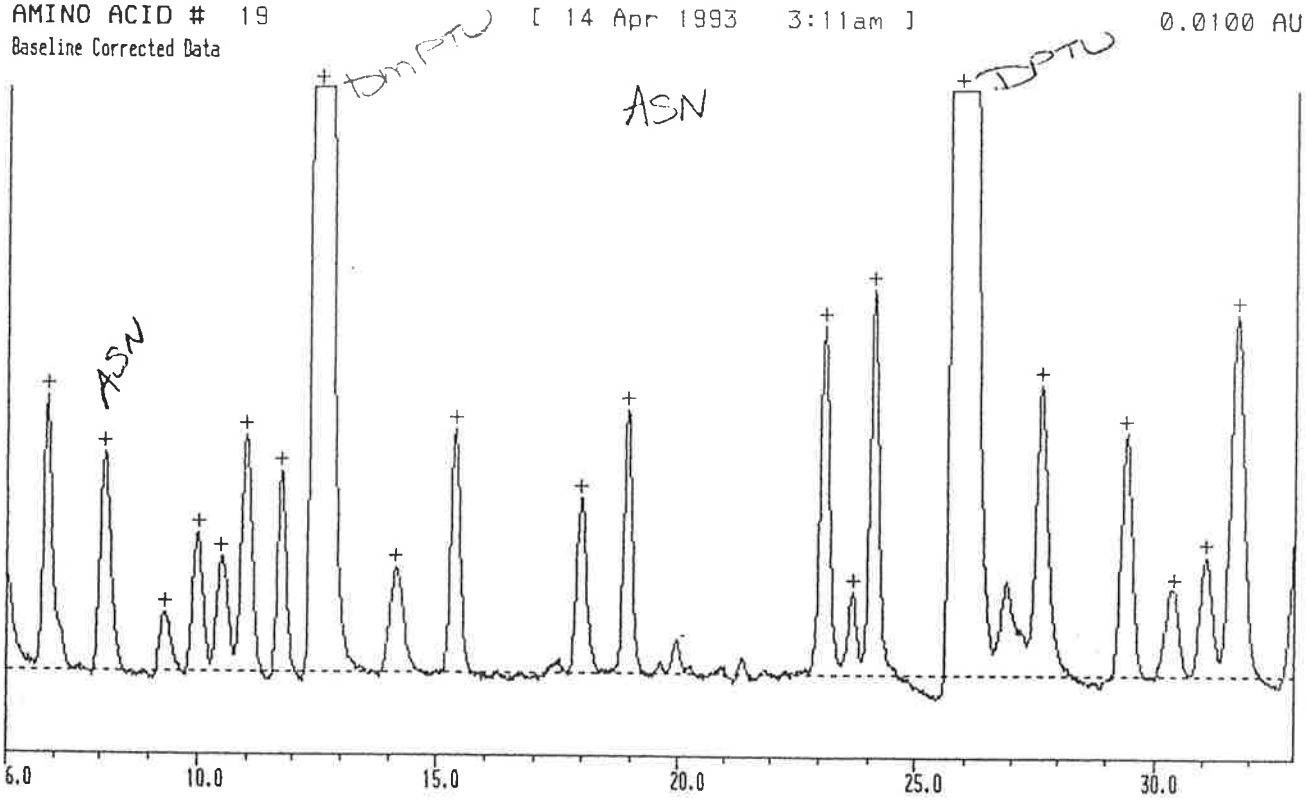
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 19 [14 Apr 1993 3:11am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		3343			19.98		1238	
	6.48		535			21.38		631	
ASP	6.85	6.83	9936	15.08	PRO	23.10	22.97	12547	15.76
ASN	8.10	8.13	7876	9.02	MET	23.68	23.53	2995	2.91
SER	9.35	9.38	2124	3.59	VAL	24.12	23.98	13857	13.87
GLN	10.02	10.03	4987	5.93	DPT	25.90	25.73	905745	1287.51
THR	10.50	10.53	4123	8.61		26.88		3386	
GLY	11.00	11.00	8546	11.99		27.15		1687	
GLU	11.77	11.73	7272	9.95	TRP	27.62	27.58	10401	10.30
DMP	12.55	12.63	207360	467.23	PHE	29.40	29.18	8719	12.41
HIS	14.15	14.27	3770	3.58	ILE	30.40	30.05	3093	5.57
ALA	15.38	15.32	8786	9.84	LYS	31.08	30.78	4264	5.35
	17.52		513		LEU	31.70	31.45	12928	21.37
ARG	17.97	18.05	6328	69.39					
TYR	18.93	18.83	9523	9.01					

Tabulation threshold : 500 uAU

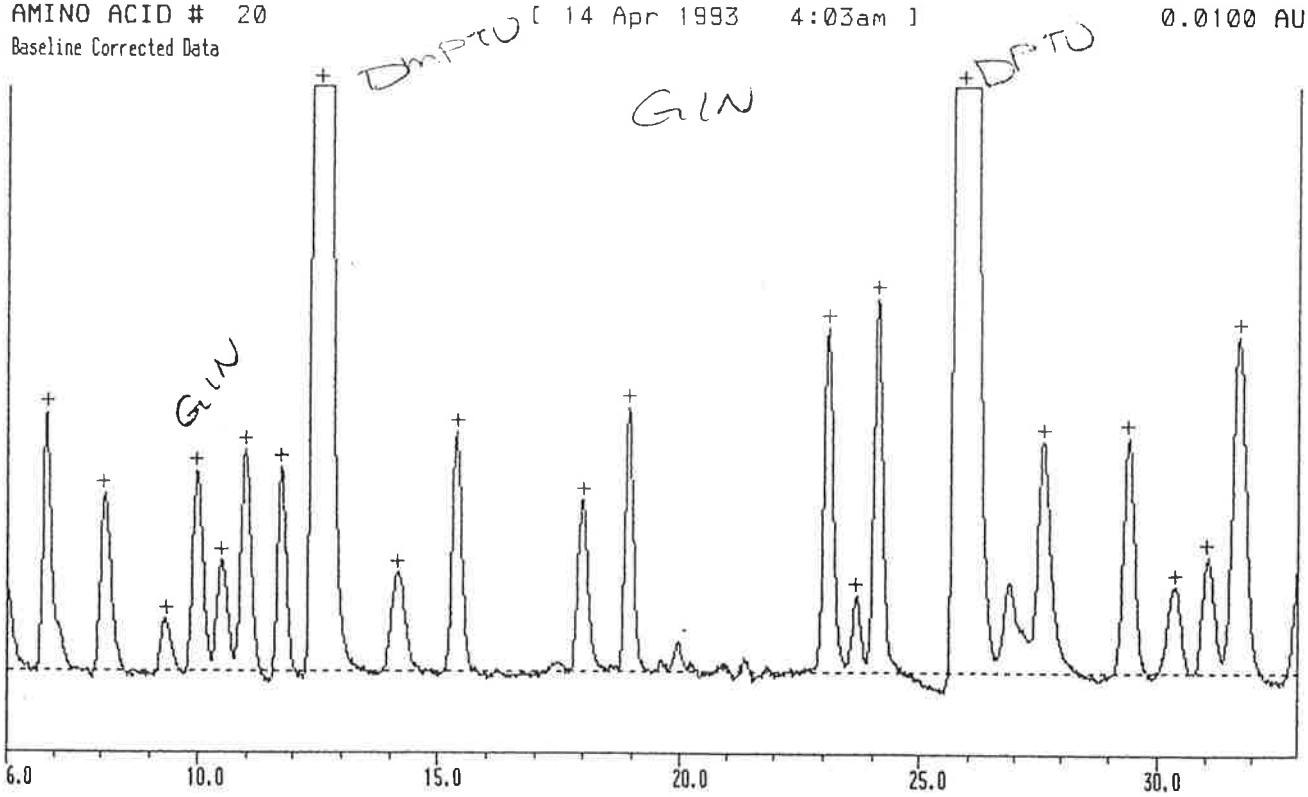
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 20 [14 Apr 1993 4:03am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2851		PRO	23.10	22.97	12405	15.58
ASP	6.85	6.83	9280	14.08	MET	23.67	23.53	2803	2.72
ASN	8.07	8.13	6417	7.35	VAL	24.12	23.98	13360	13.38
SER	9.33	9.38	1922	3.25	DPT	25.90	25.73	701964	997.83
GLN	10.00	10.03	7152	8.50		26.90		3252	
THR	10.50	10.53	3967	8.28		27.18		1584	
GLY	11.00	11.00	7980	11.19	TRP	27.62	27.58	8313	8.23
GLU	11.77	11.73	7341	10.05	PHE	29.40	29.18	8472	12.06
DMP	12.55	12.63	167284	376.93	ILE	30.38	30.05	3139	5.66
HIS	14.18	14.27	3561	3.38	LYS	31.05	30.78	4188	5.25
ALA	15.37	15.32	8647	9.68	LEU	31.70	31.45	12093	19.99
ARG	17.98	18.05	6165	67.61					
TYR	18.93	18.83	9516	9.01					
	19.98		1104						
	21.37		511						

Tabulation threshold : 500 uAU

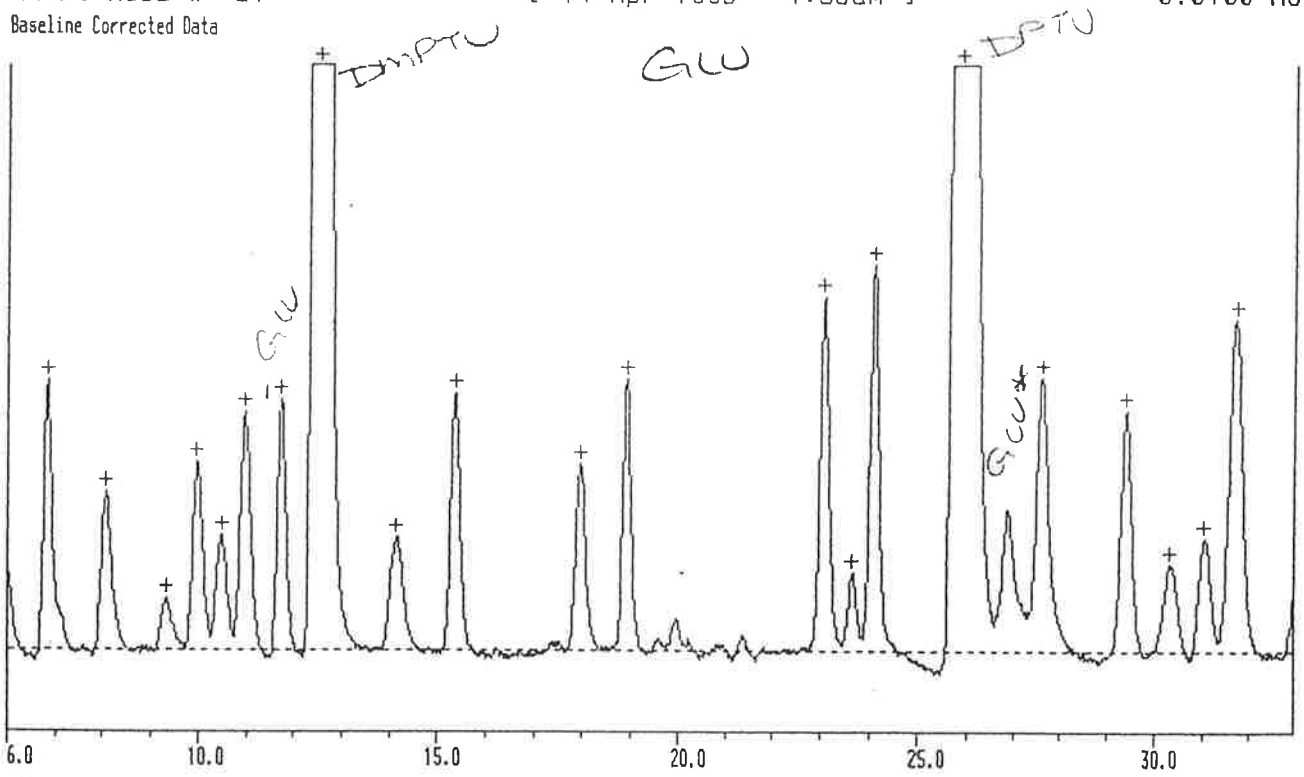
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 21 [14 Apr 1993 4:56am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2709		MET	23.67	23.53	2820	2.74
ASP	6.85	6.83	9638	14.62	VAL	24.10	23.98	13802	13.82
ASN	8.08	8.13	5664	6.48	DPT	25.88	25.73	867427	1233.04
SER	9.33	9.38	1876	3.17		26.88		5097	
GLN	10.00	10.03	6763	8.04	TRP	27.60	27.58	9794	9.70
THR	10.52	10.53	4159	8.69	PHE	29.40	29.18	8596	12.24
GLY	10.98	11.00	8587	12.04	ILE	30.33	30.05	3160	5.70
GLU	11.77	11.73	8952	12.25	LYS	31.05	30.78	4072	5.11
DMP	12.55	12.63	195583	440.69	LEU	31.70	31.45	11908	19.68
HIS	14.17	14.27	4089	3.88					
ALA	15.37	15.32	9177	10.28					
ARG	17.95	18.05	6679	73.24					
TYR	18.92	18.83	9729	9.21					
	19.98		1159						
PRO	23.08	22.97	12660	15.90					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

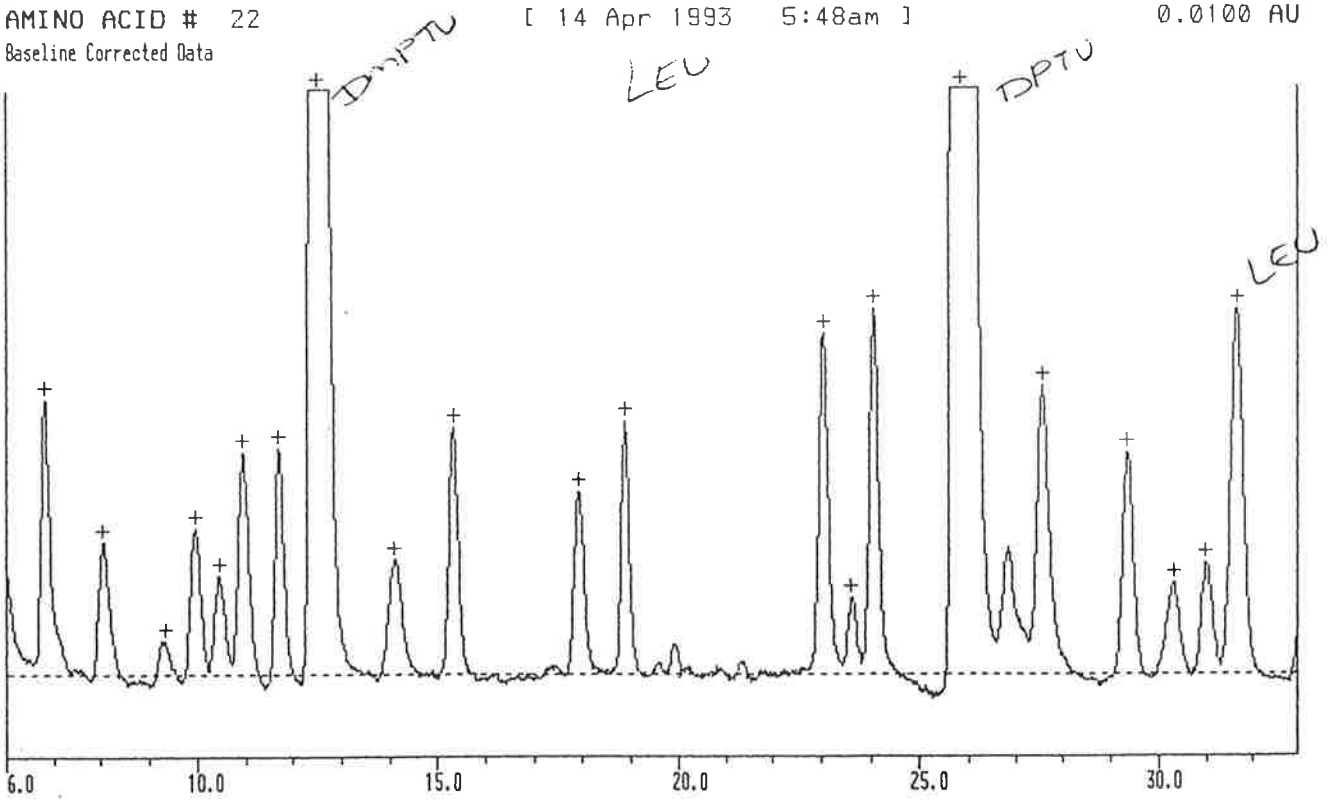
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 22 [14 Apr 1993 5:48am] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3568		PRO	23.05	22.97	12228	15.36
	6.52		585		MET	23.63	23.53	2798	2.72
ASP	6.83	6.83	9960	15.11	VAL	24.07	23.98	13132	13.15
ASN	8.07	8.13	4795	5.49	DPT	25.85	25.73	929978	1321.95
SER	9.33	9.38	1257	2.13		26.85		4564	
GLN	9.97	10.03	5316	6.32	TRP	27.57	27.58	10336	10.24
THR	10.48	10.53	3595	7.51		27.98		741	
GLY	10.97	11.00	8025	11.25	PHE	29.35	29.18	7980	11.36
GLU	11.73	11.73	8184	11.20	ILE	30.32	30.05	3273	5.90
DMP	12.52	12.63	180427	406.54	LYS	31.00	30.78	3986	5.00
HIS	14.13	14.27	4164	3.96	LEU	31.65	31.45	13068	21.60
ALA	15.33	15.32	8908	9.98					
ARG	17.92	18.05	6614	72.53					
TYR	18.88	18.83	9103	8.62					
	19.93	-	1137						

Tabulation threshold : 500 uAU

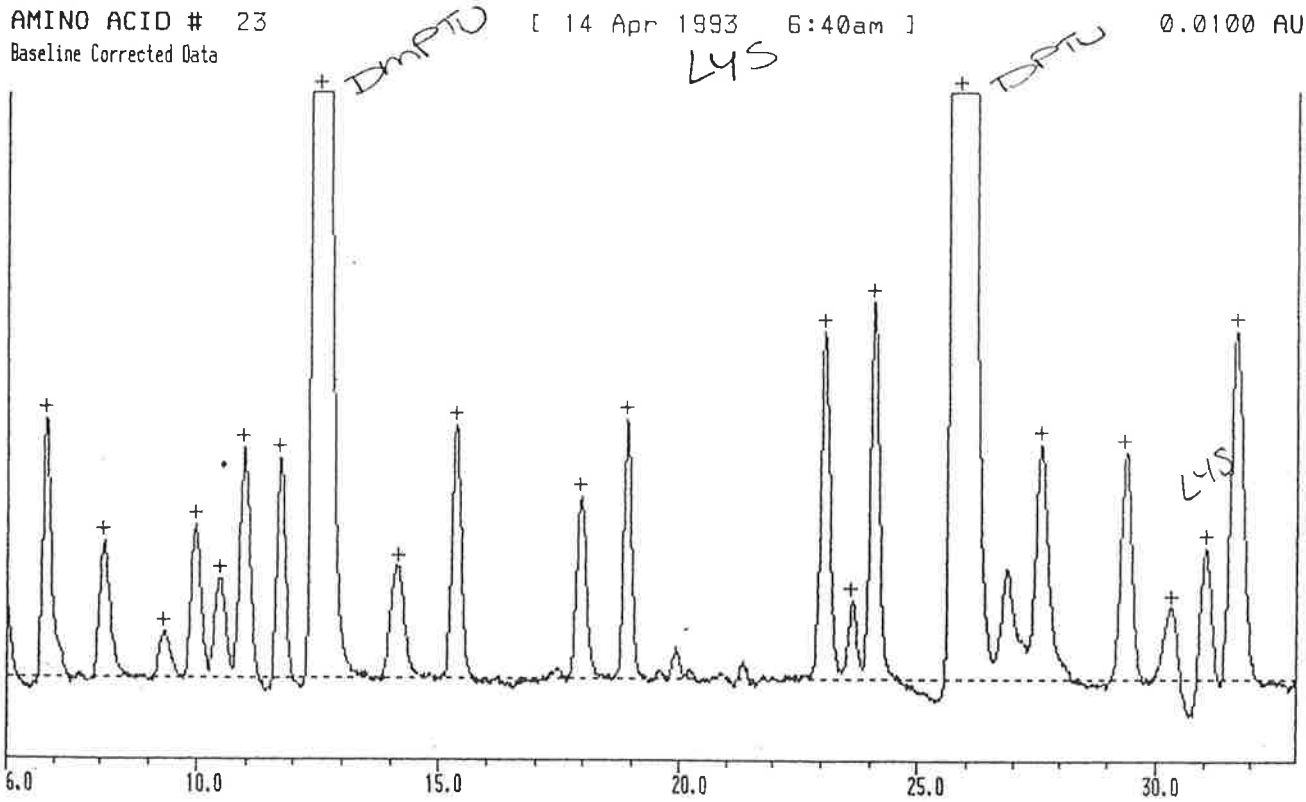
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 23 [14 Apr 1993 6:40am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2500		PRO	23.07	22.97	12381	15.55
ASP	6.83	6.83	9247	14.03	MET	23.63	23.53	2824	2.74
ASN	8.07	8.13	4958	5.68	VAL	24.08	23.98	13471	13.49
SER	9.32	9.38	1677	2.84	DPT	25.87	25.73	759856	1080.13
GLN	9.98	10.03	5532	6.57		26.85		4024	
THR	10.50	10.53	3580	7.48		27.17		1444	
GLY	10.97	11.00	8234	11.55	TRP	27.58	27.58	8431	8.35
GLU	11.73	11.73	7867	10.76	PHE	29.37	29.18	8114	11.55
DMP	12.53	12.63	162628	366.44	ILE	30.33	30.05	2544	4.58
HIS	14.17	14.27	4005	3.80	LYS	31.03	30.78	4704	5.90
ALA	15.35	15.32	9055	10.14	LEU	31.67	31.45	12415	20.52
ARG	17.93	18.05	6552	71.84					
TYR	18.90	18.83	9278	8.78					
	19.93		1147						
	21.37		655						

Tabulation threshold : 500 uAU

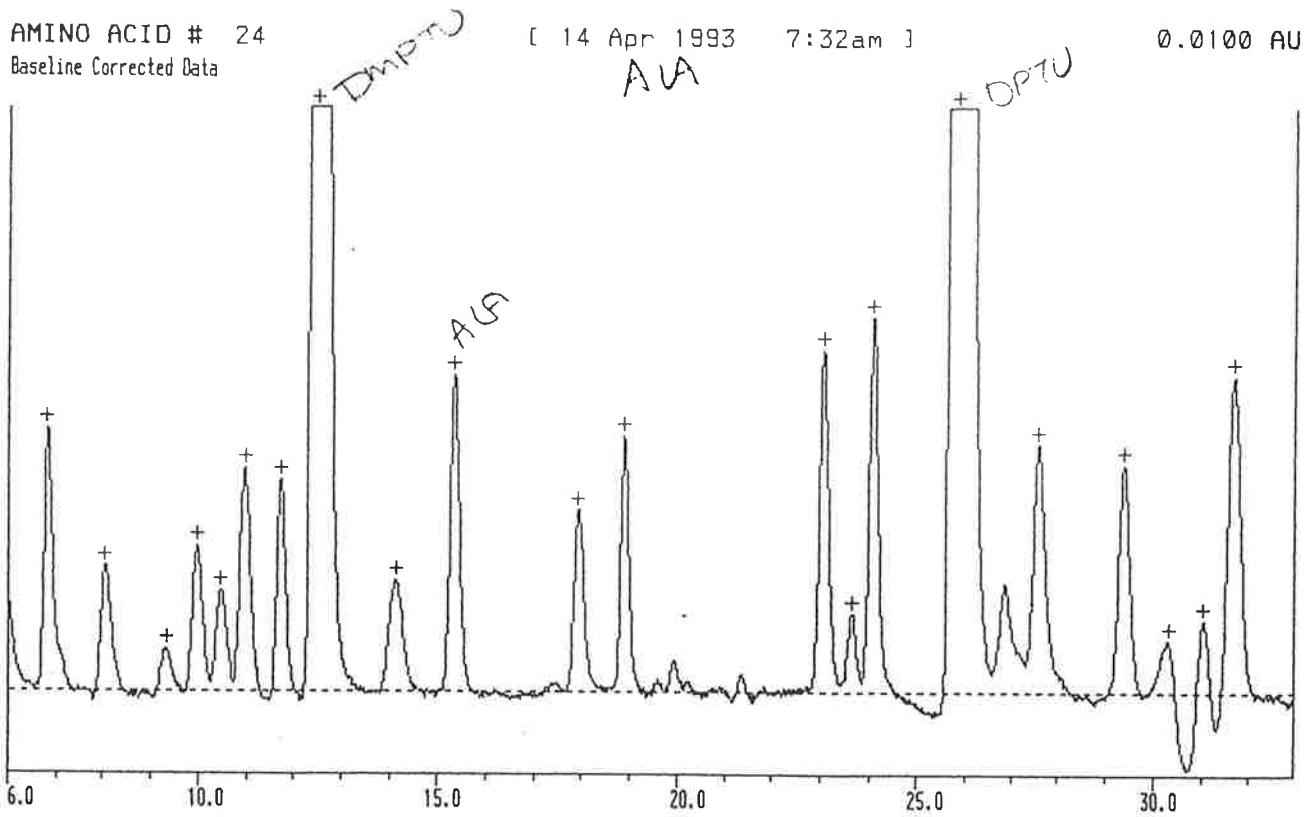
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PA0 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 24 [14 Apr 1993 7:32am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3180			21.35		643	
ASP	6.83	6.83	9429	14.31	PRO	23.07	22.97	12326	15.48
ASN	8.07	8.13	4507	5.16	MET	23.65	23.53	2872	2.79
SER	9.33	9.38	1557	2.63	VAL	24.08	23.98	13500	13.52
GLN	9.98	10.03	5248	6.24	DPT	25.87	25.73	820855	1166.84
THR	10.50	10.53	3633	7.59		26.87		3892	
GLY	10.98	11.00	8061	11.31	TRP	27.58	27.58	8940	8.85
GLU	11.75	11.73	7579	10.37		28.07		597	
DMP	12.53	12.63	184864	416.54	PHE	29.38	29.18	8205	11.68
	13.18		506		ILE	30.30	30.05	1879	3.39
HIS	14.17	14.27	3991	3.79	LYS	31.03	30.78	2656	3.33
ALA	15.35	15.32	11378	12.74	LEU	31.68	31.45	11340	18.74
ARG	17.93	18.05	6559	71.92					
TYR	18.90	18.83	9235	8.74					
	19.92		1202						

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

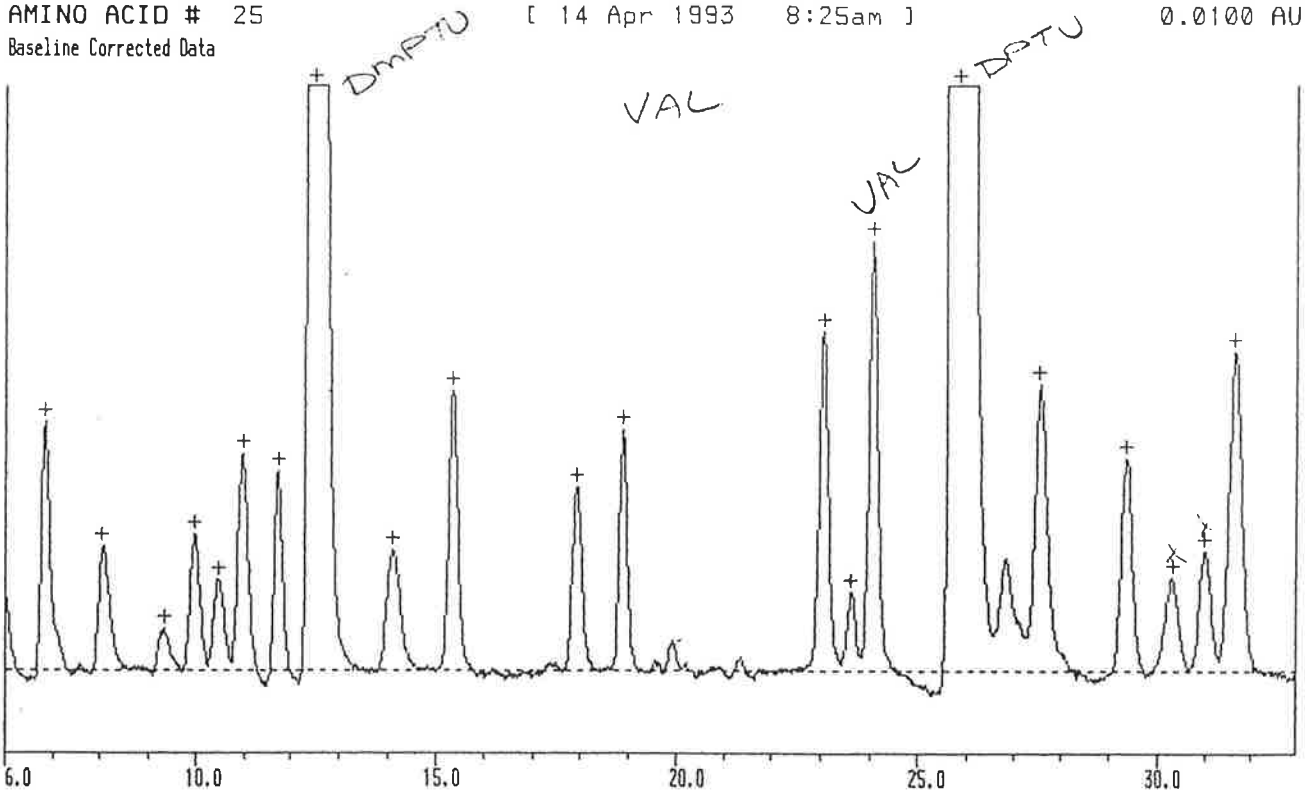
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 25 [14 Apr 1993 8:25am] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2584		PRO	23.05	22.97	12180	15.30
ASP	6.85	6.83	8980	13.63	MET	23.63	23.53	2851	2.77
ASN	8.07	8.13	4538	5.20	VAL	24.07	23.98	15410	15.43
SER	9.33	9.38	1552	2.63	DPT	25.85	25.73	977402	1389.37
GLN	10.00	10.03	4936	5.87		26.85		4077	
THR	10.48	10.53	3280	6.85	TRP	27.55	27.58	10317	10.22
GLY	10.98	11.00	7855	11.02		28.07		564	
GLU	11.75	11.73	7152	9.79	PHE	29.35	29.18	7608	10.83
DMP	12.53	12.63	190927	430.20	ILE	30.30	30.05	3340	6.02
HIS	14.13	14.27	4380	4.16	LYS	31.00	30.78	4322	5.42
ALA	15.33	15.32	10041	11.25	LEU	31.63	31.45	11438	18.90
ARG	17.92	18.05	6628	72.68					
TYR	18.88	18.83	8664	8.20					
	19.93		1125						
	21.37		554						

Tabulation threshold : 500 uAU

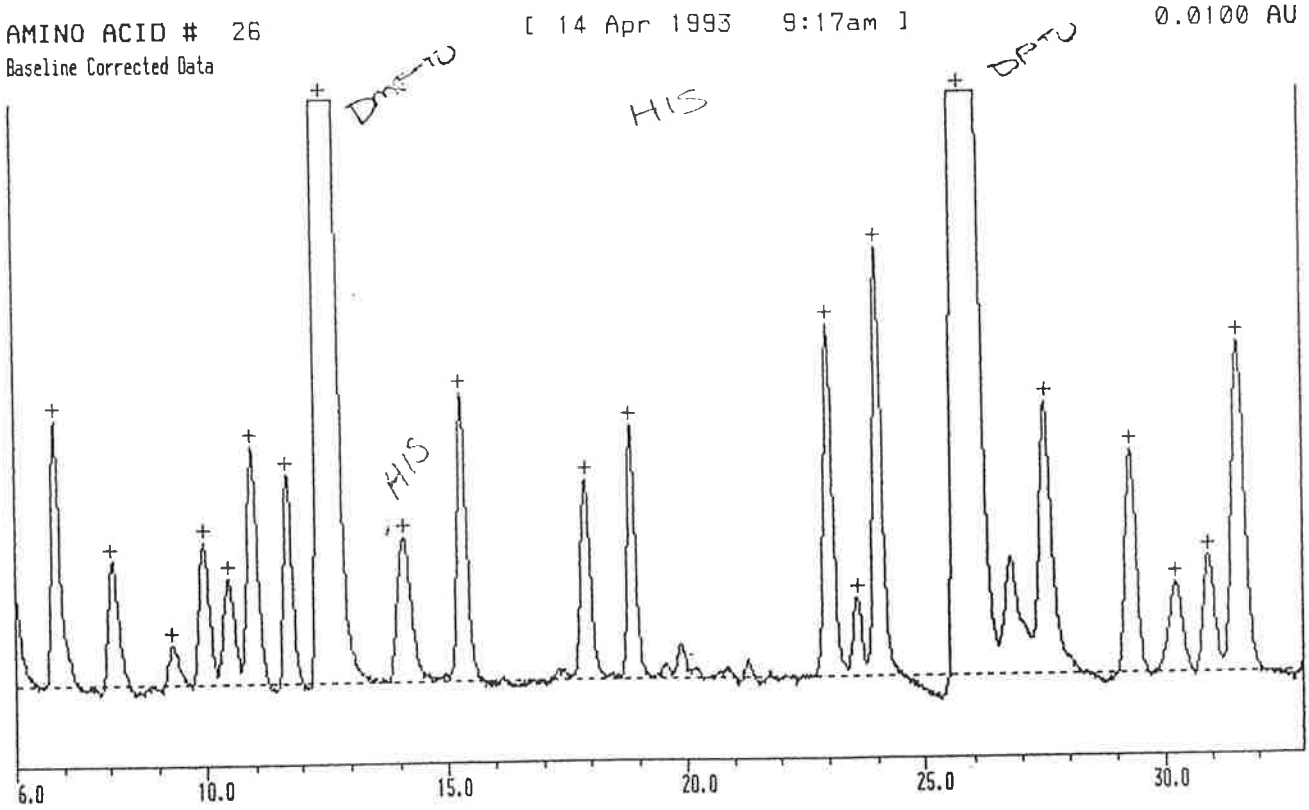
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 26 [14 Apr 1993 9:17am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3127			21.32		650	
ASP	6.85	6.83	9556	14.50	PRO	23.02	22.97	12698	15.95
ASN	8.07	8.13	4478	5.13	MET	23.60	23.53	2870	2.79
SER	9.30	9.38	1478	2.50	VAL	24.03	23.98	15410	15.43
GLN	9.98	10.03	5169	6.14	DPT	25.82	25.73	911601	1295.83
THR	10.48	10.53	3823	7.98		26.78		4209	
GLY	10.95	11.00	8539	11.97	TRP	27.52	27.58	9748	9.65
GLU	11.73	11.73	7560	10.34		28.00		631	
DMP	12.52	12.63	207355	467.22	PHE	29.30	29.18	8023	11.42
HIS	14.13	14.27	5191	4.93	ILE	30.23	30.05	3261	5.88
ALA	15.32	15.32	10351	11.59	LYS	30.93	30.78	4233	5.31
ARG	17.90	18.05	7147	78.37	LEU	31.58	31.45	11889	19.65
TYR	18.85	18.83	9103	8.62					
	19.57	–	544						
	19.88		1250						

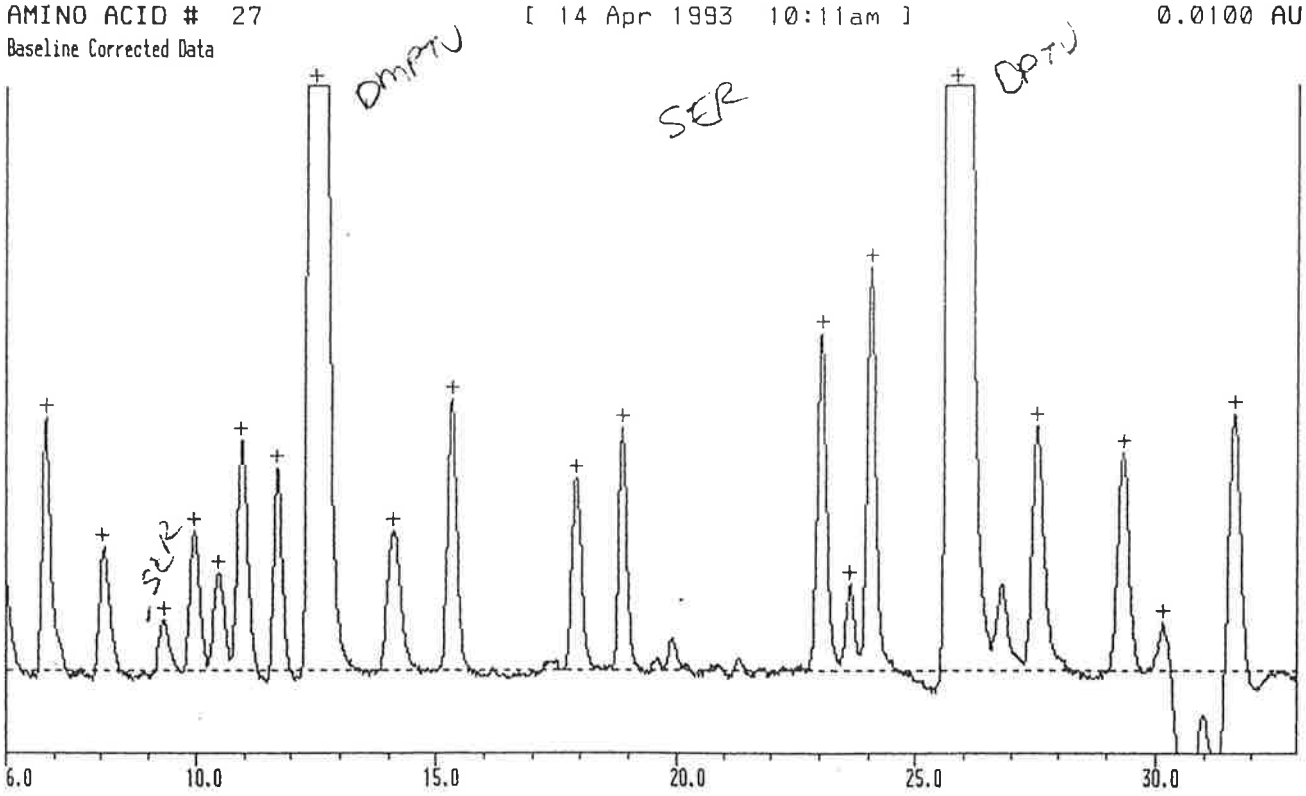
Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1
Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 27 [14 Apr 1993 10:11am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2968		MET	23.63	23.53	3103	3.01
ASP	6.85	6.83	9060	13.75	VAL	24.07	23.98	14500	14.52
ASN	8.07	8.13	4437	5.08	DPT	25.83	25.73	878268	1248.45
SER	9.33	9.38	1809	3.06		26.82		3127	
GLN	9.98	10.03	5001	5.94	TRP	27.55	27.58	8764	8.68
THR	10.48	10.53	3516	7.34		28.00		537	
GLY	10.97	11.00	8248	11.57	PHE	29.33	29.18	7838	11.16
GLU	11.73	11.73	7238	9.90	ILE	30.13	30.05	1778	3.20
DMP	12.52	12.63	184711	416.20	LEU	31.63	31.45	9216	15.23
HIS	14.13	14.27	4975	4.73					
ALA	15.33	15.32	9688	10.85					
ARG	17.92	18.05	6909	75.76					
TYR	18.88	18.83	8728	8.26					
	19.93		1156						
PRO	23.05	22.97	12115	15.21					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

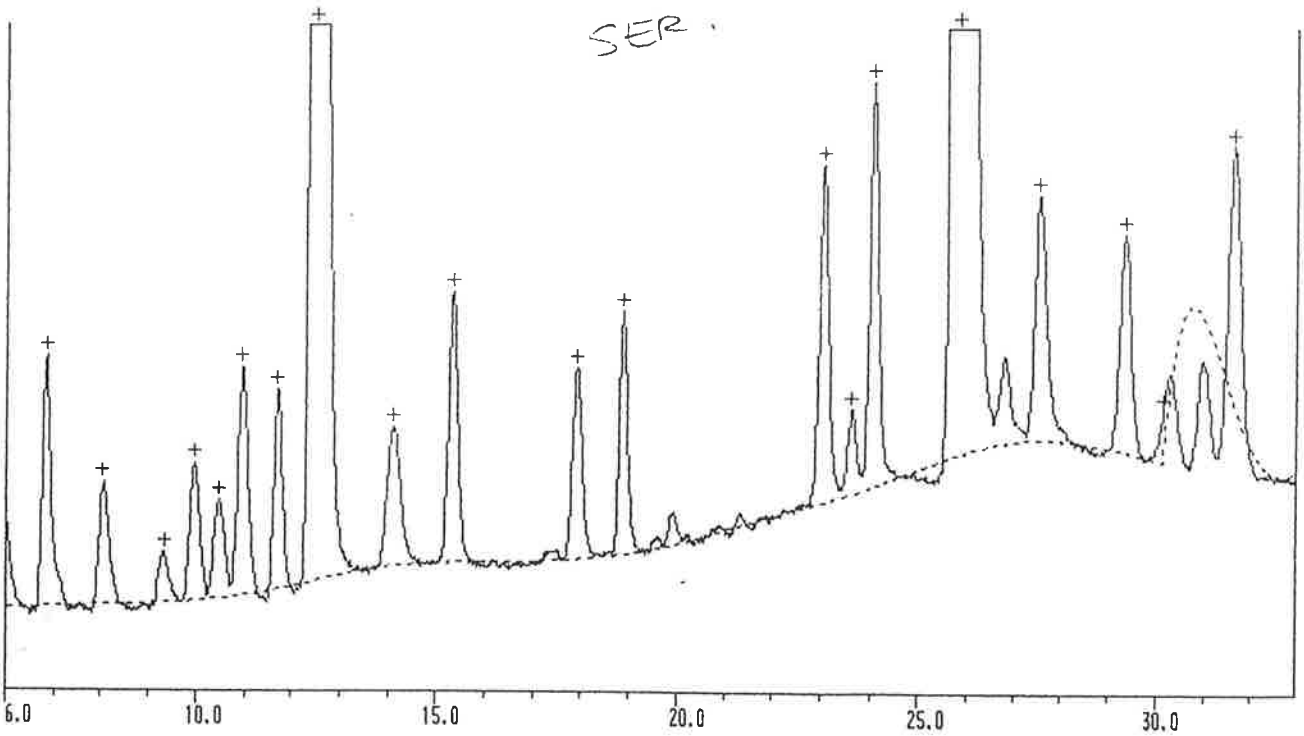
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 27 [14 Apr 1993 10:11am] 0.0100 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.05		2968		MET	23.63	23.53	3103	3.01
ASP	6.85	6.83	9060	13.75	VAL	24.07	23.98	14500	14.52
ASN	8.07	8.13	4437	5.08	DPT	25.83	25.73	878268	1248.45
SER	9.33	9.38	1809	3.06		26.82		3127	
GLN	9.98	10.03	5001	5.94	TRP	27.55	27.58	8764	8.68
THR	10.48	10.53	3516	7.34		28.00		537	
GLY	10.97	11.00	8248	11.57	PHE	29.33	29.18	7838	11.16
GLU	11.73	11.73	7238	9.90	ILE	30.13	30.05	1778	3.20
DMP	12.52	12.63	184711	416.20	LEU	31.63	31.45	9216	15.23
HIS	14.13	14.27	4975	4.73					
ALA	15.33	15.32	9688	10.85					
ARG	17.92	18.05	6909	75.76					
TYR	18.88	18.83	8728	8.26					
	19.93		1156						
PRO	23.05	22.97	12115	15.21					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

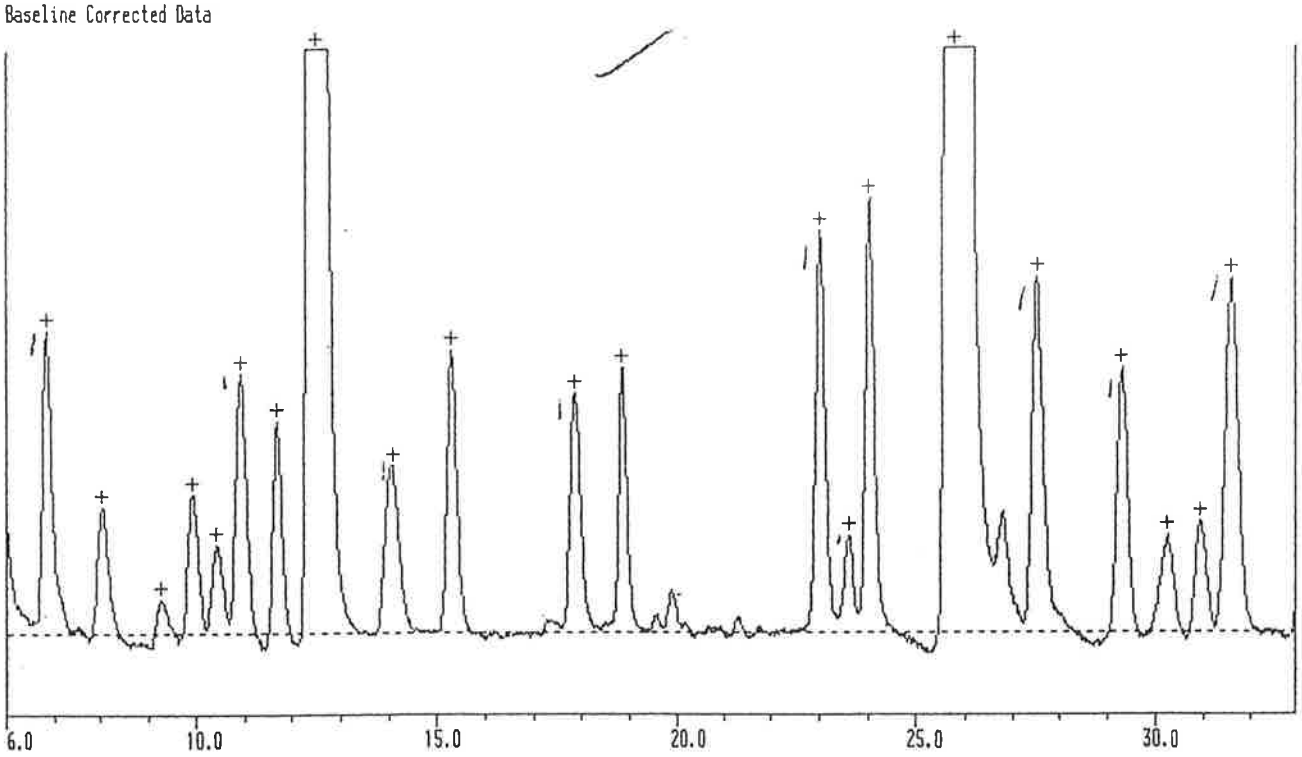
SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 28 [14 Apr 1993 11:03am] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3703			19.88		1540	
	6.27		1032			21.33		576	
ASP	6.85	6.83	10891	16.53	PRO	23.03	22.97	14409	18.10
ASN	8.05	8.13	4548	5.21	MET	23.62	23.53	3496	3.39
SER	9.25	9.38	1216	2.06	VAL	24.05	23.98	15540	15.56
GLN	9.95	10.03	5040	5.99	DPT	25.82	25.73	1293703	1838.98
THR	10.47	10.53	3204	6.69		26.80		4365	
GLY	10.95	11.00	9331	13.09	TRP	27.52	27.58	12777	12.65
GLU	11.70	11.73	7581	10.37		27.90		864	
OMP	12.50	12.63	239318	539.24		28.02		645	
HIS	14.10	14.27	5995	5.69	PHE	29.32	29.18	9463	13.47
ALA	15.30	15.32	10159	11.38	ILE	30.25	30.05	3470	6.25
ARG	17.87	18.05	8630	94.63	LYS	30.95	30.78	3962	4.97
TYR	18.87	18.83	9494	8.99	LEU	31.60	31.45	12650	20.91
	19.58		669						

Tabulation threshold : 500 uAU

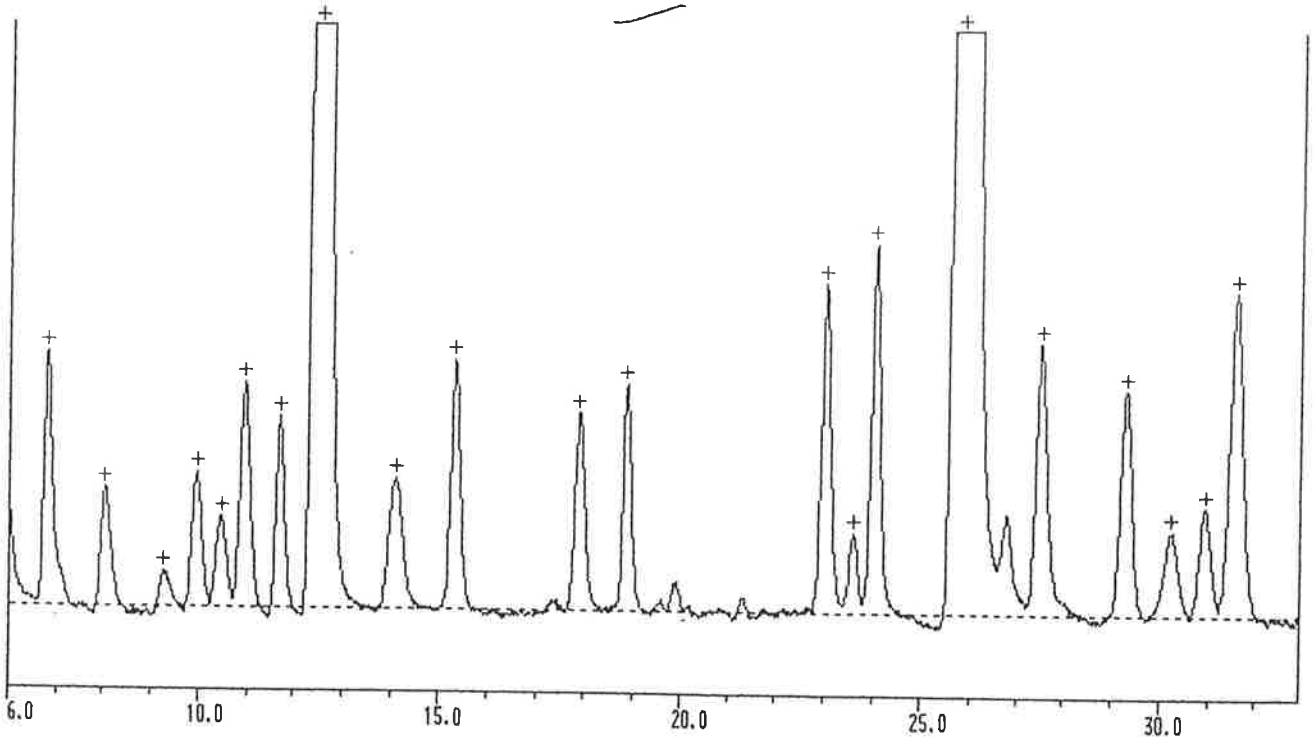
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO 11 PSE 267
[Initiated 13 Apr 1993 9:22am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 29 [14 Apr 1993 11:55am] 0.0100 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.03		3516		PRO	23.03	22.97	11894	14.94
ASP	6.82	6.83	9146	13.88	MET	23.60	23.53	2906	2.82
ASN	8.05	8.13	4308	4.93	VAL	24.05	23.98	13332	13.35
SER	9.27	9.38	1308	2.21	DPT	25.82	25.73	972019	1381.71
GLN	9.97	10.03	4838	5.75		26.78		3624	
THR	10.48	10.53	3290	6.87		27.12		652	
GLY	10.97	11.00	8152	11.43	TRP	27.52	27.58	9806	9.71
GLU	11.72	11.73	6890	9.43	PHE	29.30	29.18	8109	11.55
DMP	12.52	12.63	167352	377.08	ILE	30.25	30.05	3069	5.53
HIS	14.12	14.27	4747	4.51	LYS	30.95	30.78	3907	4.90
ALA	15.32	15.32	9014	10.10	LEU	31.57	31.45	11625	19.21
ARG	17.88	18.05	7092	77.76					
TYR	18.87	18.83	8164	7.73					
	19.93		1101						
	21.33		556						

Tabulation threshold : 500 uAU

D.3 PAO A Ref: PSE 279 Date: 17/6/93

Table D.3 *N*-Terminal Amino Acid Sequence of PSE 279 [a.]

aa no.	*1° Signal*	*2°*	*3°*	*4°*	*5°*	*6°*	*7°*	*8°*	*9°*
1	Glu		Gly/His/Arg						
2			Pro						
3	Glu		-						
4			Pro			Ala/Tyr/Thr/Leu/Phe			
5			Gly						
6			Thr			Ala/Tyr/Val			
7			Leu						
8			Pro						
9			Arg						
10			Lys						

a. Interpretation of the chromatograms

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

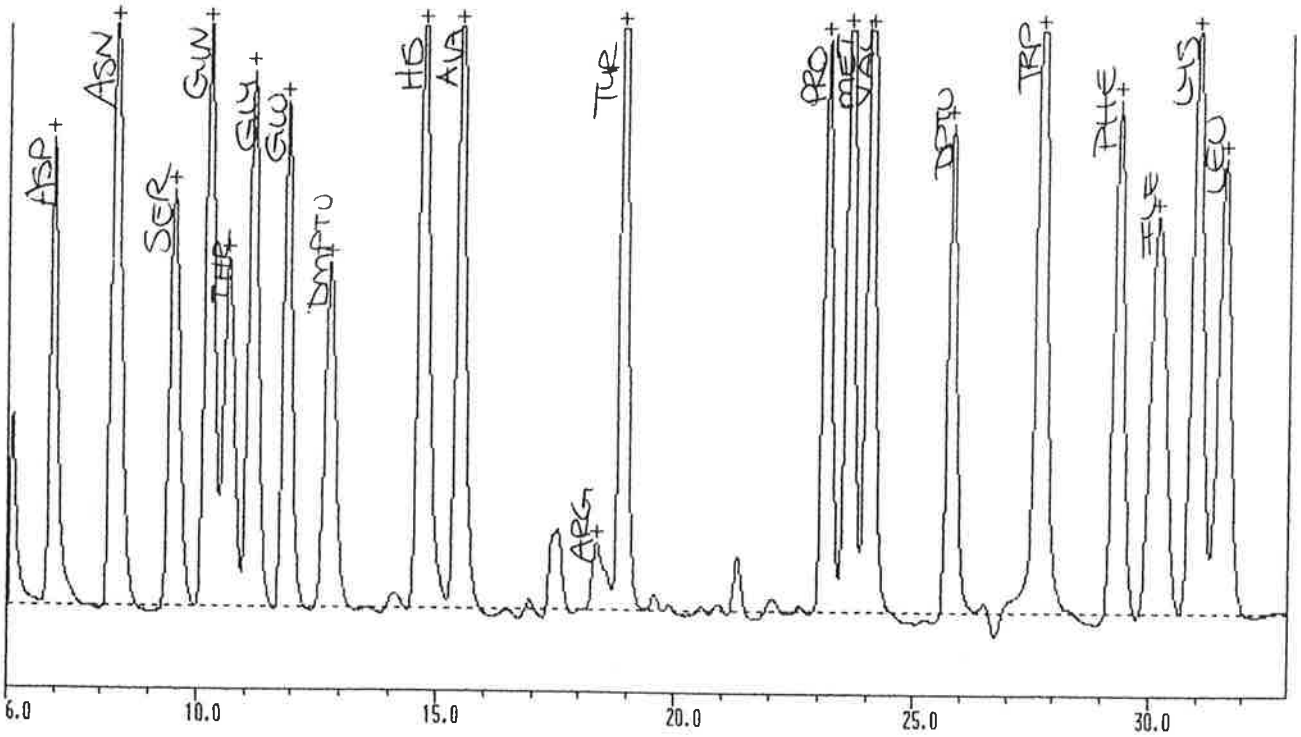
SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : BGN470-1
Conversion cycle : BGN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

CALIBRATION # 1 [15 Jun 1993 11:32am] 0.0100 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.13		6866			19.58		619	
ASP	6.97	6.97	16855	25.00		21.33		1980	
ASN	8.30	8.30	21480	25.00	PRO	23.17	23.17	20625	25.00
SER	9.52	9.52	14964	25.00	MET	23.63	23.63	27950	25.00
GLN	10.25	10.25	21180	25.00	VAL	24.07	24.07	27280	25.00
THR	10.67	10.67	12530	25.00	**DPT**	**25.78**	**25.78**	**17676**	**25.00**
GLY	11.17	11.17	19322	25.00	TRP	27.70	27.70	27369	25.00
GLU	11.90	11.90	18240	25.00	PHE	29.33	29.33	18544	25.00
DMP	12.80	12.80	12388	25.00	ILE	30.17	30.17	14344	25.00
	14.13		556		LYS	30.98	30.98	21516	25.00
HIS	14.70	14.70	21962	25.00	LEU	31.57	31.57	16447	25.00
ALA	**15.47**	**15.47**	**23145**	**25.00**					
	17.52		2887						
ARG	18.37	18.37	2416	25.00					
TYR	18.90	18.90	27969	25.00					

Tabulation threshold : 500 uAU

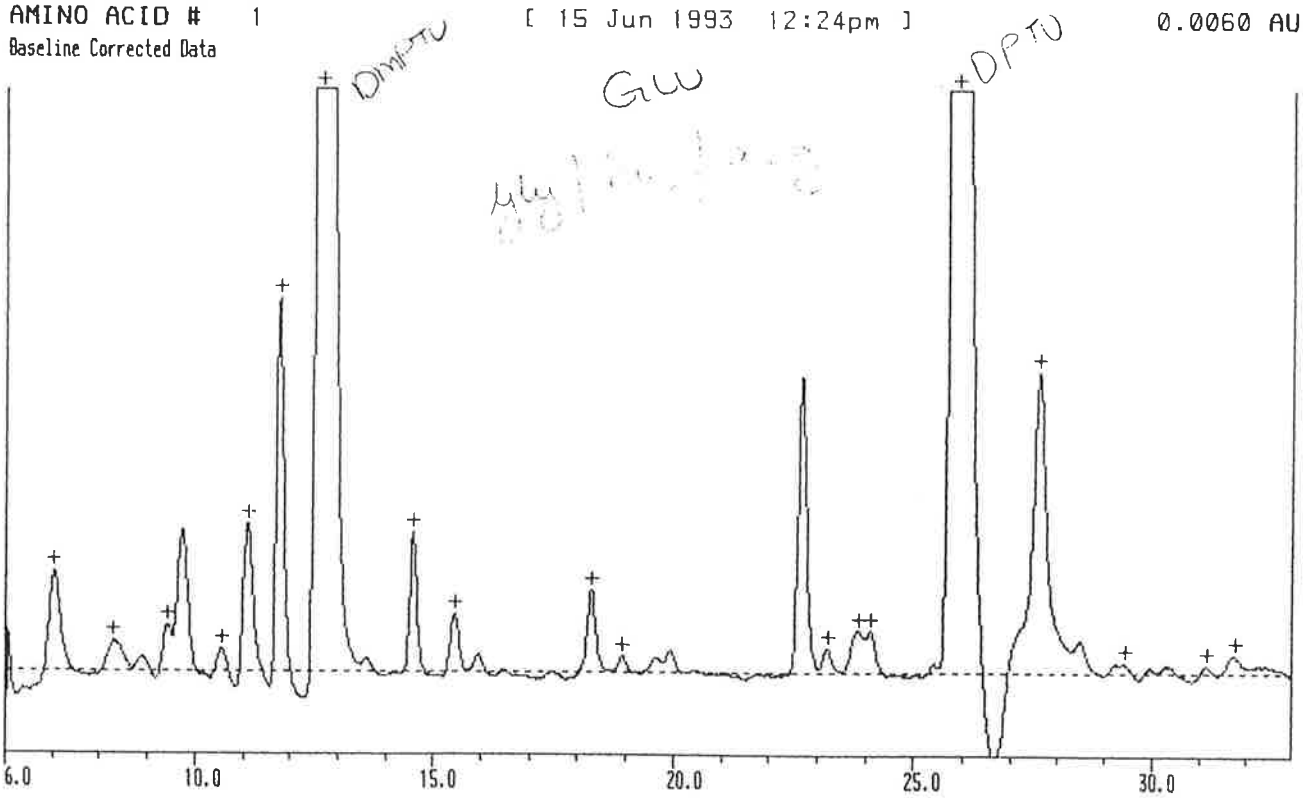
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 1 [15 Jun 1993 12:24pm] 0.0060 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		861		MET	23.85	23.63	936	0.84
ASP	7.05	6.97	2200	3.26	VAL	24.10	24.07	921	0.84
ASN	8.32	8.30	660	0.77	**DPT**	**25.88**	**25.78**	**216345**	**305.99**
SER	9.45	9.52	1017	1.70	TRP	27.58	27.70	6523	5.96
	9.75		3079			28.47		698	
THR	10.57	10.67	487	0.97	PHE	29.43	29.33	211	0.28
GLY	11.12	11.17	3208	4.15	LYS	31.15	30.98	184	0.21
GLU	11.78	11.90	8083	11.08	LEU	31.75	31.57	398	0.61
DMP	12.68	12.80	117981	238.08					
HIS	14.58	14.70	3055	3.48					
ALA	**15.45**	**15.47**	**1272**	**1.37**					
ARG	18.28	18.37	1800	18.62					
TYR	18.95	18.90	348	0.31					
	22.68		6422						
PRO	23.22	23.17	547	0.66					

Tabulation threshold : 500 uAU

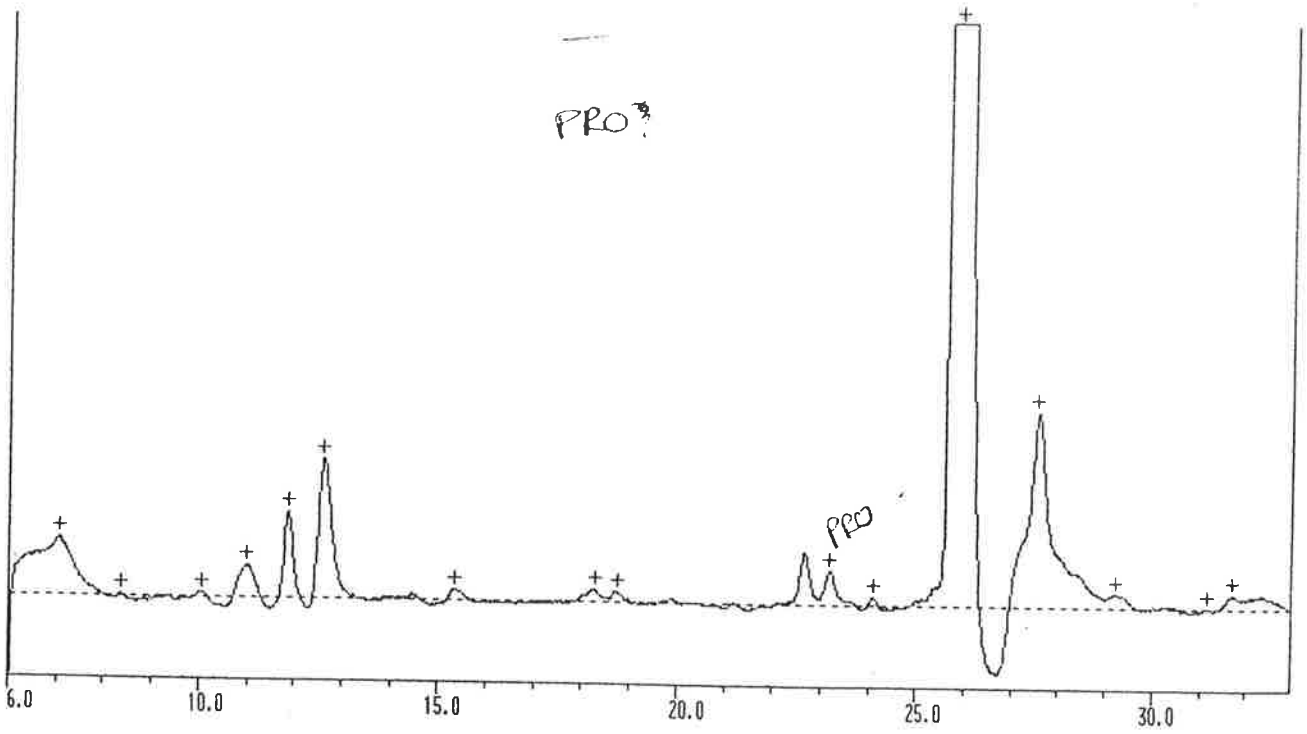
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 2 [15 Jun 1993 1:16pm] 0.0060 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
ASP	7.07	6.97	1255	1.86	LYS	31.18	30.98	31	0.04
ASN	8.38	8.30	45	0.05	LEU	31.73	31.57	295	0.45
GLN	10.05	10.25	110	0.13					
GLY	10.98	11.17	700	0.91					
GLU	11.87	11.90	1893	2.60					
DMP	12.62	12.80	3012	6.08					
ALA	15.33	15.47	242	0.26					
ARG	18.28	18.37	259	2.68					
TYR	18.75	18.90	235	0.21					
	22.67		1147						
PRO	23.20	23.17	744	0.90					
VAL	24.12	24.07	175	0.16					
DPT	25.85	25.78	178646	252.67					
TRP	27.55	27.70	4240	3.87					
PHE	29.23	29.33	316	0.43					

Tabulation threshold : 500 uAU

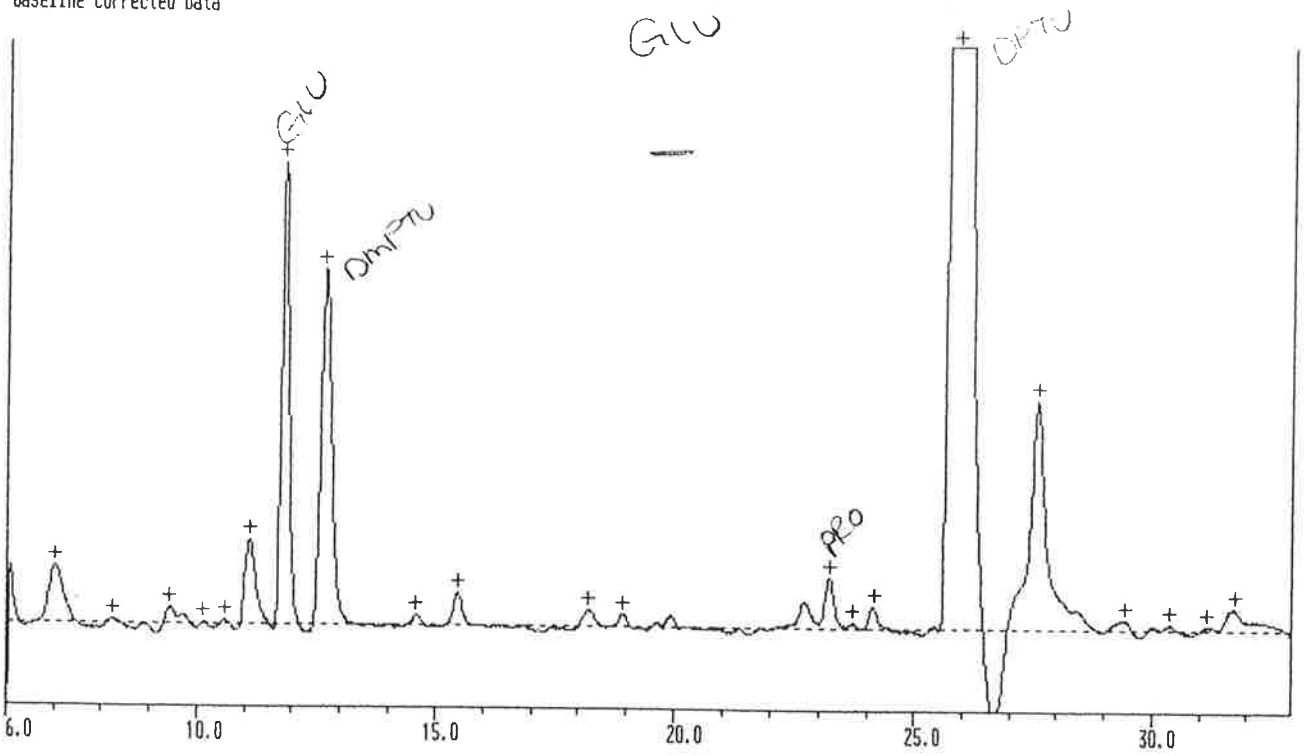
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 3 [15 Jun 1993 2:08pm] 0.0060 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.10		1207		MET	23.72	23.63	129	0.12
ASP	7.03	6.97	1228	1.82	VAL	24.15	24.07	496	0.46
ASN	8.25	8.30	98	0.11	**DPT**	**25.90**	**25.78**	**250977**	**354.97**
SER	9.43	9.52	352	0.59	TRP	27.57	27.70	4941	4.51
GLN	10.15	10.25	48	0.06	PHE	29.43	29.33	232	0.31
THR	10.62	10.67	84	0.17	ILE	30.37	30.17	132	0.23
GLY	11.12	11.17	1826	2.36	LYS	31.17	30.98	100	0.12
GLU	11.85	11.90	9986	13.69	LEU	31.75	31.57	475	0.72
DMP	12.70	12.80	7699	15.54					
HIS	14.58	14.70	240	0.27					
ALA	**15.47**	**15.47**	**708**	**0.76**					
ARG	18.23	18.37	355	3.67					
TYR	18.93	18.90	290	0.26					
	22.68		597						
PRO	23.22	23.17	1104	1.34					

Tabulation threshold : 500 uAU

- Applied Biosystems 470A Protein Sequencer Chromatogram Report -

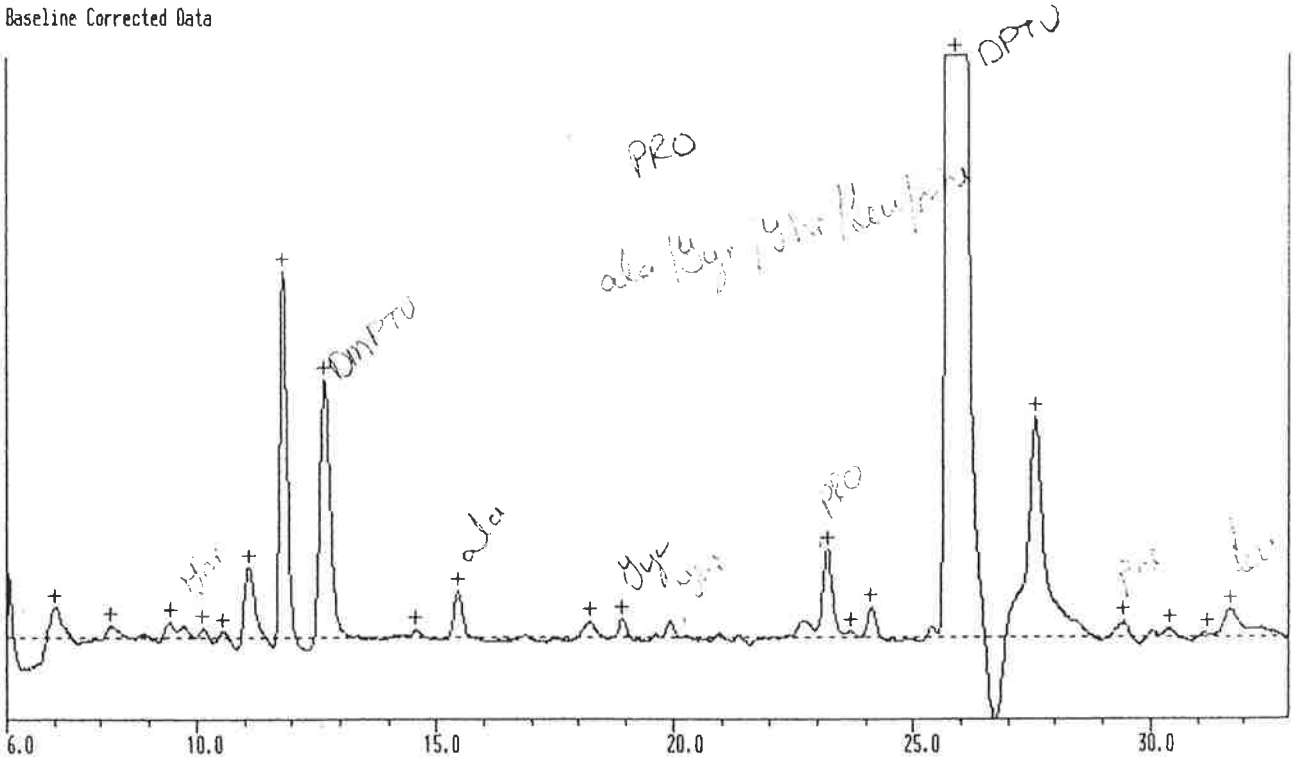
SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 4 [15 Jun 1993 3:00pm] 0.0060 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.07		1394		VAL	24.13	24.07	657	0.60
ASP	7.05	6.97	672	1.00	DPT	25.90	25.78	232778	329.23
ASN	8.23	8.30	278	0.32	TRP	27.58	27.70	4764	4.35
SER	9.47	9.52	345	0.58	PHE	29.45	29.33	338	0.46
GLN	10.17	10.25	220	0.26	ILE	30.42	30.17	170	0.30
THR	10.58	10.67	158	0.32	LYS	31.20	30.98	91	0.11
GLY	11.12	11.17	1533	1.98	LEU	31.70	31.57	612	0.93
GLU	11.85	11.90	7946	10.89					
DMP	12.70	12.80	5620	11.34					
HIS	14.60	14.70	175	0.20					
ALA	15.47	15.47	998	1.08					
ARG	18.25	18.37	345	3.57					
TYR	18.95	18.90	420	0.38					
PRO	23.22	23.17	1922	2.33					
MET	23.68	23.63	158	0.14					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

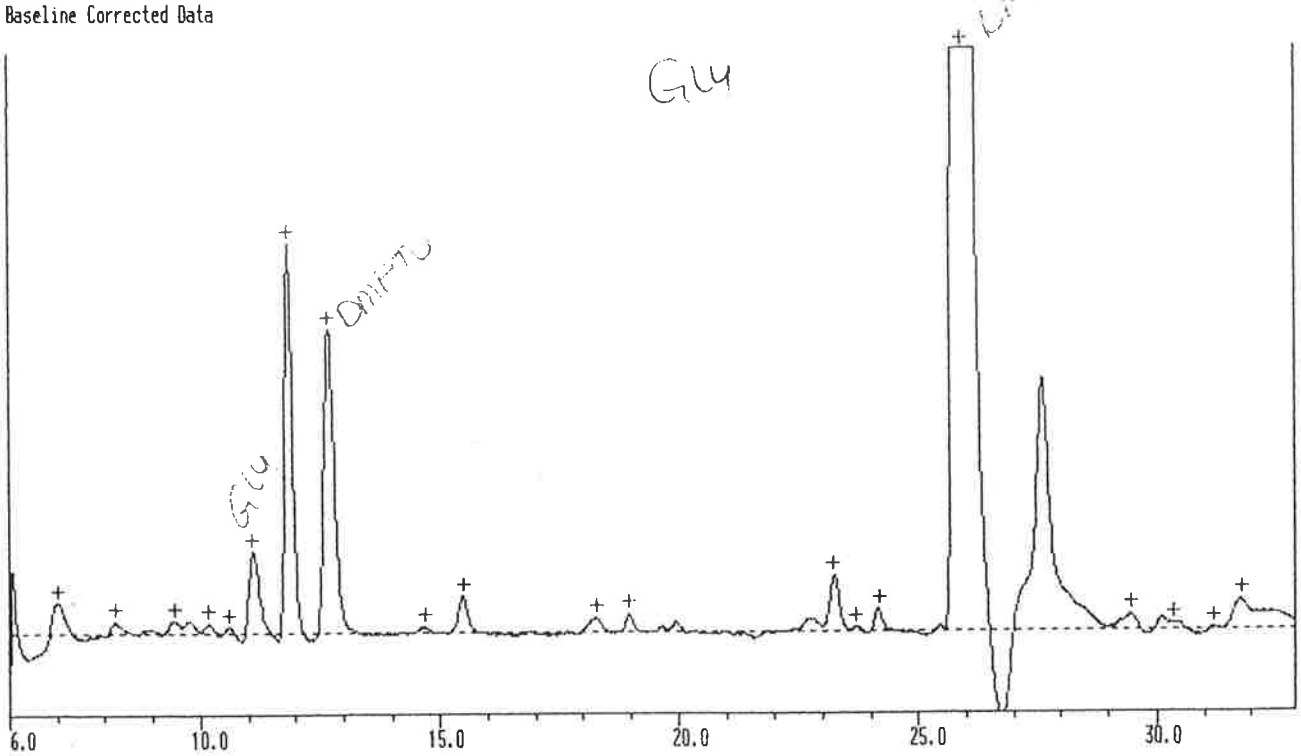
SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 5 [15 Jun 1993 3:52pm] 0.0060 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.10		1360		VAL	24.18	24.07	484	0.44
ASP	7.02	6.97	684	1.01	DPT	25.95	25.78	247994	350.75
ASN	8.25	8.30	261	0.30		27.62		5460	
SER	9.50	9.52	292	0.49	PHE	29.48	29.33	345	0.47
GLN	10.20	10.25	211	0.25	ILE	30.37	30.17	153	0.27
THR	10.62	10.67	146	0.29	LYS	31.20	30.98	60	0.07
GLY	11.13	11.17	1771	2.29	LEU	31.78	31.57	643	0.98
GLU	11.88	11.90	8412	11.53					
DMP	12.73	12.80	6583	13.28					
HIS	14.70	14.70	129	0.15					
ALA	15.50	15.47	775	0.84					
ARG	18.28	18.37	321	3.33					
TYR	18.97	18.90	384	0.34					
PRO	23.25	23.17	1243	1.51					
MET	23.72	23.63	120	0.11					

Tabulation threshold : 500 uAU

– Applied Biosystems 4758 Protein Sequencer Chromatogram Report –

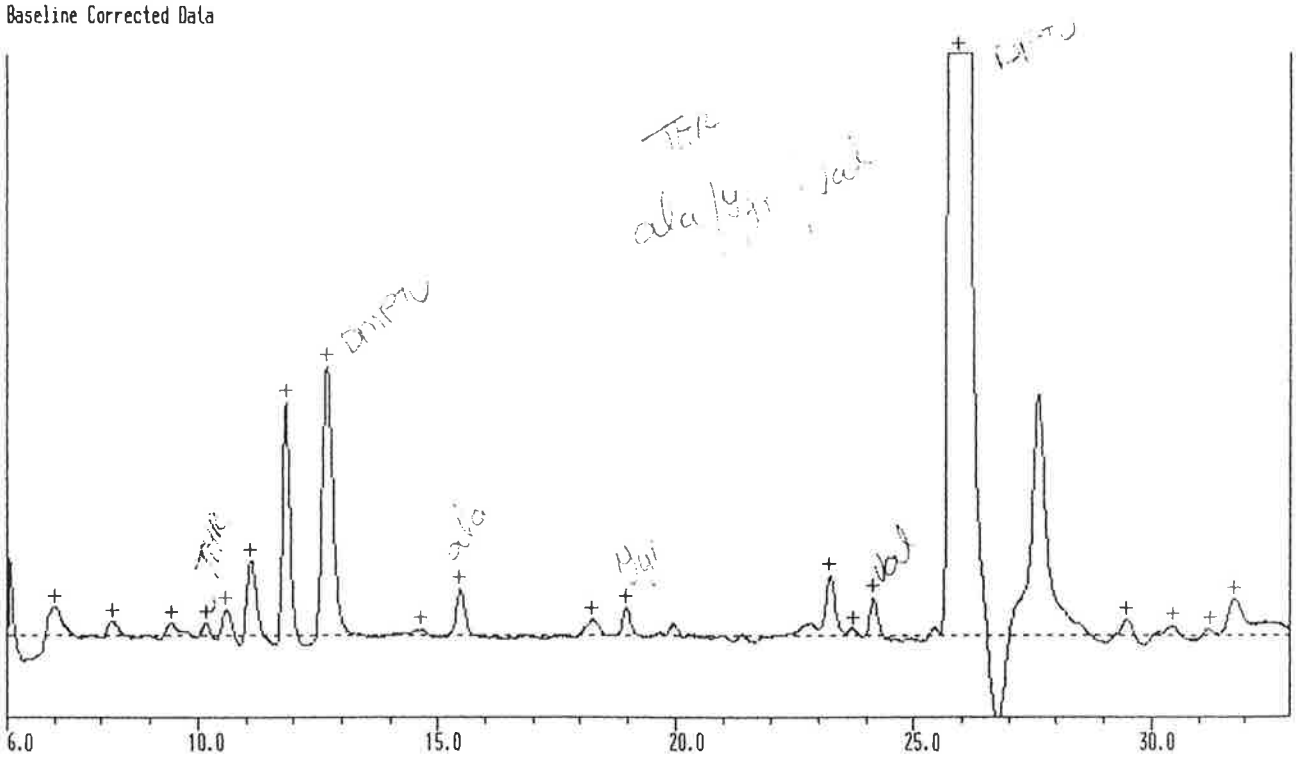
SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 6 [15 Jun 1993 4:44pm] 0.0060 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		1658		VAL	24.17	24.07	837	0.77
ASP	7.05	6.97	621	0.92	DPT	25.95	25.78	237160	335.43
ASN	8.25	8.30	321	0.37		27.62		5236	
SER	9.48	9.52	285	0.48	PHE	29.48	29.33	355	0.48
GLN	10.20	10.25	261	0.31	ILE	30.43	30.17	208	0.36
THR	10.63	10.67	571	1.14	LYS	31.23	30.98	151	0.18
GLY	11.13	11.17	1612	2.09	LEU	31.77	31.57	801	1.22
GLU	11.87	11.90	5023	6.88					
DMP	12.72	12.80	5822	11.75					
HIS	14.65	14.70	141	0.16					
ALA	15.48	15.47	998	1.08					
ARG	18.27	18.37	357	3.70					
TYR	18.98	18.90	626	0.56					
PRO	23.25	23.17	1310	1.59					
MET	23.75	23.63	158	0.14					

Tabulation threshold : 500 uAU

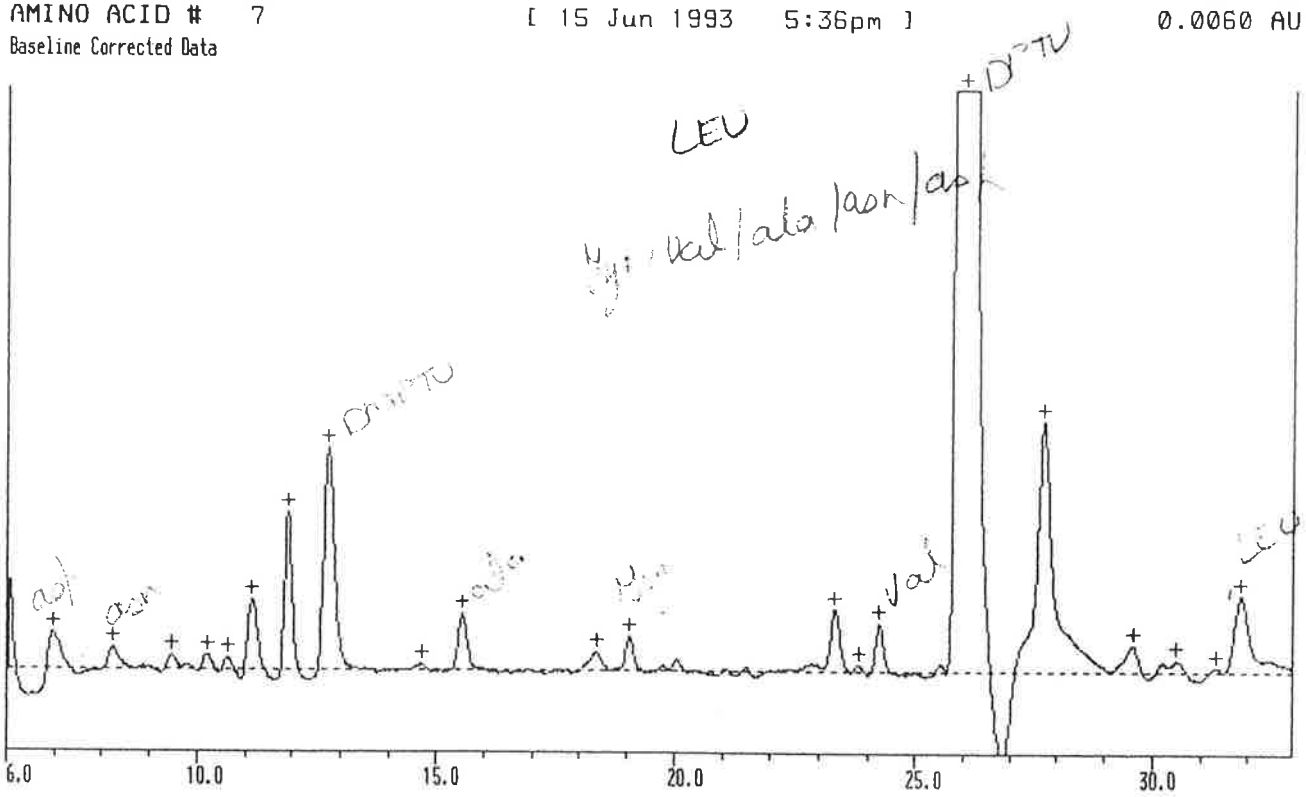
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 7 [15 Jun 1993 5:36pm] 0.0060 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		1927		VAL	24.25	24.07	1044	0.96
ASP	6.98	6.97	789	1.17	**DPT**	**26.03**	**25.78**	**241802**	**341.99**
ASN	8.25	8.30	470	0.55	TRP	27.72	27.70	5431	4.96
SER	9.48	9.52	300	0.50	PHE	29.60	29.33	576	0.78
GLN	10.23	10.25	336	0.40	ILE	30.48	30.17	256	0.45
THR	10.67	10.67	256	0.51	LYS	31.32	30.98	117	0.14
GLY	11.17	11.17	1540	1.99	LEU	31.83	31.57	1653	2.51
GLU	11.93	11.90	3444	4.72					
DMP	12.77	12.80	4836	9.76					
HIS	14.72	14.70	124	0.14					
ALA	**15.55**	**15.47**	**1212**	**1.31**					
ARG	18.38	18.37	412	4.27					
TYR	19.07	18.90	751	0.67					
PRO	23.33	23.17	1370	1.66					
MET	23.82	23.63	163	0.15					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

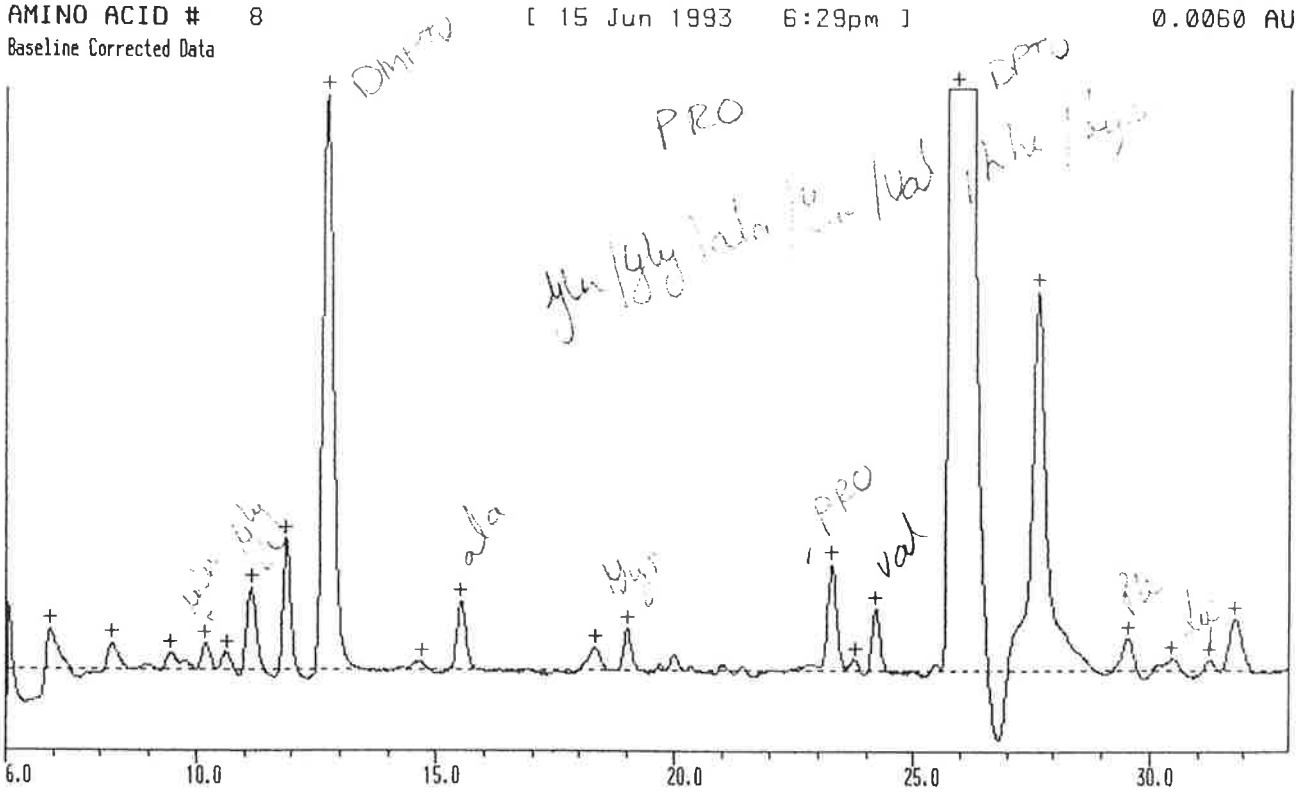
SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 36.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 8 [15 Jun 1993 6:29pm] 0.0060 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.08		1440		VAL	24.22	24.07	1356	1.24
ASP	6.93	6.97	868	1.29	DPT	25.95	25.78	448041	633.69
ASN	8.25	8.30	561	0.65	TRP	27.67	27.70	8227	7.51
SER	9.50	9.52	352	0.59	PHE	29.55	29.33	717	0.97
GLN	10.22	10.25	561	0.66	ILE	30.45	30.17	280	0.49
THR	10.67	10.67	376	0.75	LYS	31.27	30.98	242	0.28
GLY	11.15	11.17	1776	2.30	LEU	31.80	31.57	1152	1.75
GLU	11.90	11.90	2829	3.88					
DMP	12.75	12.80	12427	25.08					
HIS	14.70	14.70	175	0.20					
ALA	15.52	15.47	1483	1.60					
ARG	18.33	18.37	494	5.11					
TYR	19.02	18.90	926	0.83					
PRO	23.28	23.17	2320	2.81					
MET	23.78	23.63	244	0.22					

Tabulation threshold : 500 uAU

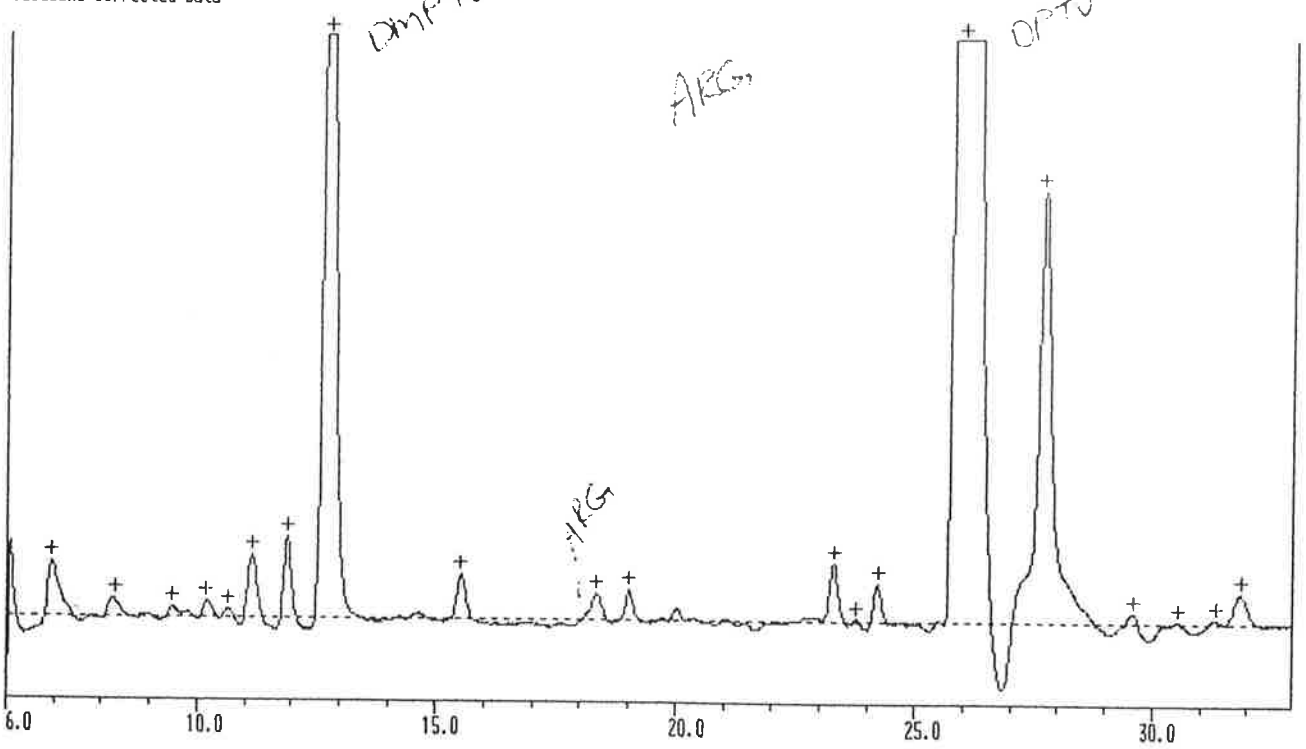
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 9 [15 Jun 1993 7:21pm] 0.0060 AU
Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.10		1600		DPT	25.97	25.78	552117	780.89
ASP	6.95	6.97	1190	1.77	TRP	27.68	27.70	9348	8.54
ASN	8.28	8.30	405	0.47	PHE	29.58	29.33	249	0.34
SER	9.48	9.52	220	0.37	ILE	30.55	30.17	50	0.09
GLN	10.22	10.25	367	0.43	LYS	31.32	30.98	110	0.13
THR	10.67	10.67	194	0.39	LEU	31.87	31.57	648	0.98
GLY	11.17	11.17	1348	1.75					
GLU	11.92	11.90	1730	2.37					
DMP	12.75	12.80	20268	40.90					
ALA	15.53	15.47	960	1.04					
ARG	18.35	18.37	564	5.83					
TYR	19.02	18.90	645	0.58					
PRO	23.30	23.17	1260	1.53					
MET	23.80	23.63	69	0.06					
VAL	24.23	24.07	818	0.75					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

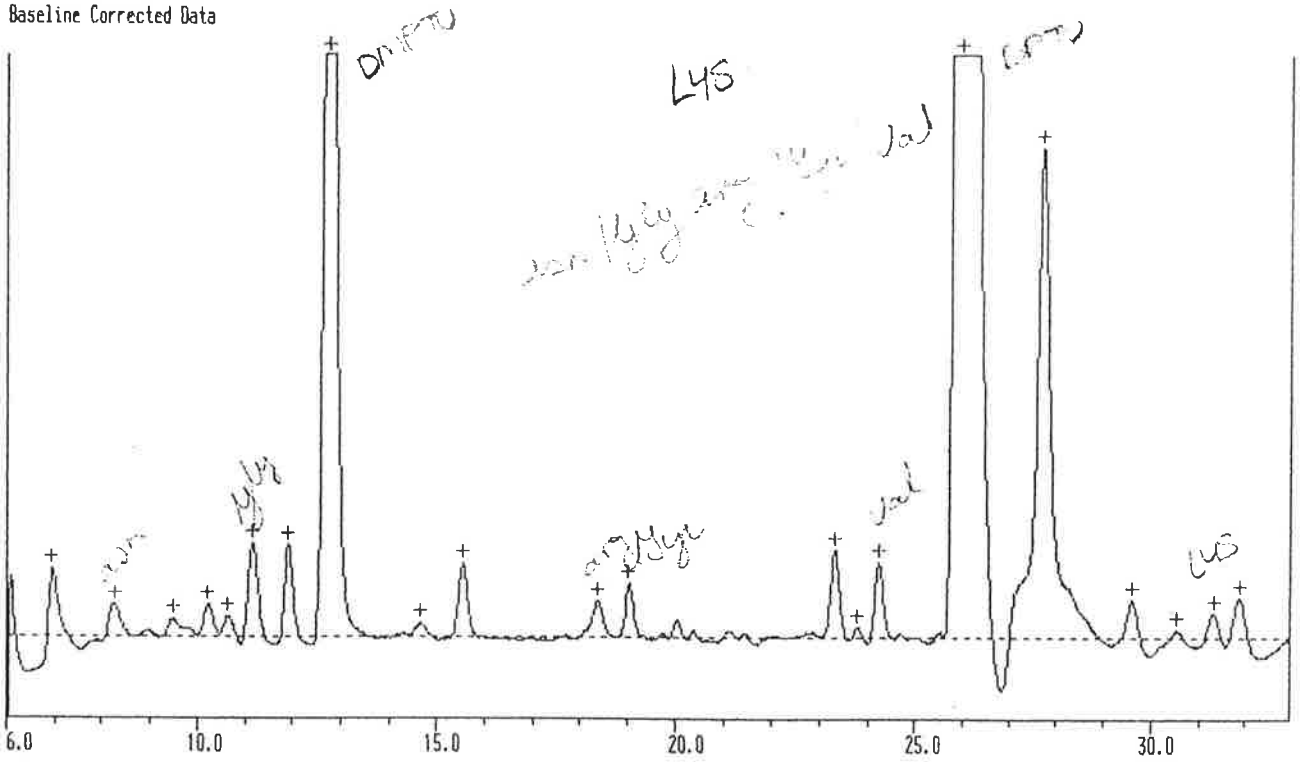
SAMPLE : JAMES STORER PAO.A. PSE 279
[Initiated 15 Jun 1993 10:14am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 36.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 ul

AMINO ACID # 10 [15 Jun 1993 8:13pm] 0.0060 AU

Baseline Corrected Data



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	6.12		1298		VAL	24.23	24.07	1675	1.54
ASP	6.97	6.97	1471	2.18	DPT	25.98	25.78	619816	876.64
ASN	8.28	8.30	724	0.84	TRP	27.70	27.70	10612	9.69
SER	9.52	9.52	410	0.69	PHE	29.60	29.33	816	1.10
GLN	10.25	10.25	727	0.86	ILE	30.52	30.17	182	0.32
THR	10.67	10.67	441	0.88	LYS	31.32	30.98	535	0.62
GLY	11.18	11.17	2054	2.66	LEU	31.87	31.57	873	1.33
GLU	11.93	11.90	2016	2.76					
DMP	12.77	12.80	24261	48.96					
HIS	14.67	14.70	297	0.34					
ALA	15.55	15.47	1605	1.73					
ARG	18.38	18.37	794	8.22					
TYR	19.03	18.90	1180	1.06					
PRO	23.32	23.17	1939	2.35					
MET	23.80	23.63	235	0.21					

Tabulation threshold : 500 uAU

D.4 PAO *Ref: PSE 201* *Date: 13/7/92*

Table D.4 *N*-Terminal Amino Acid Sequence of PSE 201 [a.]

aa no.	*1° Signal*	*2°*	*3°*	*4°*	*5°*	*6°*	*7°*	*8°*	*9°*
1	Ser			Thr/Glu					
2	Pro					Leu			
3	-					Val/Ser			
4	Pro					Val/Ala/Glu			
5	Gly					Tyr/Glu			
6	Thr			Arg/Glu/Phe/Ala/Tyr/Val/Pro					
7	Leu			Phe		Ile			
8	Pro					Tyr/Val			
9	Arg								
10	Lys								
11	Ala								
12	Gly								
13	Val								
14	Phe								
15	Ser								
16	Asp/Glu								
17	Leu								
18	Ser/Leu								
19	Asn								
20	Gln?								
21	Glu/Gly								
22	-								
23	Phe/Glu								
24	-								
25	Val								
26	Pro								
27	Asn								
28	-								

a. Interpretation of the chromatograms

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

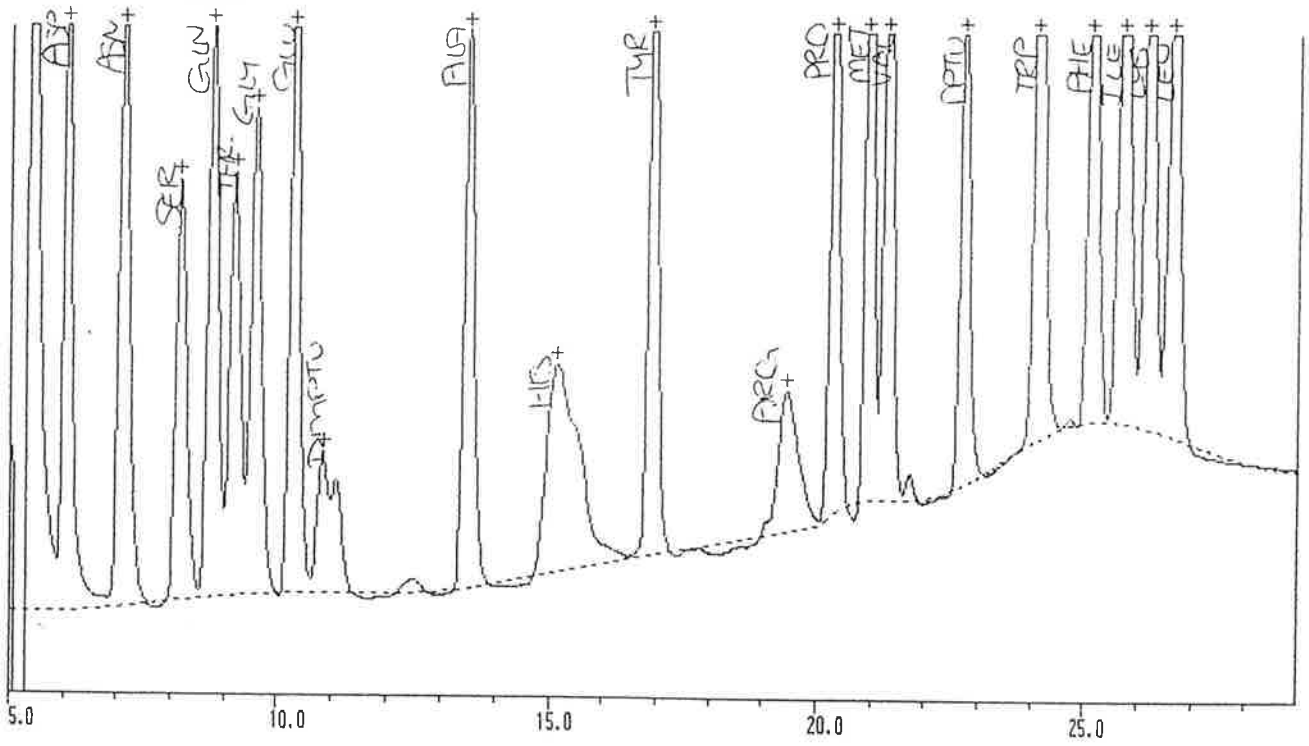
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : BGN470-1
Conversion cycle : BGN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

CALIBRATION # 1 [13 Jul 1992 10:44am] 0.0100 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.43		42338		PRO	20.30	20.30	20328	25.00
ASP	6.07	6.07	28214	25.00	MET	20.93	20.93	26143	25.00
ASN	7.12	7.12	22699	25.00	VAL	21.30	21.30	25929	25.00
SER	8.18	8.18	15014	25.00		21.72		972	
GLN	8.77	8.77	21321	25.00	DPT	22.72	22.72	17503	25.00
THR	9.20	9.20	15141	25.00	TRP	24.12	24.12	30331	25.00
GLY	9.58	9.58	17395	25.00	PHE	25.13	25.13	22274	25.00
GLU	10.32	10.32	23824	25.00	ILE	25.72	25.72	18458	25.00
DMP	10.85	10.85	5023	25.00	LYS	26.18	26.18	23443	25.00
	11.08		4041		LEU	26.62	26.62	20164	25.00
	12.50		516						
ALA	13.50	13.50	20107	25.00					
HIS	15.15	15.15	7430	25.00					
TYR	16.92	16.92	25346	25.00					
ARG	19.45	19.45	5068	25.00					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

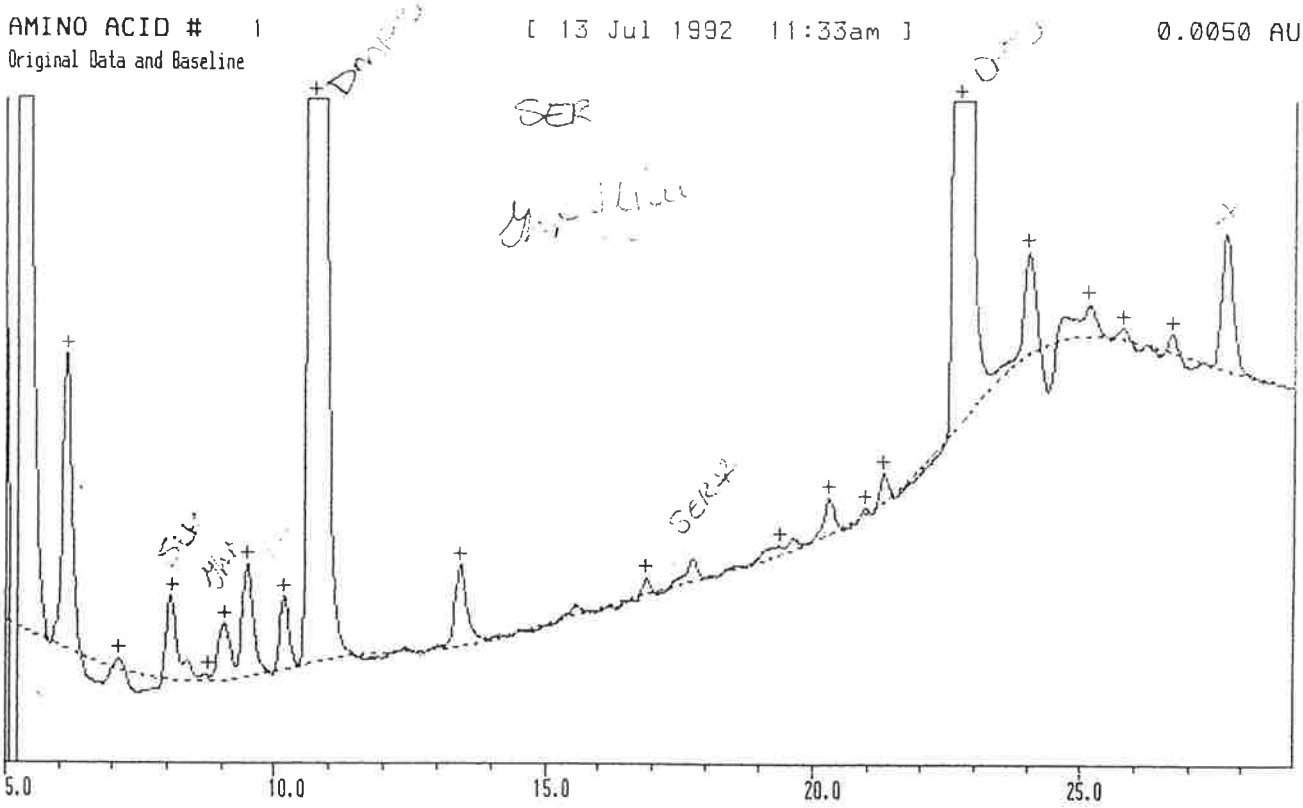
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 1 [13 Jul 1992 11:33am] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.35		58267		DPT	22.72	22.72	89731	128.16
ASP	6.17	6.07	5340	4.73	TRP	24.00	24.12	1852	1.53
ASN	7.12	7.12	201	0.22	PHE	25.13	25.13	564	0.63
SER	8.08	8.18	1531	2.55	ILE	25.77	25.72	196	0.27
GLN	8.75	8.77	105	0.12	LEU	26.65	26.62	338	0.42
THR	9.07	9.20	1046	1.73		27.67		2457	
GLY	9.48	9.58	2068	2.97					
GLU	10.20	10.32	1332	1.40					
DMP	10.77	10.85	52596	261.77					
ALA	13.42	13.50	1526	1.90					
TYR	16.88	16.92	295	0.29					
ARG	19.38	19.45	156	0.77					
PRO	20.28	20.30	636	0.78					
MET	20.95	20.93	120	0.11					
VAL	21.30	21.30	508	0.49					

Tabulation threshold : 500 uAU

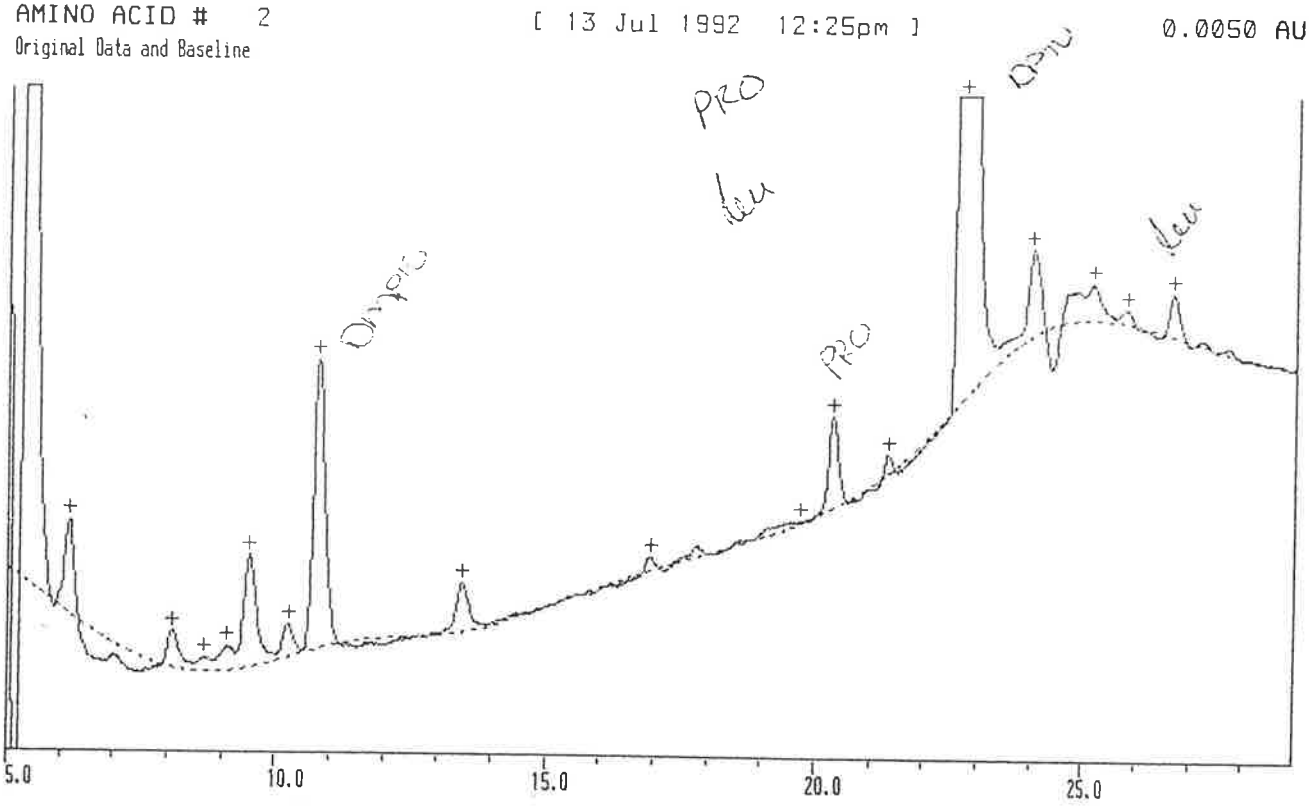
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 2 [13 Jul 1992 12:25pm] 0.0050 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.07		4704		TRP	24.00	24.12	1562	1.29
	5.37		57772			24.75		528	
ASP	6.18	6.07	1696	1.50	PHE	25.15	25.13	645	0.72
SER	8.10	8.18	672	1.12	ILE	25.78	25.72	259	0.35
GLN	8.68	8.77	266	0.31	LEU	26.65	26.62	758	0.94
THR	9.12	9.20	444	0.73					
GLY	9.52	9.58	2020	2.90					
GLU	10.25	10.32	614	0.64					
DMP	10.78	10.85	5167	25.72					
ALA	13.45	13.50	897	1.12					
TYR	16.93	16.92	256	0.25					
ARG	19.72	19.45	43	0.21					
PRO	20.30	20.30	1634	2.01					
VAL	21.32	21.30	355	0.34					
DPT	22.73	22.72	112027	160.01					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

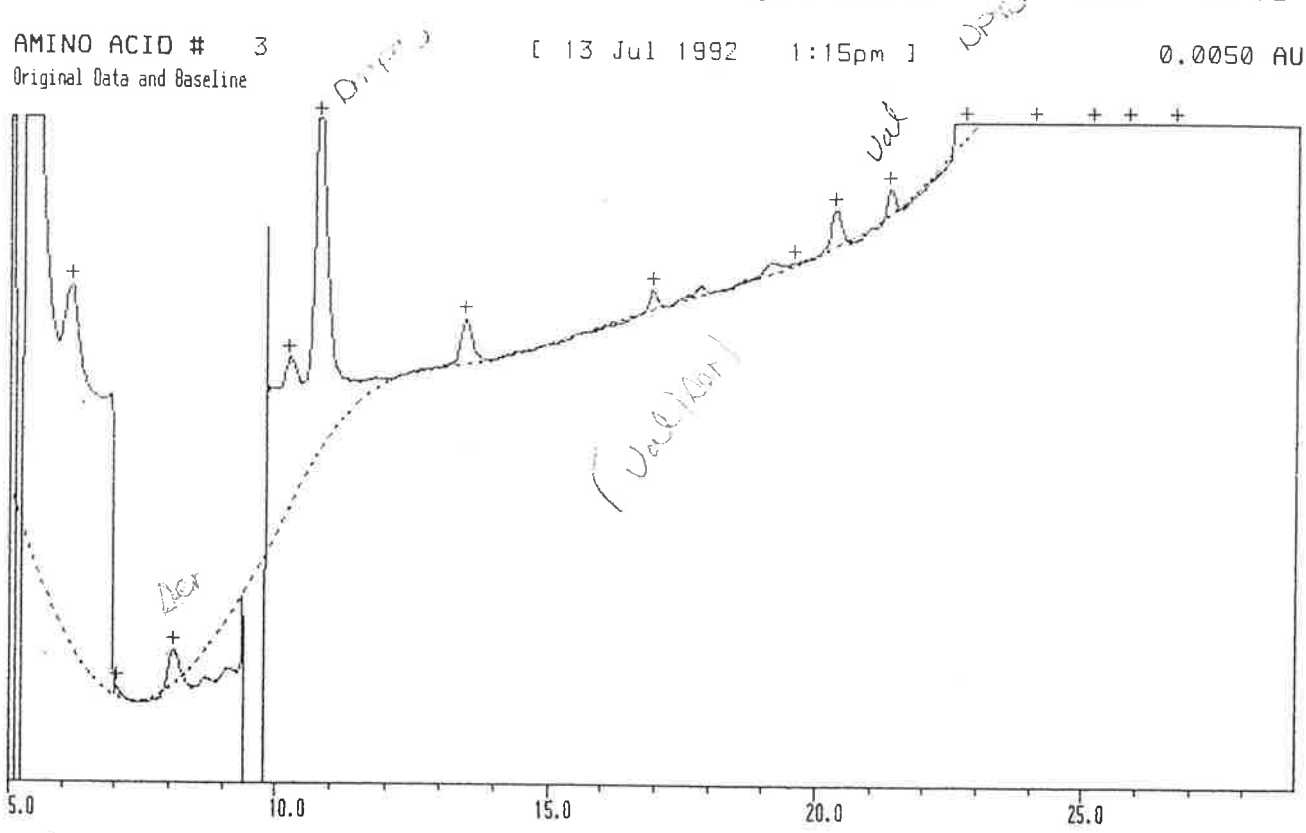
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 3 [13 Jul 1992 1:15pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.07		10922		PHE	25.17	25.13	590	0.66
	5.38		53493		ILE	25.83	25.72	189	0.26
ASP	6.18	6.07	6496	5.76	LEU	26.70	26.62	691	0.86
	6.93		5376						
ASN	7.05	7.12	172	0.19					
SER	8.10	8.18	631	1.05					
GLU	10.23	10.32	2726	2.86					
DMP	10.80	10.85	6321	31.46					
ALA	13.47	13.50	799	0.99					
TYR	16.95	16.92	343	0.34					
ARG	19.57	19.45	48	0.24					
PRO	20.33	20.30	645	0.79					
VAL	21.33	21.30	458	0.44					
DPT	22.75	22.72	95582	136.52					
TRP	24.05	24.12	1171	0.97					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

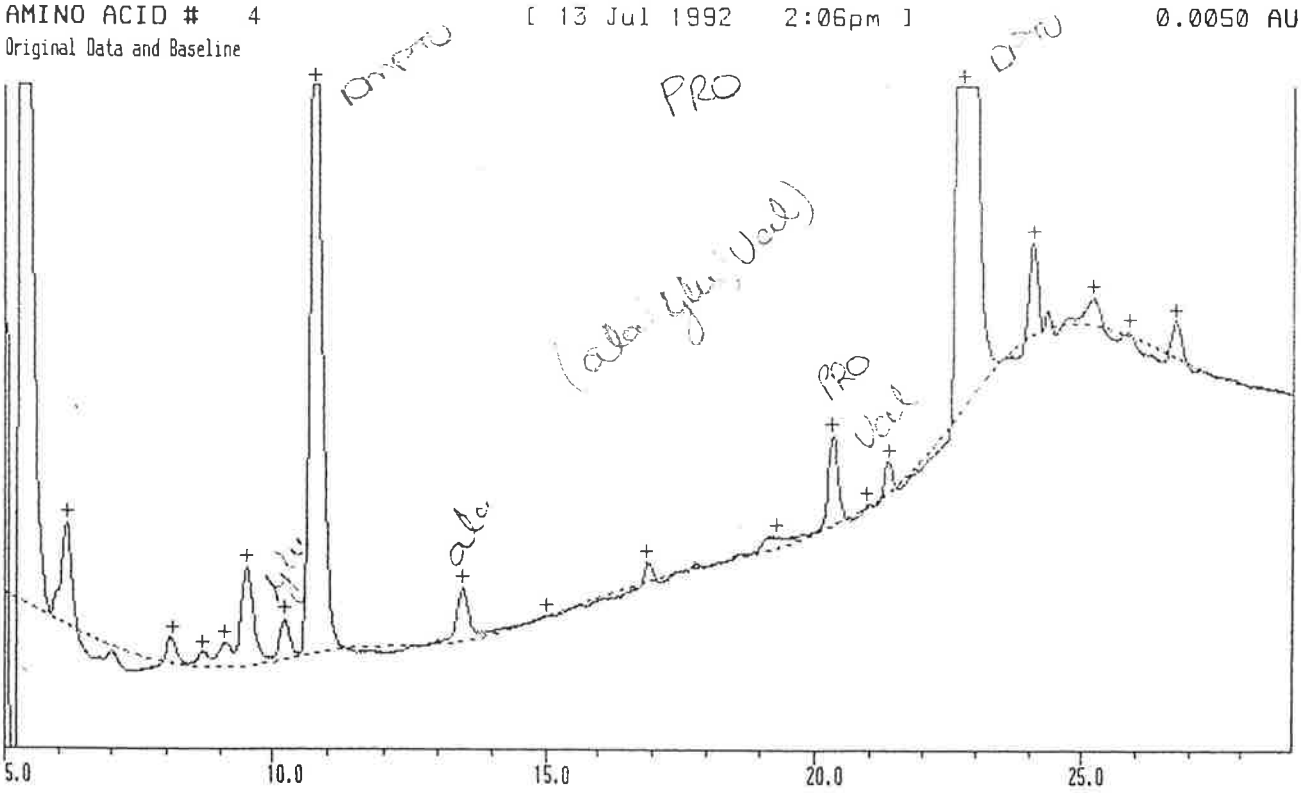
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 4 [13 Jul 1992 2:06pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.37		58600		DPT	22.77	22.72	148034	211.44
ASP	6.18	6.07	1862	1.65	TRP	24.05	24.12	1634	1.35
SER	8.12	8.18	446	0.74	PHE	25.20	25.13	484	0.54
GLN	8.70	8.77	276	0.32	ILE	25.85	25.72	76	0.10
THR	9.10	9.20	451	0.74	LEU	26.73	26.62	669	0.83
GLY	9.52	9.58	1800	2.59					
GLU	10.23	10.32	741	0.78					
DMP	10.80	10.85	11800	58.73					
ALA	13.45	13.50	981	1.22					
HIS	15.03	15.15	12	0.04					
TYR	16.92	16.92	336	0.33					
ARG	19.32	19.45	187	0.92					
PRO	20.33	20.30	1584	1.95					
MET	20.97	20.93	50	0.05					
VAL	21.35	21.30	561	0.54					

Tabulation threshold : 500 uAU

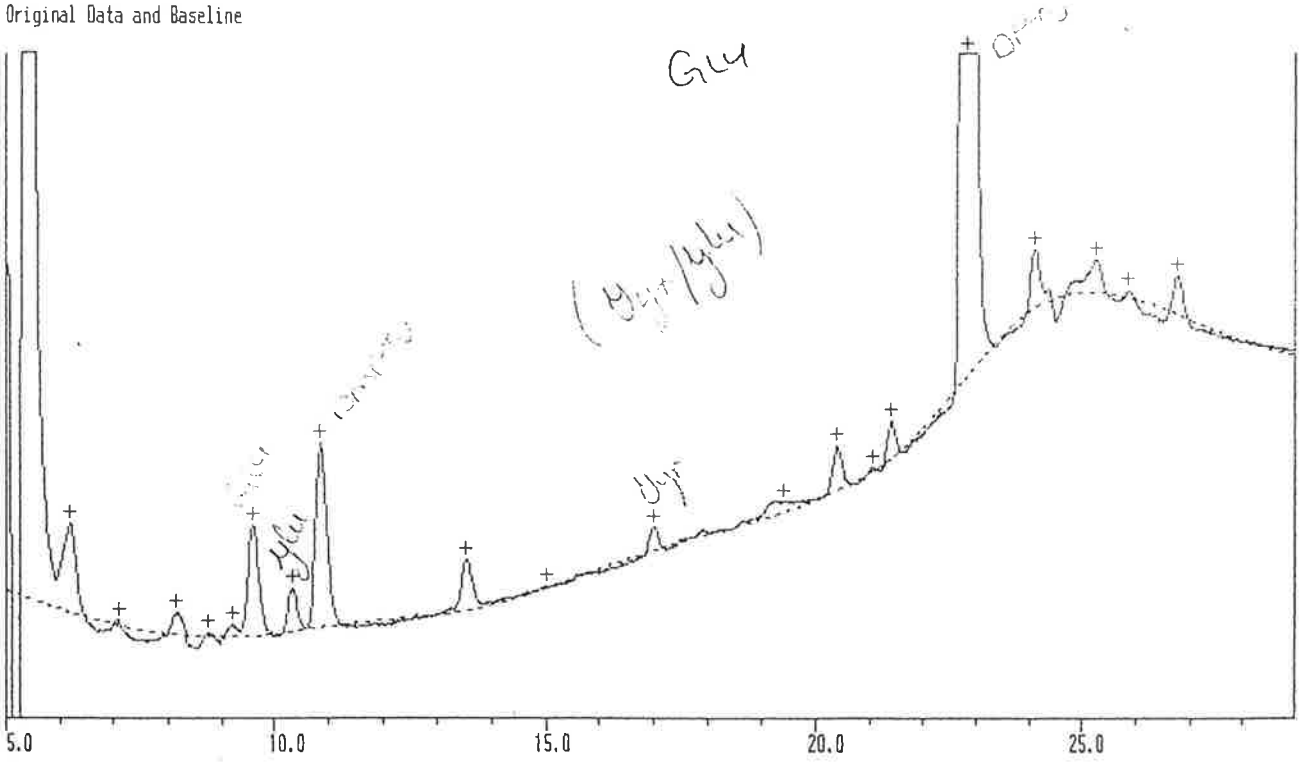
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1
Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 5 [13 Jul 1992 2:58pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.03		5824		MET	21.05	20.93	76	0.07
	5.42		53880		VAL	21.40	21.30	657	0.63
ASP	6.23	6.07	1608	1.42	DPT	22.82	22.72	63628	90.88
ASN	7.10	7.12	108	0.12	TRP	24.10	24.12	1068	0.88
SER	8.18	8.18	424	0.71	PHE	25.25	25.13	583	0.65
GLN	8.75	8.77	81	0.10	ILE	25.88	25.72	120	0.16
THR	9.22	9.20	216	0.36	LEU	26.78	26.62	686	0.85
GLY	9.60	9.58	1975	2.84					
GLU	10.35	10.32	770	0.81					
DMP	10.87	10.85	3312	16.48					
ALA	13.53	13.50	928	1.15					
HIS	15.02	15.15	16	0.06					
TYR	17.03	16.92	393	0.39					
ARG	19.43	19.45	201	0.99					
PRO	20.40	20.30	784	0.97					

Tabulation threshold : 500 uAU

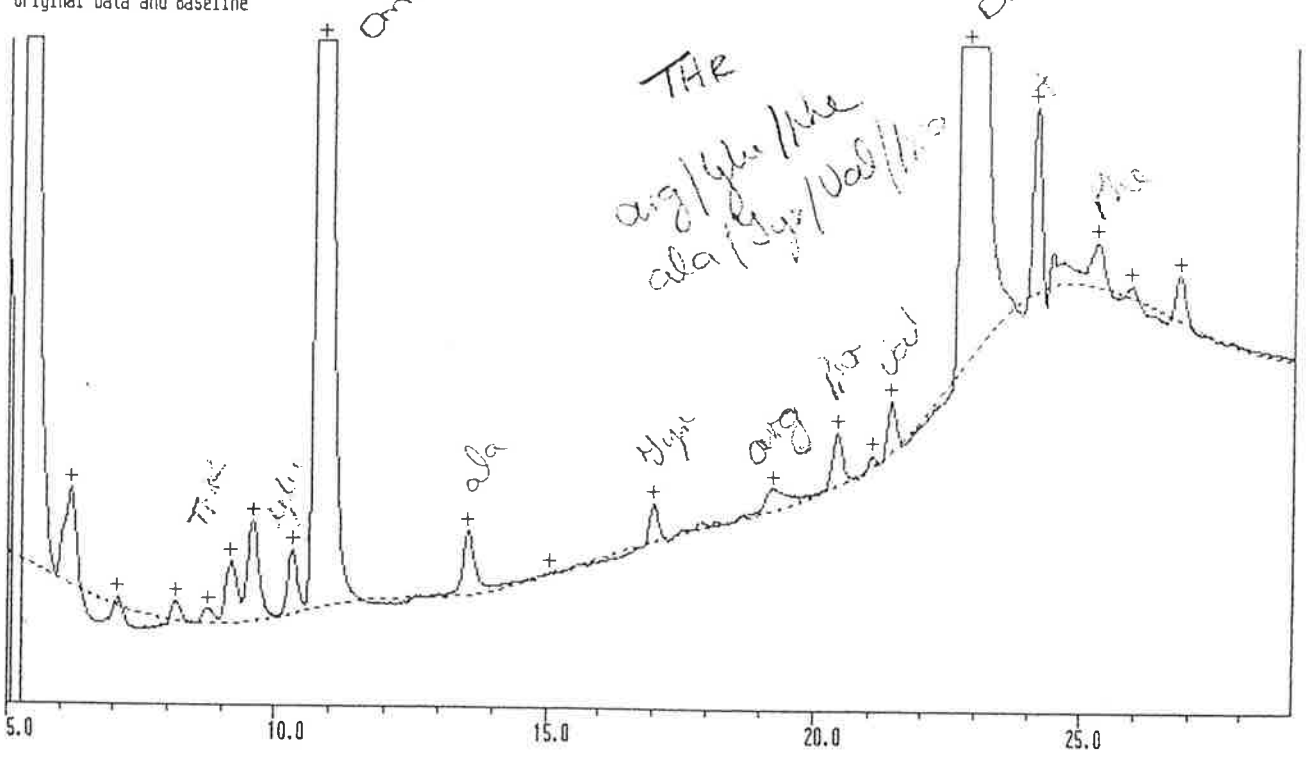
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 6 [13 Jul 1992 3:49pm] 0.0050 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		65412		VAL	21.42	21.30	909	0.88
ASP	6.22	6.07	1749	1.55	DPT	22.83	22.72	365258	521.70
ASN	7.08	7.12	148	0.16	TRP	24.12	24.12	3321	2.74
SER	8.18	8.18	364	0.61		24.42		578	
GLN	8.78	8.77	278	0.33	PHE	25.27	25.13	753	0.85
THR	9.18	9.20	1106	1.83	ILE	25.92	25.72	206	0.28
GLY	9.60	9.58	1814	2.61	LEU	26.80	26.62	813	1.01
GLU	10.33	10.32	1144	1.20					
DMP	10.87	10.85	38440	191.32					
ALA	13.53	13.50	1149	1.43					
HIS	15.07	15.15	55	0.19					
TYR	17.02	16.92	674	0.67					
ARG	19.23	19.45	405	2.00					
PRO	20.42	20.30	938	1.15					
MET	21.05	20.93	204	0.20					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

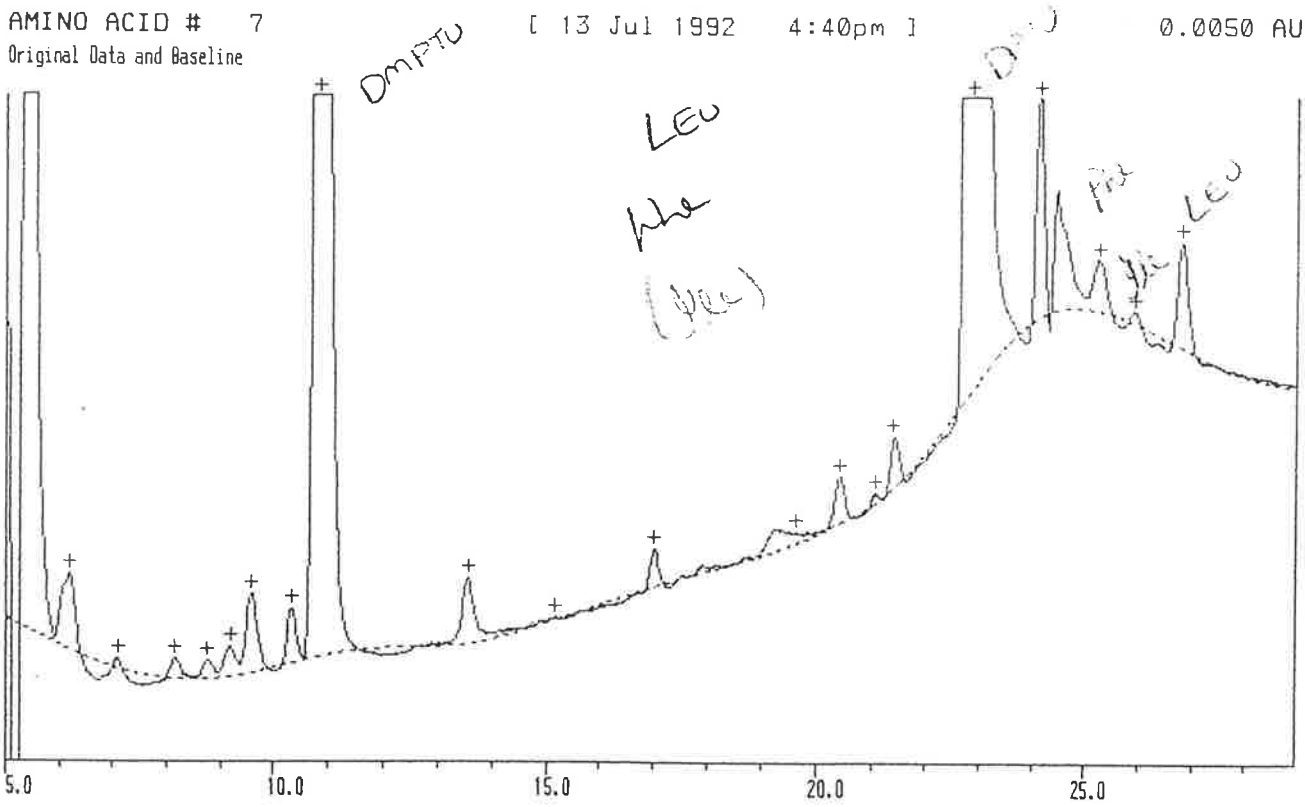
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 7 [13 Jul 1992 4:40pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		59304		VAL	21.42	21.30	931	0.90
ASP	6.22	6.07	1365	1.21	DPT	22.85	22.72	415020	592.78
ASN	7.10	7.12	160	0.18	TRP	24.13	24.12	4039	3.33
SER	8.18	8.18	374	0.62		24.45		2148	
GLN	8.78	8.77	328	0.39	PHE	25.27	25.13	916	1.03
THR	9.20	9.20	525	0.87	ILE	25.93	25.72	216	0.29
GLY	9.60	9.58	1396	2.01	LEU	26.80	26.62	1900	2.36
GLU	10.35	10.32	972	1.02					
DMP	10.87	10.85	45830	228.09					
ALA	13.55	13.50	1166	1.45					
HIS	15.18	15.15	31	0.10					
TYR	17.02	16.92	676	0.67					
ARG	19.65	19.45	201	0.99					
PRO	20.42	20.30	844	1.04					
MET	21.07	20.93	163	0.16					

Tabulation threshold : 500 uAU

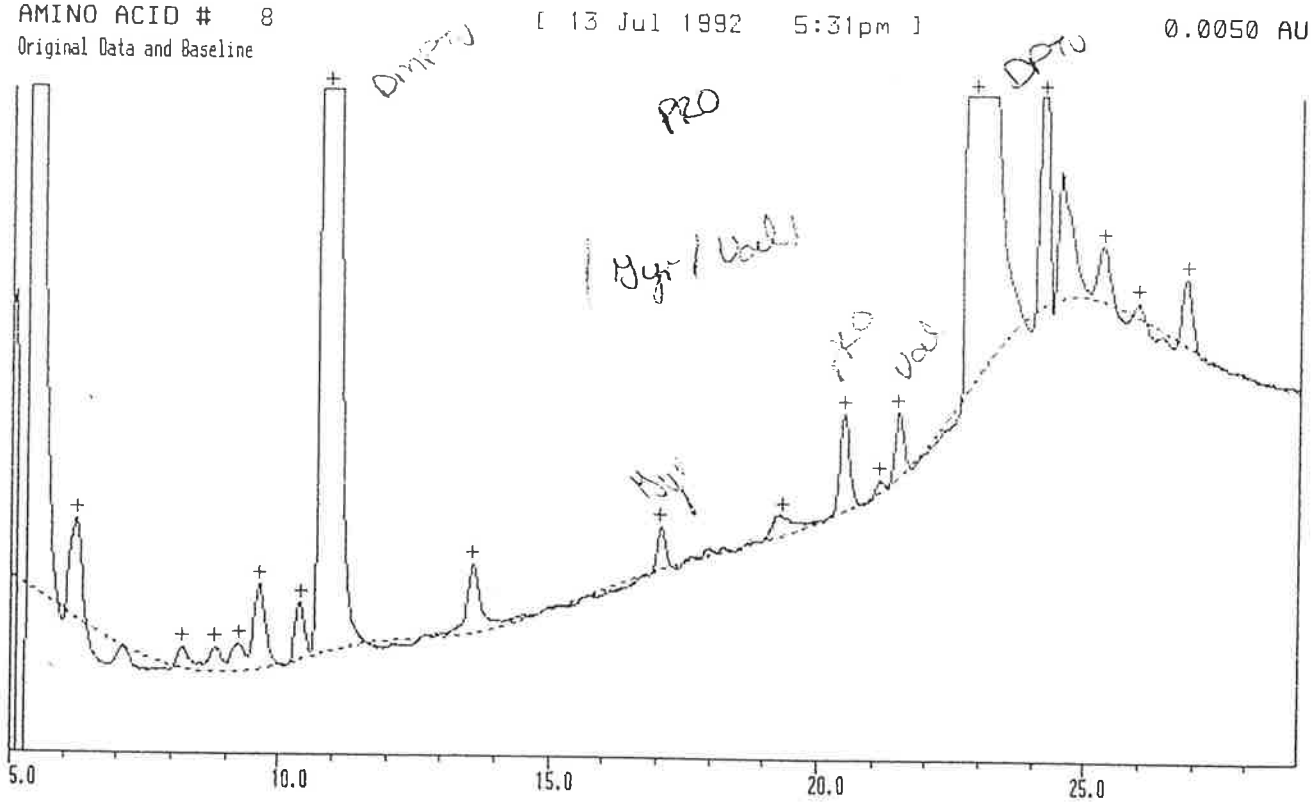
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 8 [13 Jul 1992 5:31pm] 0.0050 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.10		5152		DPT	22.88	22.72	555496	793.42
	5.43		59532		TRP	24.17	24.12	5068	4.18
ASP	6.25	6.07	1814	1.61		24.48		2275	
SER	8.22	8.18	384	0.64	PHE	25.30	25.13	986	1.11
GLN	8.82	8.77	451	0.53	ILE	25.95	25.72	230	0.31
THR	9.25	9.20	511	0.84	LEU	26.83	26.62	1200	1.49
GLY	9.63	9.58	1545	2.22					
GLU	10.40	10.32	1046	1.10					
DMP	10.92	10.85	50601	251.84					
ALA	13.58	13.50	1248	1.55					
TYR	17.07	16.92	789	0.78					
ARG	19.33	19.45	348	1.72					
PRO	20.47	20.30	1730	2.13					
MET	21.10	20.93	228	0.22					
VAL	21.45	21.30	1161	1.12					

Tabulation threshold : 500 uAU

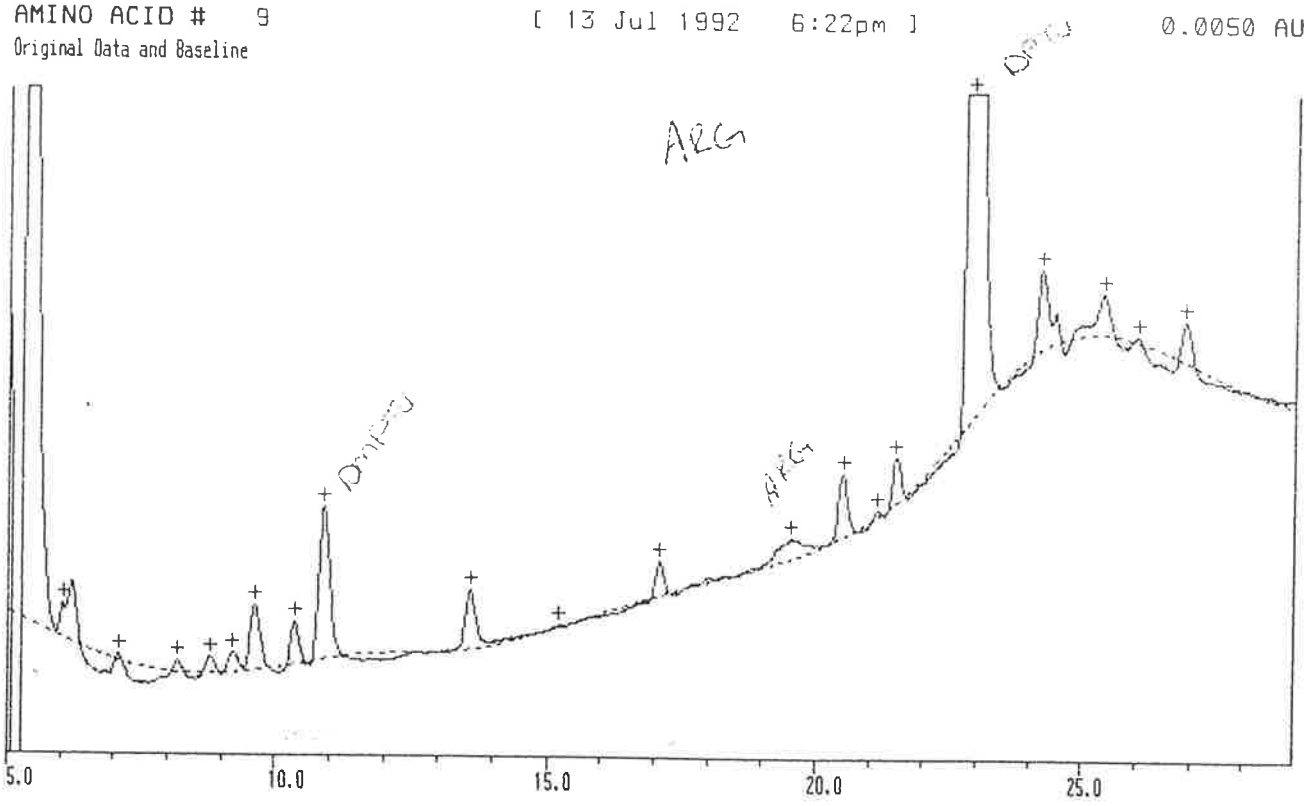
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 9 [13 Jul 1992 6:22pm] 0.0050 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		50191		MET	21.15	20.93	110	0.11
ASP	6.05	6.07	600	0.53	VAL	21.48	21.30	823	0.79
	6.23		1080		DPT	22.90	22.72	80179	114.52
ASN	7.12	7.12	110	0.12	TRP	24.20	24.12	1430	1.18
SER	8.20	8.18	199	0.33		24.45		540	
GLN	8.80	8.77	314	0.37	PHE	25.37	25.13	736	0.83
THR	9.23	9.20	352	0.58	ILE	26.02	25.72	110	0.15
GLY	9.63	9.58	1178	1.69	LEU	26.88	26.62	748	0.93
GLU	10.37	10.32	756	0.79					
DMP	10.90	10.85	2740	13.64					
ALA	13.60	13.50	1051	1.31					
HIS	15.23	15.15	28	0.10					
TYR	17.10	16.92	612	0.60					
ARG	19.53	19.45	364	1.80					
PRO	20.50	20.30	1101	1.35					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

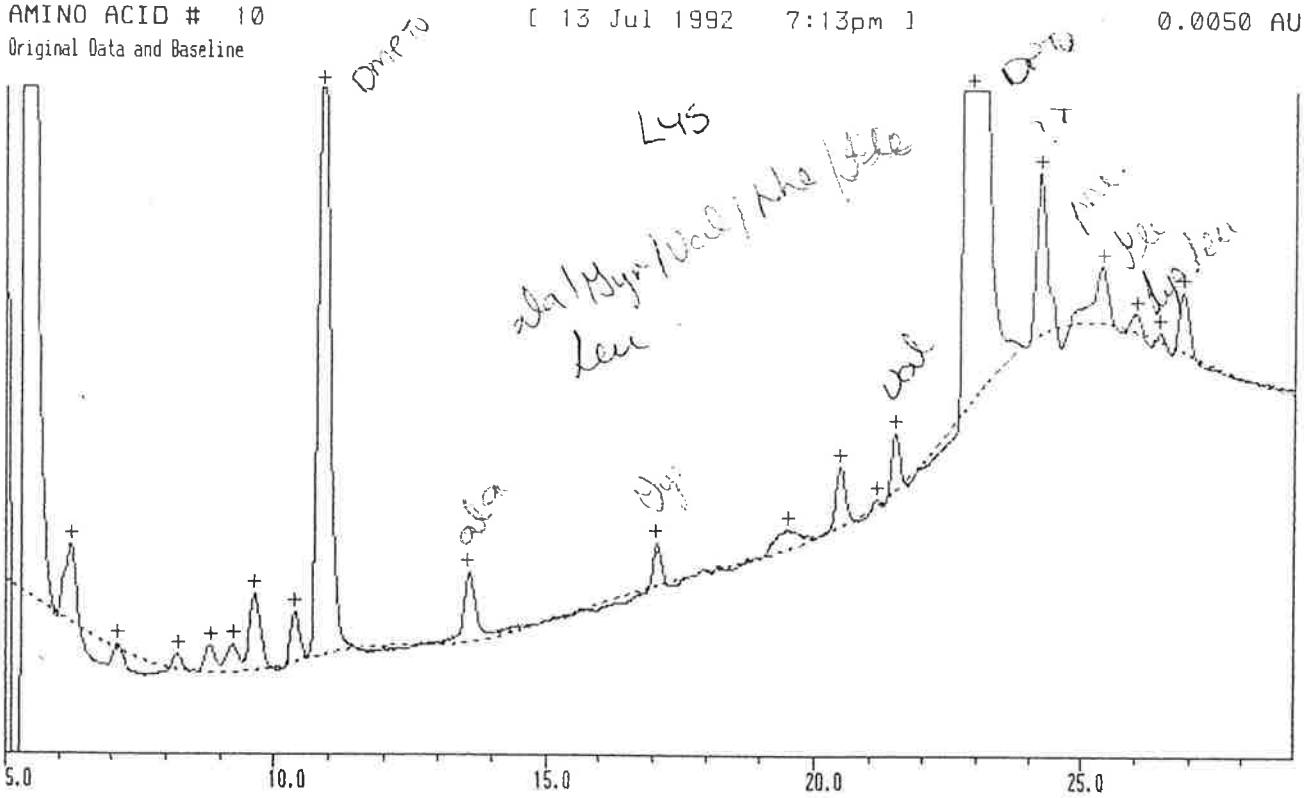
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 10 [13 Jul 1992 7:13pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.42		56469		DPT	22.90	22.72	232989	332.78
ASP	6.25	6.07	1389	1.23	TRP	24.20	24.12	2887	2.38
ASN	7.13	7.12	81	0.09	PHE	25.37	25.13	1027	1.15
SER	8.23	8.18	273	0.46	ILE	26.00	25.72	324	0.44
GLN	8.83	8.77	487	0.57	LYS	26.43	26.18	160	0.17
THR	9.25	9.20	496	0.82	LEU	26.87	26.62	1068	1.32
GLY	9.63	9.58	1384	1.99					
GLU	10.38	10.32	902	0.95					
DMP	10.90	10.85	11642	57.94					
ALA	13.58	13.50	1243	1.55					
TYR	17.07	16.92	760	0.75					
ARG	19.52	19.45	367	1.81					
PRO	20.47	20.30	1072	1.32					
MET	21.13	20.93	117	0.11					
VAL	21.48	21.30	1015	0.98					

Tabulation threshold : 500 uAU

- Applied Biosystems 475A Protein Sequencer Chromatogram Report -

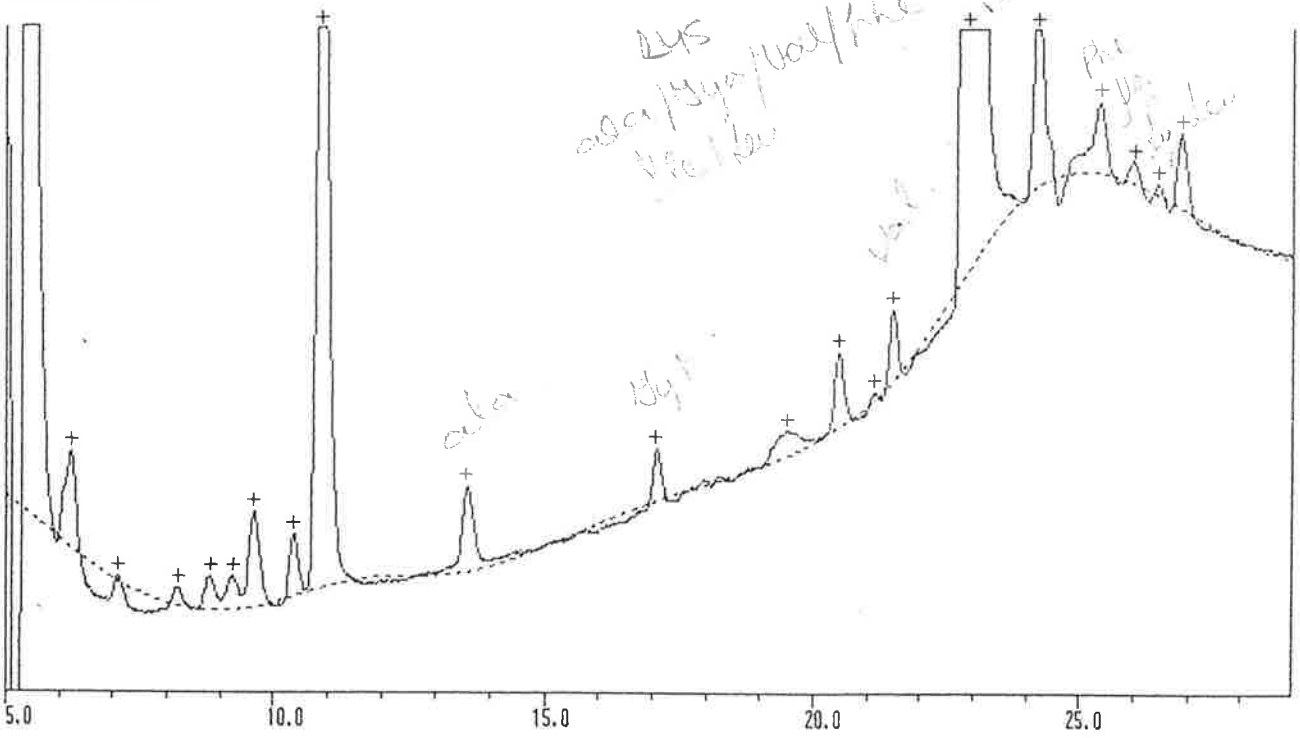
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 10 [13 Jul 1992 7:13pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.42		56469		DPT	22.90	22.72	232989	332.78
ASP	6.25	6.07	1389	1.23	TRP	24.20	24.12	2887	2.38
ASN	7.13	7.12	81	0.09	PHE	25.37	25.13	1027	1.15
SER	8.23	8.18	273	0.46	ILE	26.00	25.72	324	0.44
GLN	8.83	8.77	487	0.57	LYS	26.43	26.18	160	0.17
THR	9.25	9.20	496	0.82	LEU	26.87	26.62	1068	1.32
GLY	9.63	9.58	1384	1.99					
GLU	10.38	10.32	902	0.95					
DMP	10.90	10.85	11642	57.94					
ALA	13.58	13.50	1243	1.55					
TYR	17.07	16.92	760	0.75					
ARG	19.52	19.45	367	1.81					
PRO	20.47	20.30	1072	1.32					
MET	21.13	20.93	117	0.11					
VAL	21.48	21.30	1015	0.98					

Tabulation threshold : 500 uAU

- Applied Biosystems 475A Protein Sequencer Chromatogram Report -

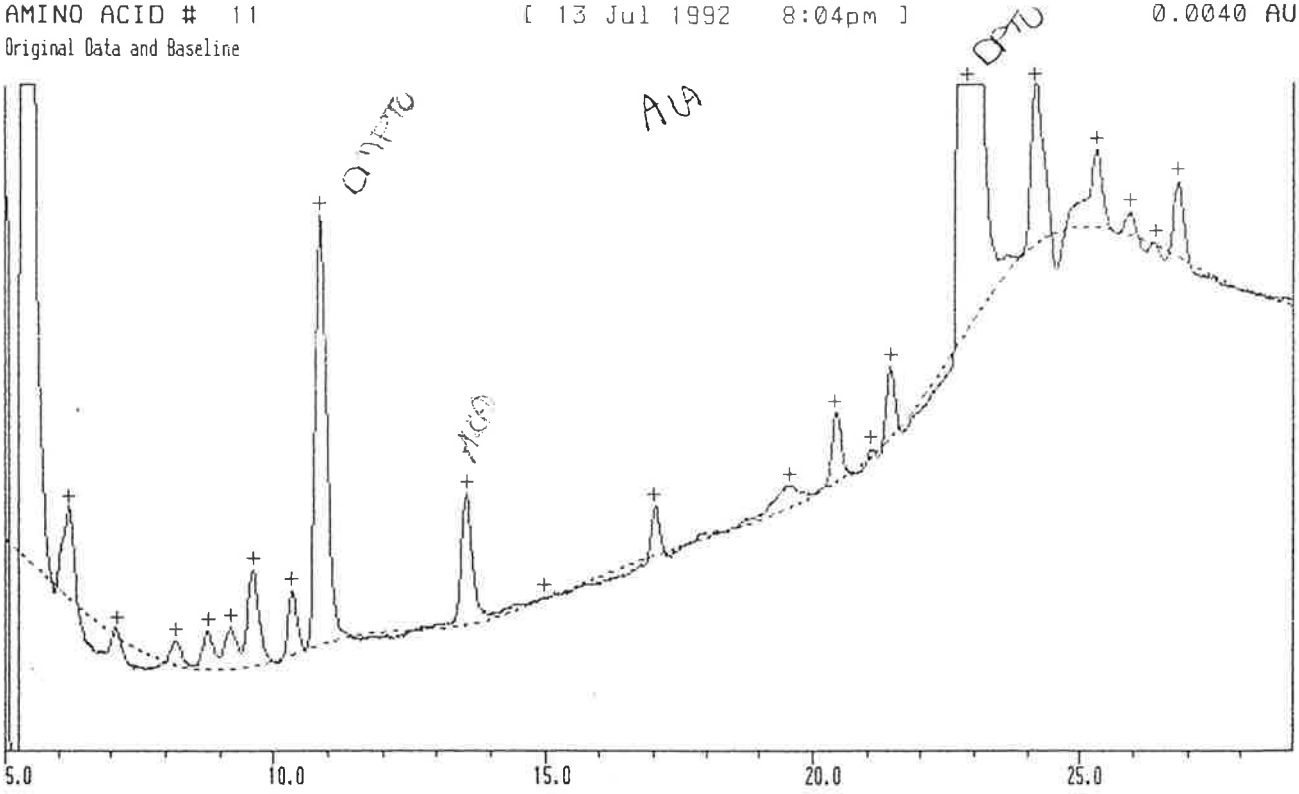
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 11 [13 Jul 1992 8:04pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		53032		VAL	21.43	21.30	1029	0.99
ASP	6.23	6.07	1308	1.16	DPT	22.87	22.72	177048	252.88
ASN	7.12	7.12	110	0.12	TRP	24.15	24.12	2479	2.04
SER	8.20	8.18	343	0.57	PHE	25.32	25.13	1096	1.23
GLN	8.80	8.77	547	0.64	ILE	25.95	25.72	316	0.43
THR	9.22	9.20	590	0.97	LYS	26.38	26.18	28	0.03
GLY	9.62	9.58	1358	1.95	LEU	26.82	26.62	1075	1.33
GLU	10.35	10.32	919	0.96					
DMP	10.88	10.85	6168	30.70					
ALA	13.55	13.50	1879	2.34					
HIS	15.00	15.15	26	0.09					
TYR	17.03	16.92	715	0.71					
ARG	19.55	19.45	331	1.63					
PRO	20.43	20.30	988	1.22					
MET	21.07	20.93	108	0.10					

Tabulation threshold : 500 uAU

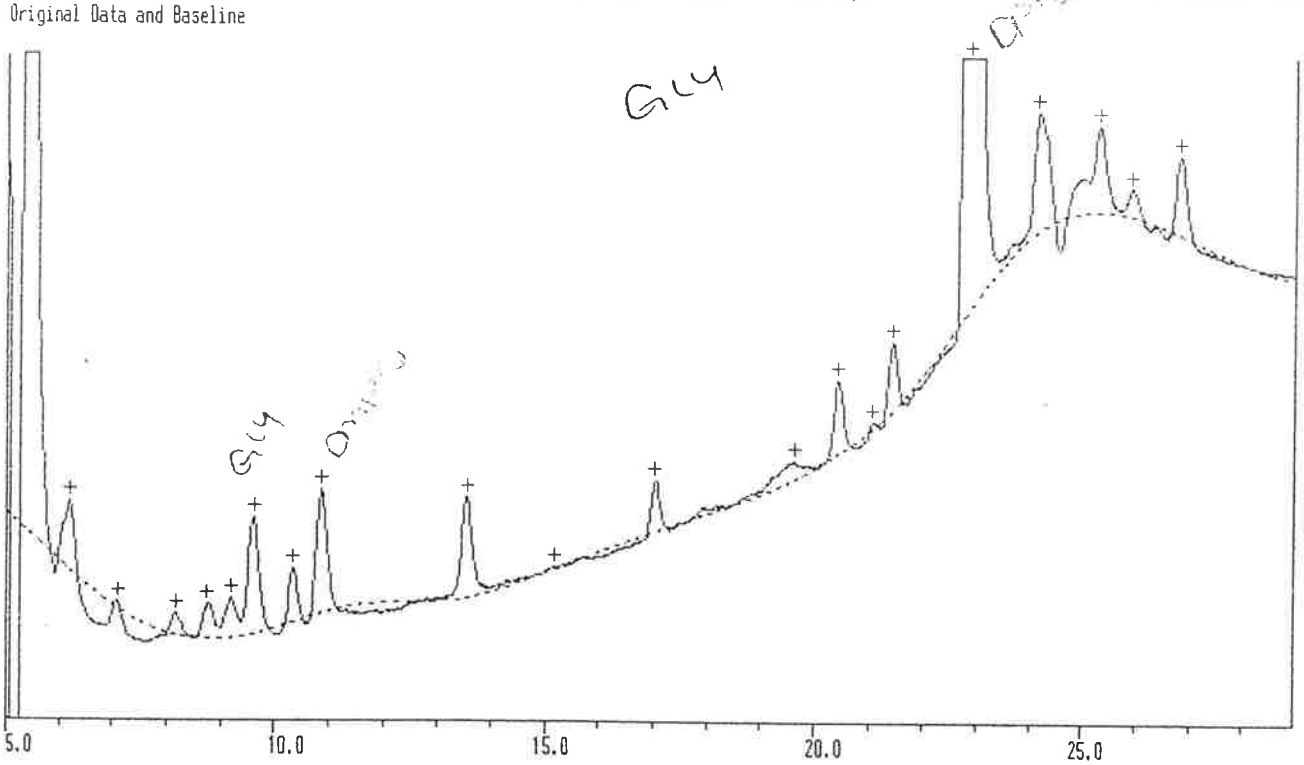
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 12 [13 Jul 1992 8:55pm] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		50733		VAL	21.43	21.30	998	0.96
ASP	6.22	6.07	998	0.88	DPT	22.85	22.72	85209	121.71
ASN	7.10	7.12	103	0.11	TRP	24.15	24.12	1725	1.42
SER	8.20	8.18	302	0.50		25.02		504	
GLN	8.78	8.77	508	0.60	PHE	25.30	25.13	1236	1.39
THR	9.20	9.20	554	0.92	ILE	25.92	25.72	422	0.57
GLY	9.62	9.58	1660	2.39	LEU	26.80	26.62	1116	1.38
GLU	10.35	10.32	787	0.83					
DMP	10.87	10.85	1780	8.86					
ALA	13.55	13.50	1449	1.80					
HIS	15.18	15.15	40	0.14					
TYR	17.03	16.92	722	0.71					
ARG	19.63	19.45	249	1.23					
PRO	20.43	20.30	1051	1.29					
MET	21.05	20.93	93	0.09					

Tabulation threshold : 500 uAU

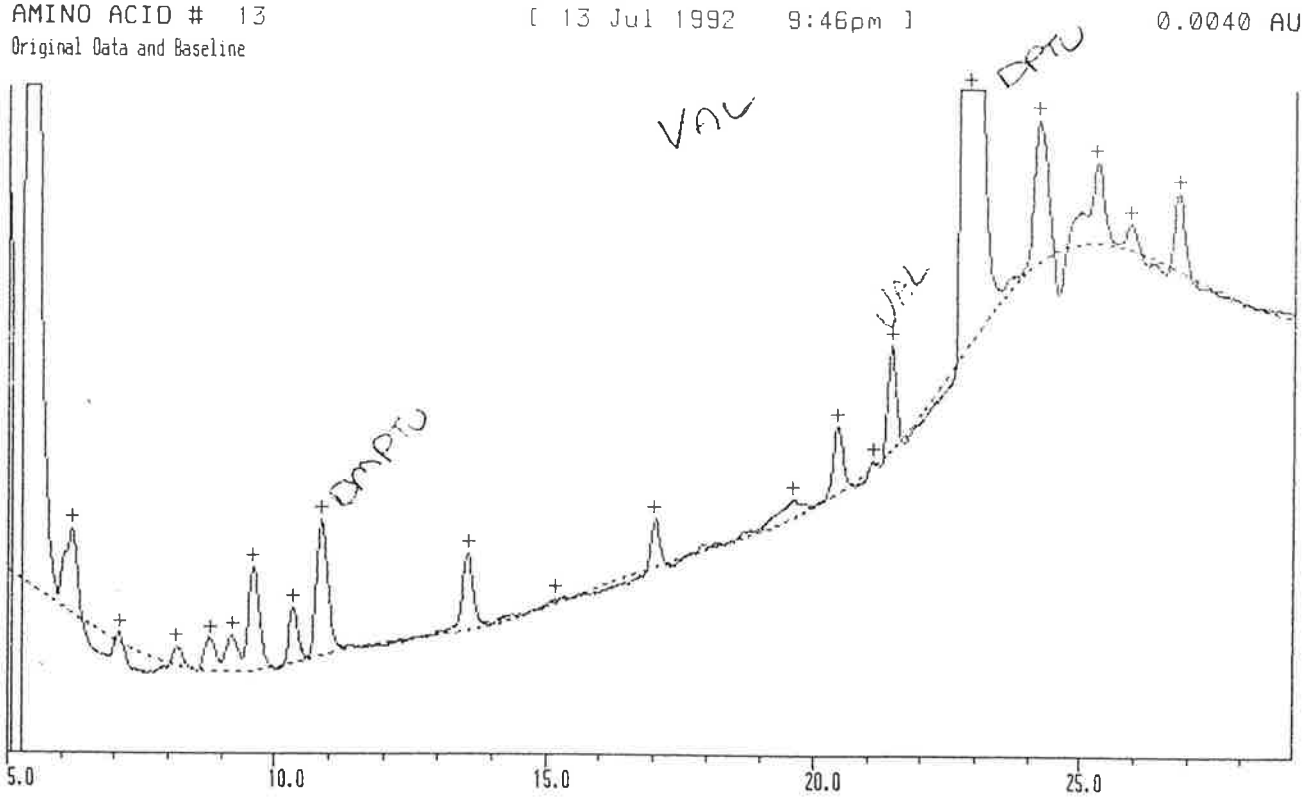
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 13 [13 Jul 1992 9:46pm] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		52118		VAL	21.45	21.30	1480	1.43
ASP	6.22	6.07	1236	1.10	DPT	22.87	22.72	108355	154.76
ASN	7.10	7.12	136	0.15	TRP	24.17	24.12	2035	1.68
SER	8.17	8.18	271	0.45	PHE	25.28	25.13	1166	1.31
GLN	8.82	8.77	456	0.53	ILE	25.92	25.72	369	0.50
THR	9.22	9.20	520	0.86	LEU	26.80	26.62	1104	1.37
GLY	9.60	9.58	1502	2.16					
GLU	10.35	10.32	801	0.84					
DMP	10.87	10.85	1977	9.84					
ALA	13.55	13.50	1108	1.38					
HIS	15.18	15.15	76	0.26					
TYR	17.03	16.92	700	0.69					
ARG	19.60	19.45	271	1.34					
PRO	20.43	20.30	955	1.17					
MET	21.10	20.93	79	0.08					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

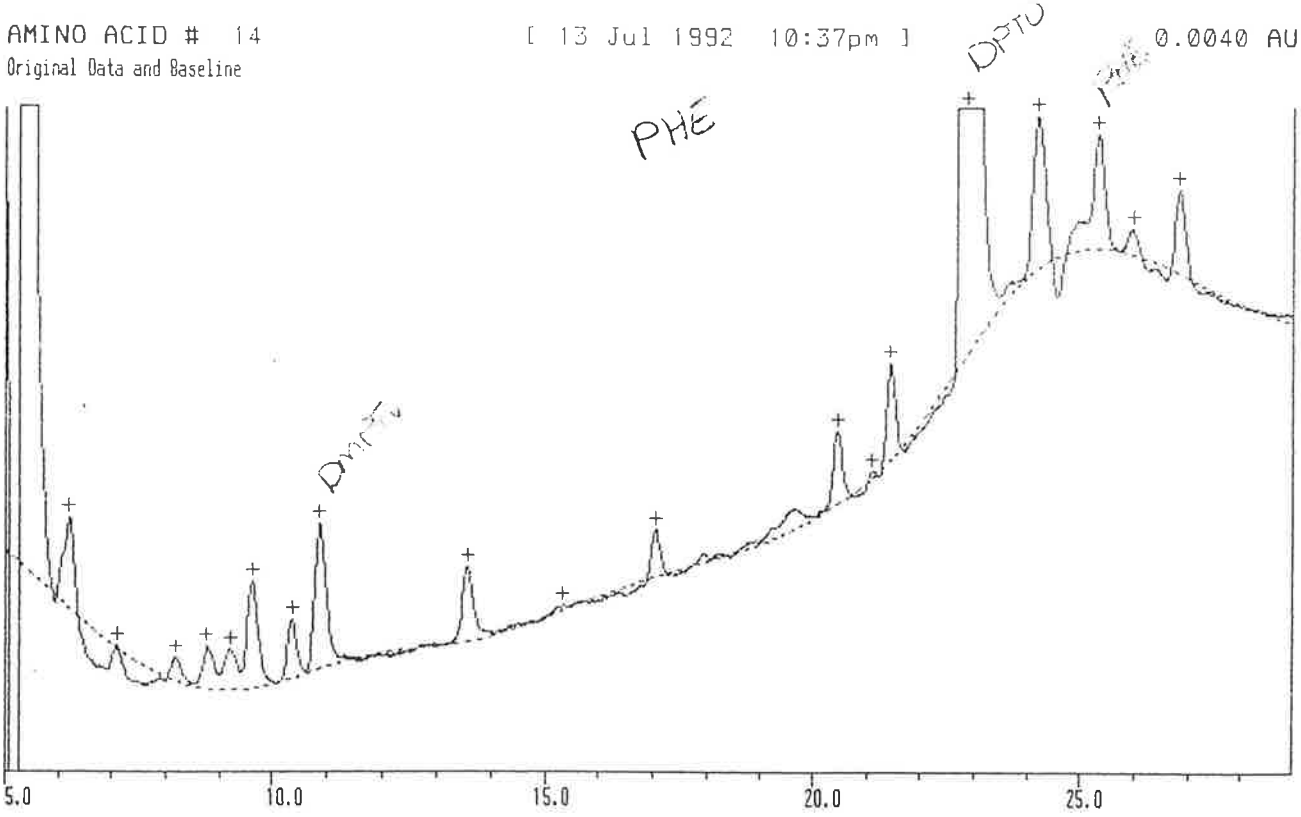
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 14 [13 Jul 1992 10:37pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		59736		DPT	22.87	22.72	116988	167.10
ASP	6.23	6.07	1327	1.18	TRP	24.18	24.12	2186	1.80
ASN	7.12	7.12	43	0.05	PHE	25.32	25.13	1634	1.83
SER	8.22	8.18	345	0.58	ILE	25.97	25.72	362	0.49
GLN	8.78	8.77	619	0.73	LEU	26.82	26.62	1207	1.50
THR	9.22	9.20	588	0.97					
GLY	9.62	9.58	1548	2.22					
GLU	10.37	10.32	856	0.90					
DMP	10.88	10.85	2085	10.38					
ALA	13.55	13.50	1075	1.34					
HIS	15.33	15.15	72	0.24					
TYR	17.05	16.92	698	0.69					
PRO	20.45	20.30	1039	1.28					
MET	21.10	20.93	105	0.10					
VAL	21.45	21.30	1389	1.34					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

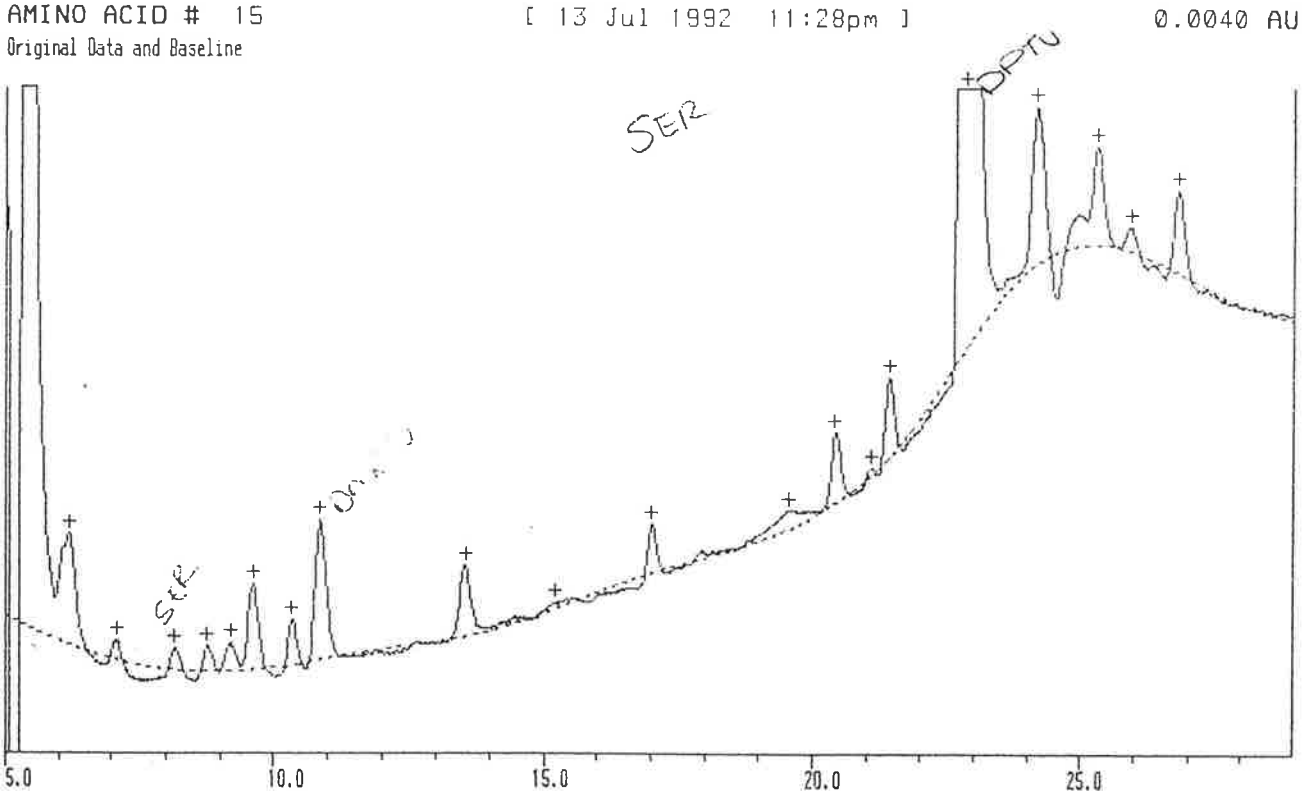
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 15 [13 Jul 1992 11:28pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		50649		VAL	21.43	21.30	1156	1.12
ASP	6.22	6.07	1598	1.42	DPT	22.87	22.72	121831	174.01
ASN	7.12	7.12	278	0.31	TRP	24.18	24.12	2265	1.87
SER	8.18	8.18	300	0.50	PHE	25.30	25.13	1418	1.59
GLN	8.78	8.77	369	0.43	ILE	25.95	25.72	348	0.47
THR	9.20	9.20	408	0.67	LEU	26.80	26.62	1173	1.46
GLY	9.62	9.58	1214	1.75					
GLU	10.35	10.32	650	0.68					
DMP	10.88	10.85	2006	9.99					
ALA	13.55	13.50	1027	1.28					
HIS	15.23	15.15	76	0.26					
TYR	17.03	16.92	712	0.70					
ARG	19.57	19.45	276	1.36					
PRO	20.43	20.30	1012	1.25					
MET	21.10	20.93	105	0.10					

Tabulation threshold : 500 uAU

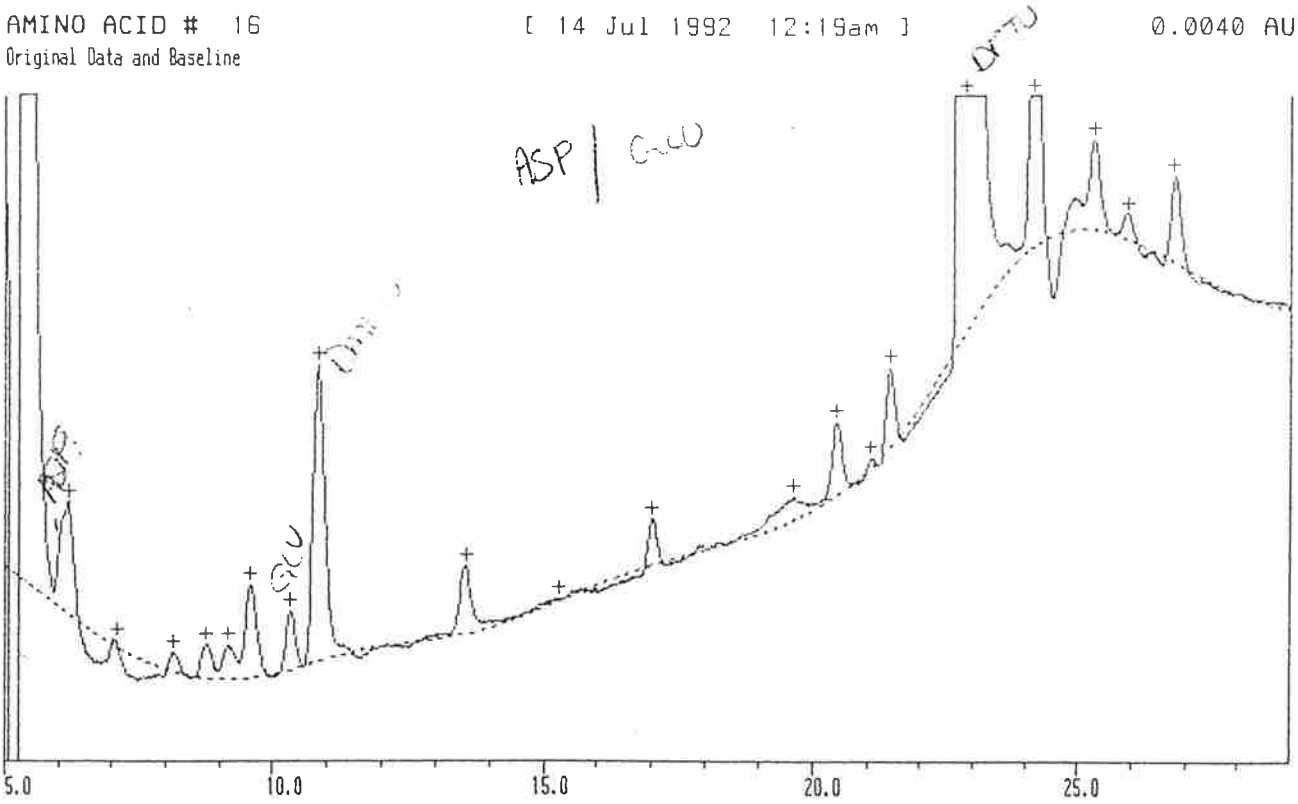
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 16 [14 Jul 1992 12:19am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		51139		VAL	21.43	21.30	1147	1.11
ASP	6.22	6.07	1615	1.43	DPT	22.85	22.72	262932	375.55
ASN	7.10	7.12	72	0.08	TRP	24.17	24.12	3583	2.95
SER	8.18	8.18	285	0.48	PHE	25.30	25.13	1291	1.45
GLN	8.78	8.77	494	0.58	ILE	25.95	25.72	369	0.50
THR	9.18	9.20	499	0.82	LEU	26.78	26.62	1226	1.52
GLY	9.60	9.58	1351	1.94					
GLU	10.35	10.32	861	0.90					
DMP	10.87	10.85	4257	21.19					
ALA	13.55	13.50	972	1.21					
HIS	15.28	15.15	28	0.10					
TYR	17.02	16.92	686	0.68					
ARG	19.65	19.45	321	1.59					
PRO	20.43	20.30	1034	1.27					
MET	21.07	20.93	144	0.14					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

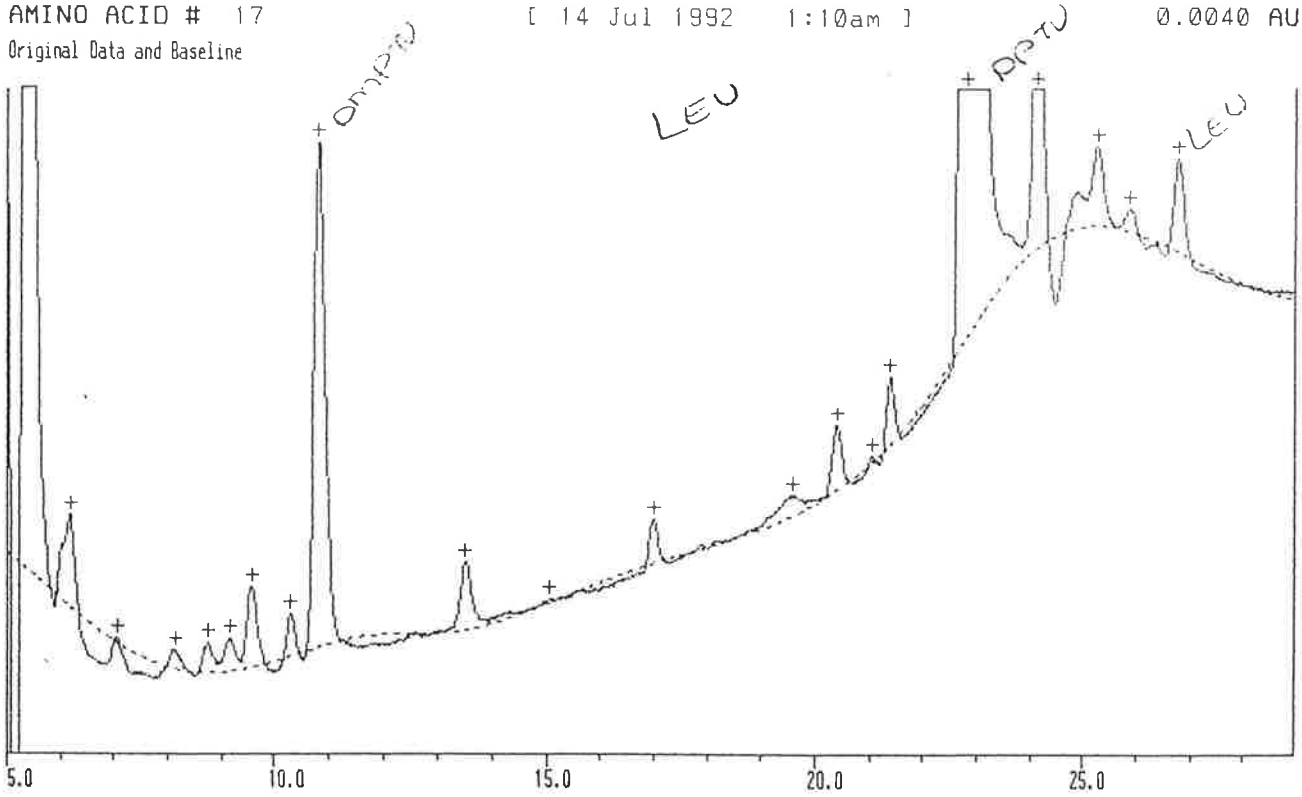
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 17 [14 Jul 1992 1:10am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.37		49190		VAL	21.40	21.30	998	0.96
ASP	6.20	6.07	1320	1.17	DPT	22.83	22.72	333314	476.08
ASN	7.07	7.12	79	0.09	TRP	24.13	24.12	4048	3.34
SER	8.15	8.18	259	0.43		24.87		523	
GLN	8.77	8.77	436	0.51	PHE	25.25	25.13	1140	1.28
THR	9.17	9.20	472	0.78	ILE	25.88	25.72	326	0.44
GLY	9.58	9.58	1152	1.66	LEU	26.75	26.62	1334	1.65
GLU	10.32	10.32	621	0.65					
DMP	10.83	10.85	7243	36.05					
ALA	13.50	13.50	964	1.20					
HIS	15.08	15.15	55	0.19					
TYR	17.00	16.92	636	0.63					
ARG	19.62	19.45	285	1.41					
PRO	20.42	20.30	931	1.15					
MET	21.05	20.93	108	0.10					

Tabulation threshold : 500 uAU

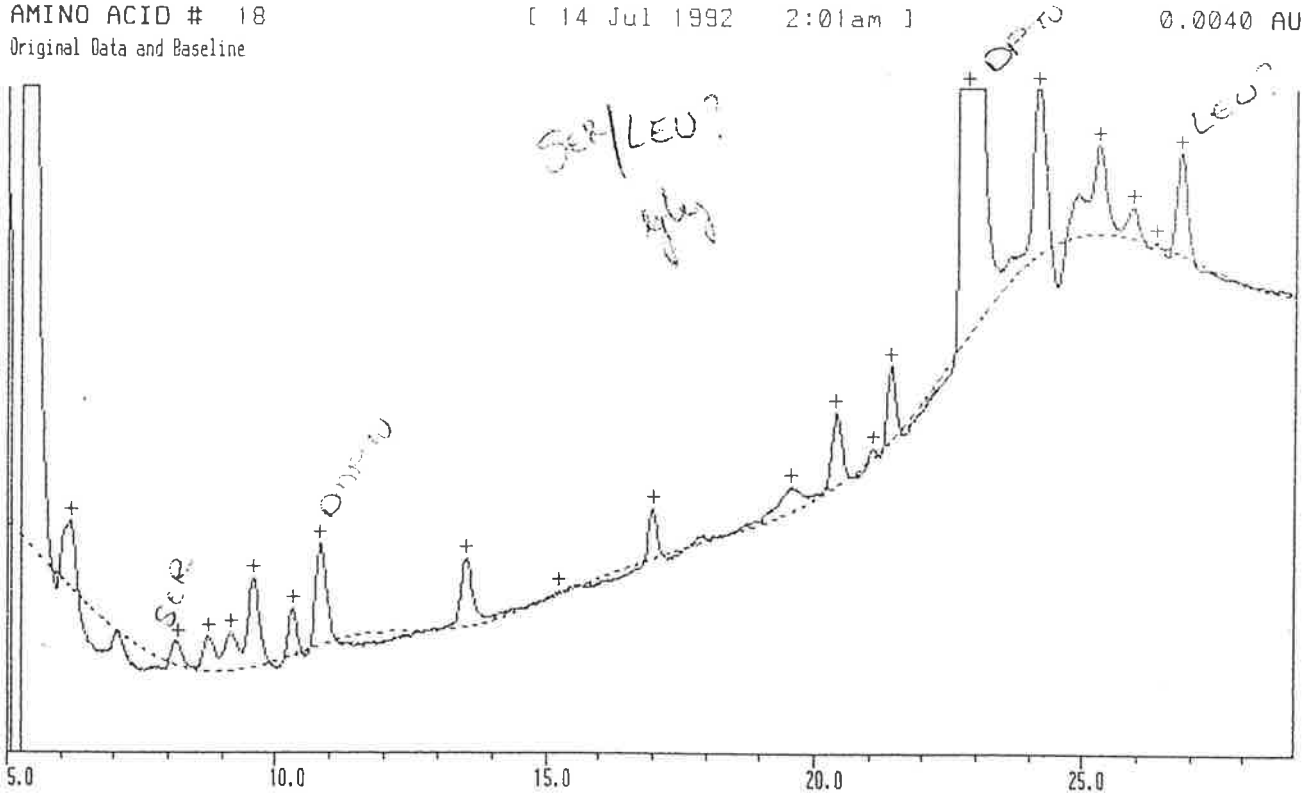
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 18 [14 Jul 1992 2:01am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		50781		DPT	22.83	22.72	125354	179.04
ASP	6.20	6.07	967	0.86	TRP	24.13	24.12	2712	2.24
SER	8.20	8.18	326	0.54		24.87		597	
GLN	8.75	8.77	499	0.59	PHE	25.25	25.13	1255	1.41
THR	9.18	9.20	549	0.91	ILE	25.92	25.72	415	0.56
GLY	9.60	9.58	1269	1.82	LYS	26.33	26.18	43	0.05
GLU	10.33	10.32	664	0.70	LEU	26.77	26.62	1459	1.81
DMP	10.83	10.85	1459	7.26					
ALA	13.52	13.50	979	1.22					
HIS	15.25	15.15	43	0.15					
TYR	17.00	16.92	717	0.71					
ARG	19.58	19.45	336	1.66					
PRO	20.40	20.30	1046	1.29					
MET	21.05	20.93	146	0.14					
VAL	21.40	21.30	1080	1.04					

Tabulation threshold : 500 uAU

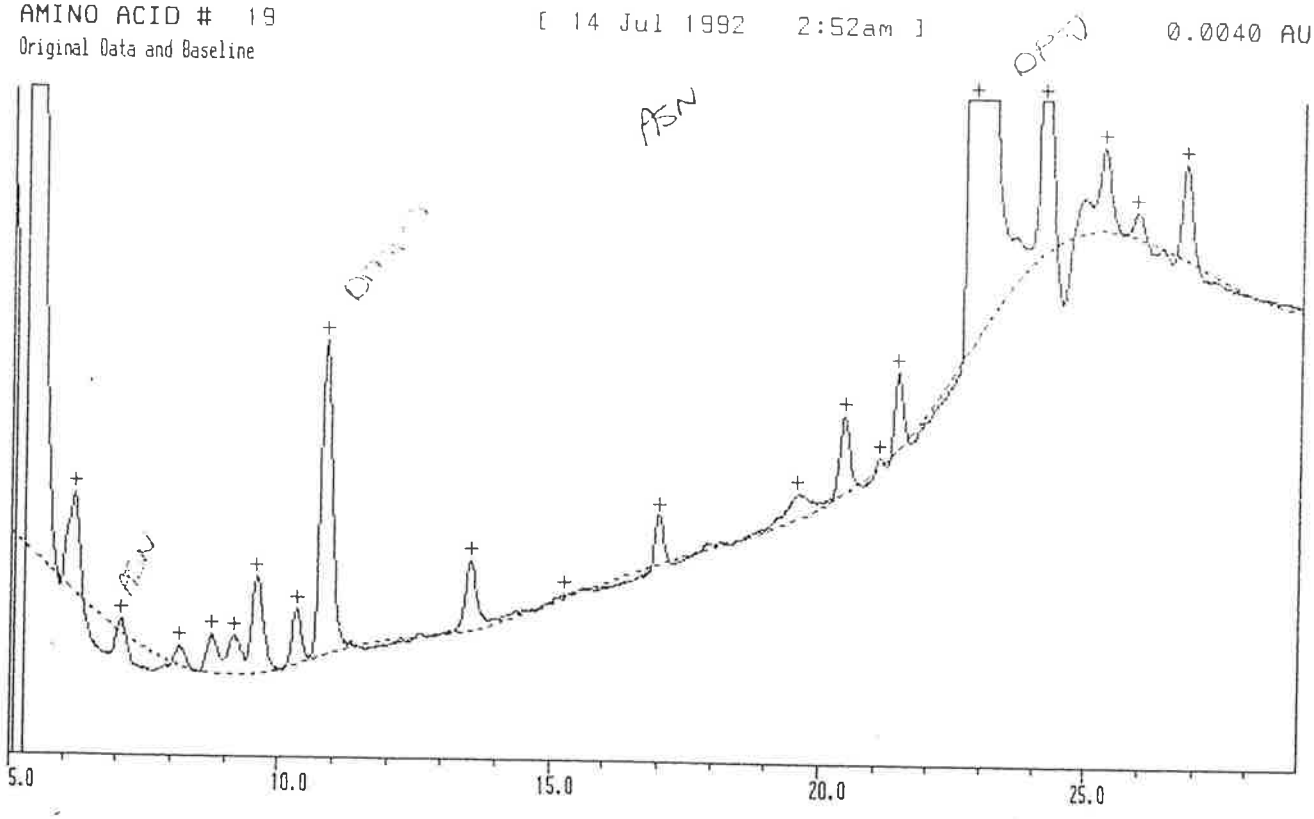
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 19 [14 Jul 1992 2:52am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		53961		VAL	21.42	21.30	1111	1.07
ASP	6.22	6.07	1444	1.28	DPT	22.83	22.72	277922	396.96
ASN	7.08	7.12	192	0.21	TRP	24.13	24.12	3892	3.21
SER	8.18	8.18	285	0.48		24.92		506	
GLN	8.77	8.77	566	0.66	PHE	25.27	25.13	1192	1.34
THR	9.18	9.20	564	0.93	ILE	25.88	25.72	374	0.51
GLY	9.60	9.58	1384	1.99	LEU	26.77	26.62	1356	1.68
GLU	10.33	10.32	789	0.83					
DMP	10.85	10.85	4540	22.60					
ALA	13.52	13.50	1005	1.25					
HIS	15.27	15.15	62	0.21					
TYR	17.00	16.92	722	0.71					
ARG	19.58	19.45	364	1.80					
PRO	20.42	20.30	1123	1.38					
MET	21.05	20.93	148	0.14					

Tabulation threshold : 500 uAU

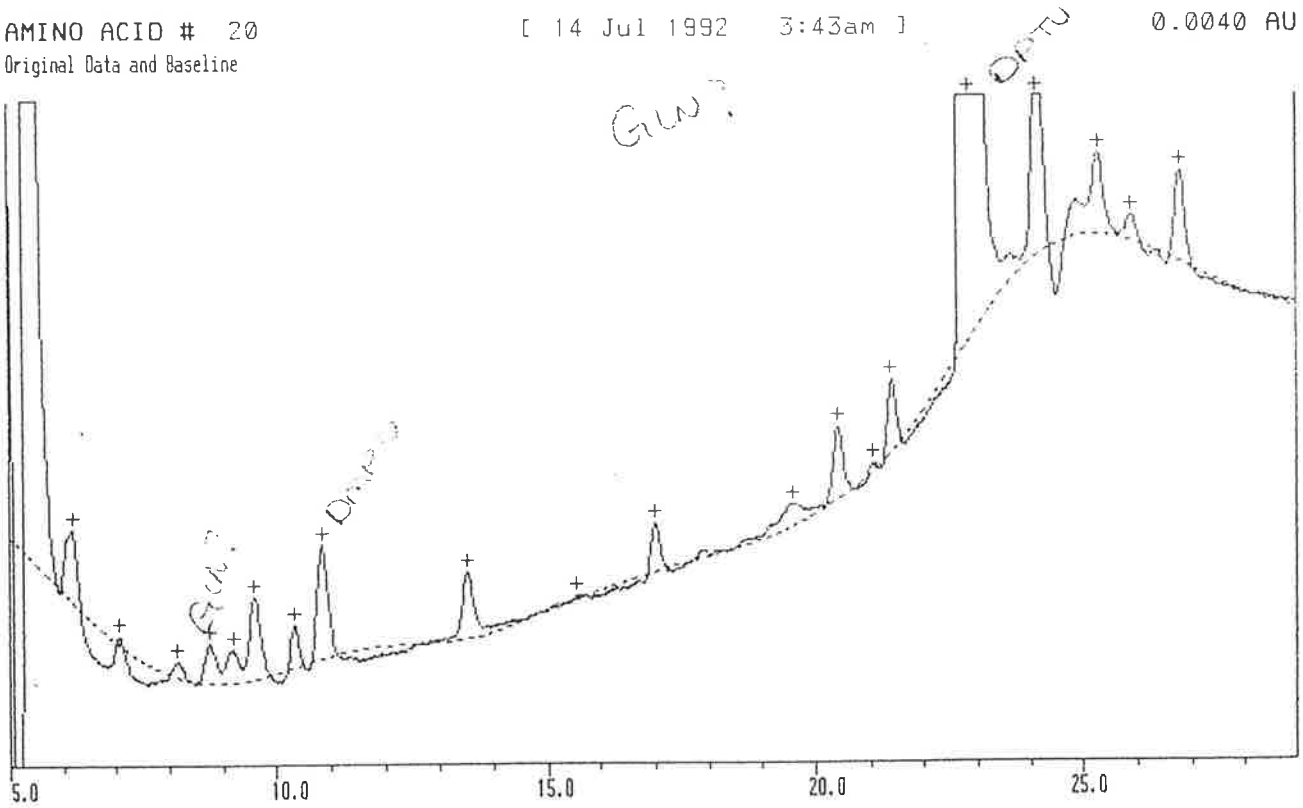
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 20 [14 Jul 1992 3:43am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.38		48936		VAL	21.42	21.30	1044	1.01
ASP	6.20	6.07	1046	0.93	DPT	22.85	22.72	185788	265.36
ASN	7.08	7.12	124	0.14	TRP	24.15	24.12	3343	2.76
SER	8.18	8.18	244	0.41		24.88		518	
GLN	8.78	8.77	580	0.68	PHE	25.30	25.13	1164	1.31
THR	9.20	9.20	477	0.79	ILE	25.92	25.72	374	0.51
GLY	9.60	9.58	1195	1.72	LEU	26.80	26.62	1305	1.62
GLU	10.35	10.32	609	0.64					
DMP	10.87	10.85	1639	8.16					
ALA	13.52	13.50	955	1.19					
HIS	15.55	15.15	52	0.18					
TYR	17.02	16.92	700	0.69					
ARG	19.60	19.45	331	1.63					
PRO	20.43	20.30	1044	1.28					
MET	21.07	20.93	136	0.13					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

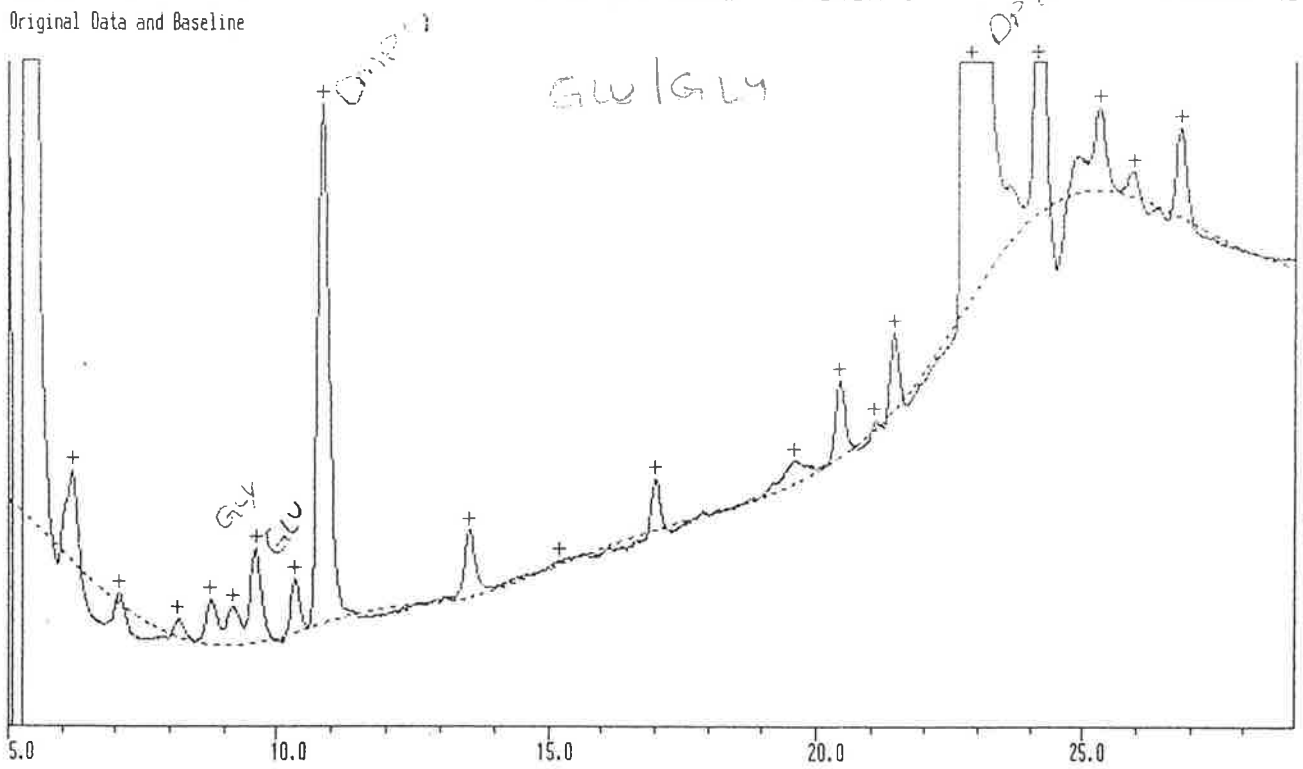
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 21 [14 Jul 1992 4:34am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.38		53649		VAL	21.43	21.30	1111	1.07
ASP	6.22	6.07	1312	1.16	DPT	22.87	22.72	336849	481.13
ASN	7.08	7.12	151	0.17	TRP	24.15	24.12	4406	3.63
SER	8.18	8.18	268	0.45		24.90		530	
GLN	8.78	8.77	657	0.77	PHE	25.30	25.13	1195	1.34
THR	9.18	9.20	561	0.93	ILE	25.93	25.72	357	0.48
GLY	9.62	9.58	1346	1.94	LEU	26.80	26.62	1279	1.59
GLU	10.35	10.32	792	0.83					
DMP	10.87	10.85	7456	37.11					
ALA	13.53	13.50	960	1.19					
HIS	15.23	15.15	64	0.22					
TYR	17.02	16.92	732	0.72					
ARG	19.60	19.45	331	1.63					
PRO	20.43	20.30	1094	1.35					
MET	21.08	20.93	117	0.11					

Tabulation threshold : 500 uAU

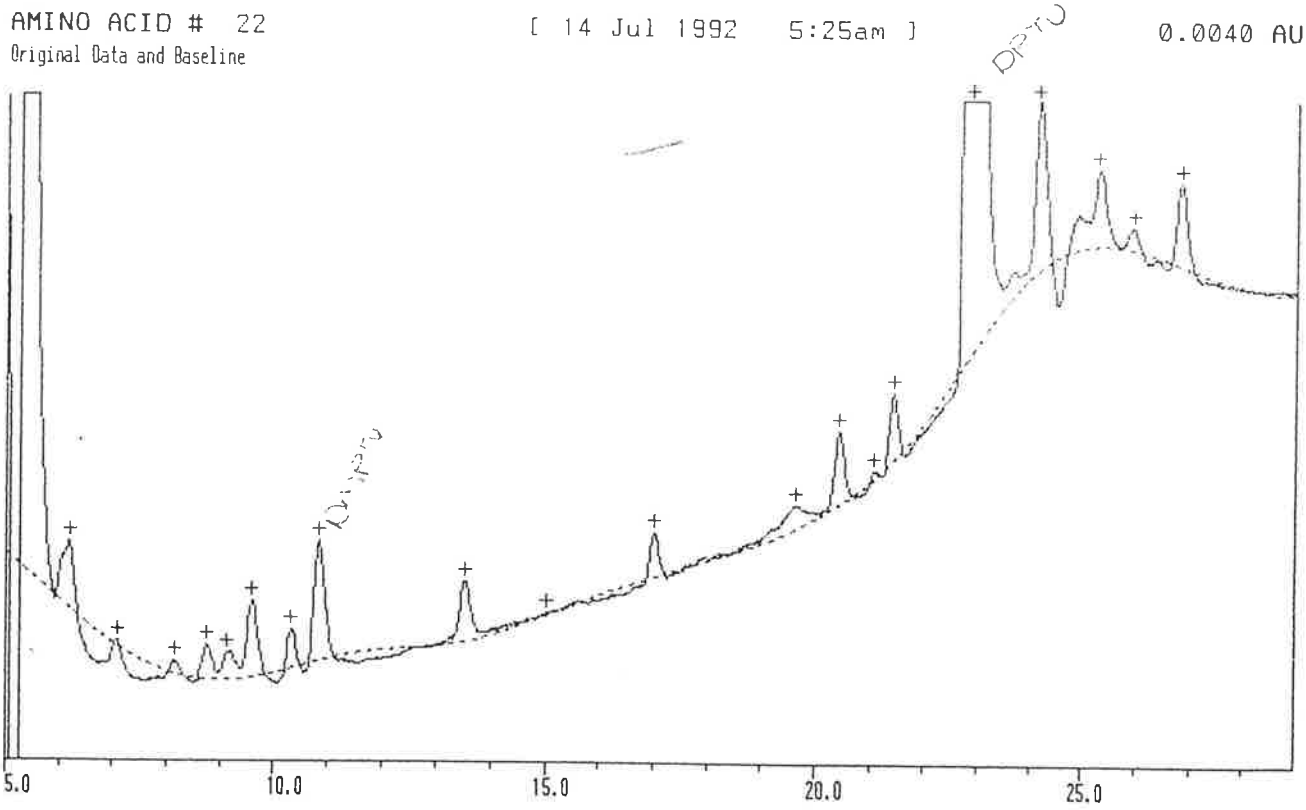
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 22 [14 Jul 1992 5:25am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		47469		VAL	21.43	21.30	986	0.95
ASP	6.20	6.07	957	0.85	DPT	22.85	22.72	133524	190.71
ASN	7.12	7.12	74	0.08	TRP	24.15	24.12	2539	2.09
SER	8.15	8.18	182	0.30		24.88		506	
GLN	8.78	8.77	484	0.57	PHE	25.28	25.13	1099	1.23
THR	9.15	9.20	396	0.65	ILE	25.93	25.72	331	0.45
GLY	9.60	9.58	1094	1.57	LEU	26.80	26.62	1180	1.46
GLU	10.33	10.32	552	0.58					
DMP	10.87	10.85	1718	8.55					
ALA	13.53	13.50	864	1.07					
HIS	15.02	15.15	40	0.14					
TYR	17.02	16.92	633	0.62					
ARG	19.65	19.45	336	1.66					
PRO	20.43	20.30	1044	1.28					
MET	21.08	20.93	120	0.11					

Tabulation threshold : 500 uAU

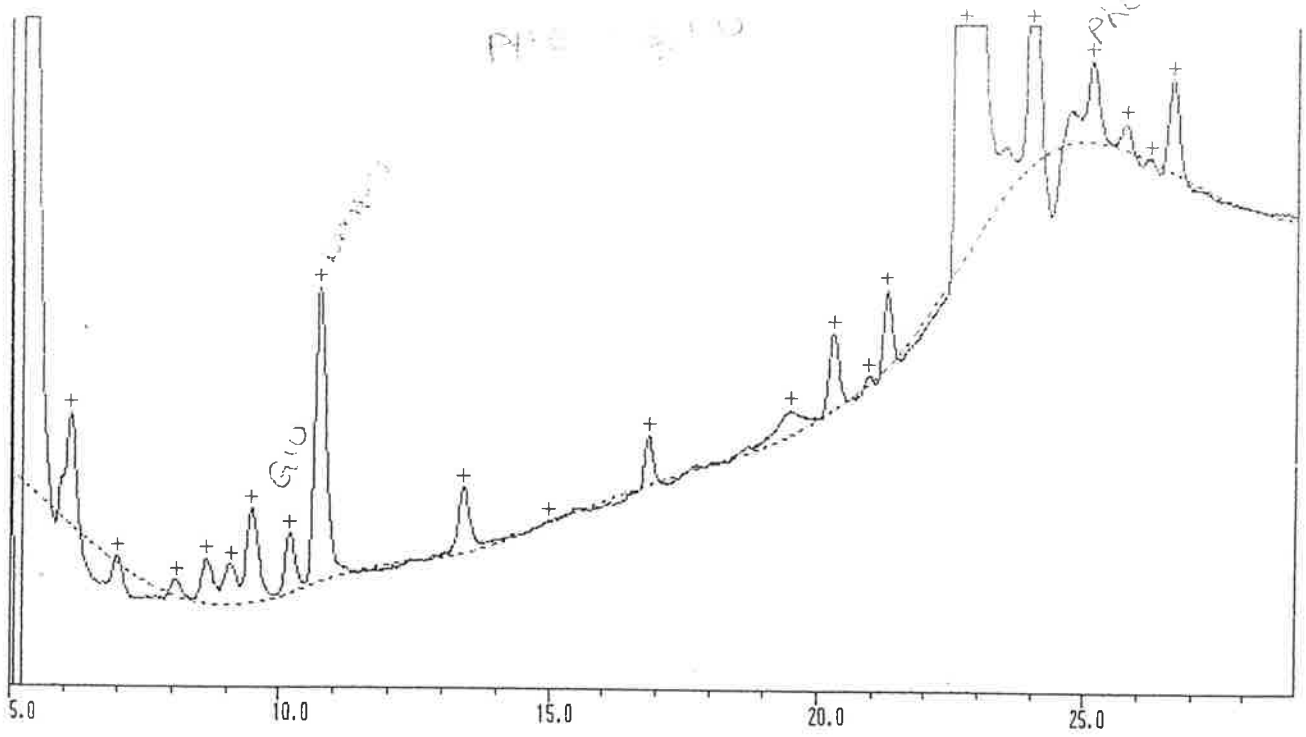
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 23 [14 Jul 1992 6:16am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.35		49864		VAL	21.27	21.30	1111	1.07
ASP	6.15	6.07	1608	1.42	DPT	22.70	22.72	264518	377.81
ASN	7.02	7.12	103	0.11		23.47		691	
SER	8.08	8.18	244	0.41	TRP	23.98	24.12	3628	2.99
GLN	8.65	8.77	640	0.75	PHE	25.12	25.13	1168	1.31
THR	9.12	9.20	585	0.97	ILE	25.75	25.72	374	0.51
GLY	9.48	9.58	1377	1.98	LYS	26.20	26.18	38	0.04
GLU	10.20	10.32	878	0.92	LEU	26.62	26.62	1327	1.65
DMP	10.75	10.85	4248	21.14					
ALA	13.38	13.50	960	1.19					
HIS	15.00	15.15	45	0.15					
TYR	16.85	16.92	732	0.72					
ARG	19.48	19.45	355	1.75					
PRO	20.27	20.30	1101	1.35					
MET	20.92	20.93	129	0.12					

Tabulation threshold : 500 uAU

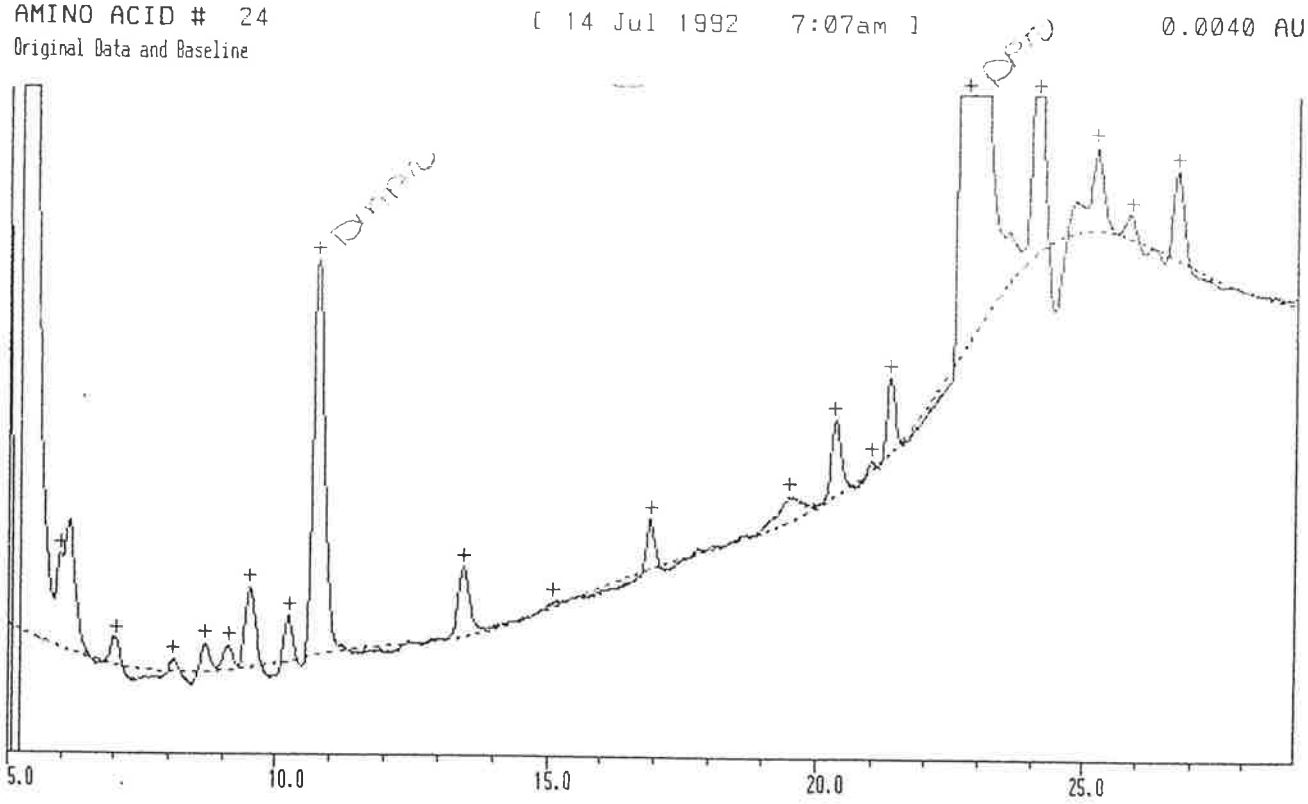
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 24 [14 Jul 1992 7:07am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.35		53616		MET	21.00	20.93	146	0.14
ASP	6.00	6.07	1336	1.18	VAL	21.33	21.30	1080	1.04
	6.17		1872		DPT	22.77	22.72	305104	435.78
ASN	7.03	7.12	376	0.41	TRP	24.05	24.12	4101	3.38
SER	8.10	8.18	180	0.30	PHE	25.20	25.13	1207	1.35
GLN	8.68	8.77	398	0.47	ILE	25.85	25.72	343	0.46
THR	9.13	9.20	336	0.55	LEU	26.70	26.62	1281	1.59
GLY	9.52	9.58	1144	1.65					
GLU	10.25	10.32	655	0.69					
DMP	10.78	10.85	5664	28.19					
ALA	13.45	13.50	1015	1.26					
HIS	15.12	15.15	72	0.24					
TYR	16.93	16.92	715	0.71					
ARG	19.48	19.45	372	1.83					
PRO	20.33	20.30	1099	1.35					

Tabulation threshold : 500 uAU

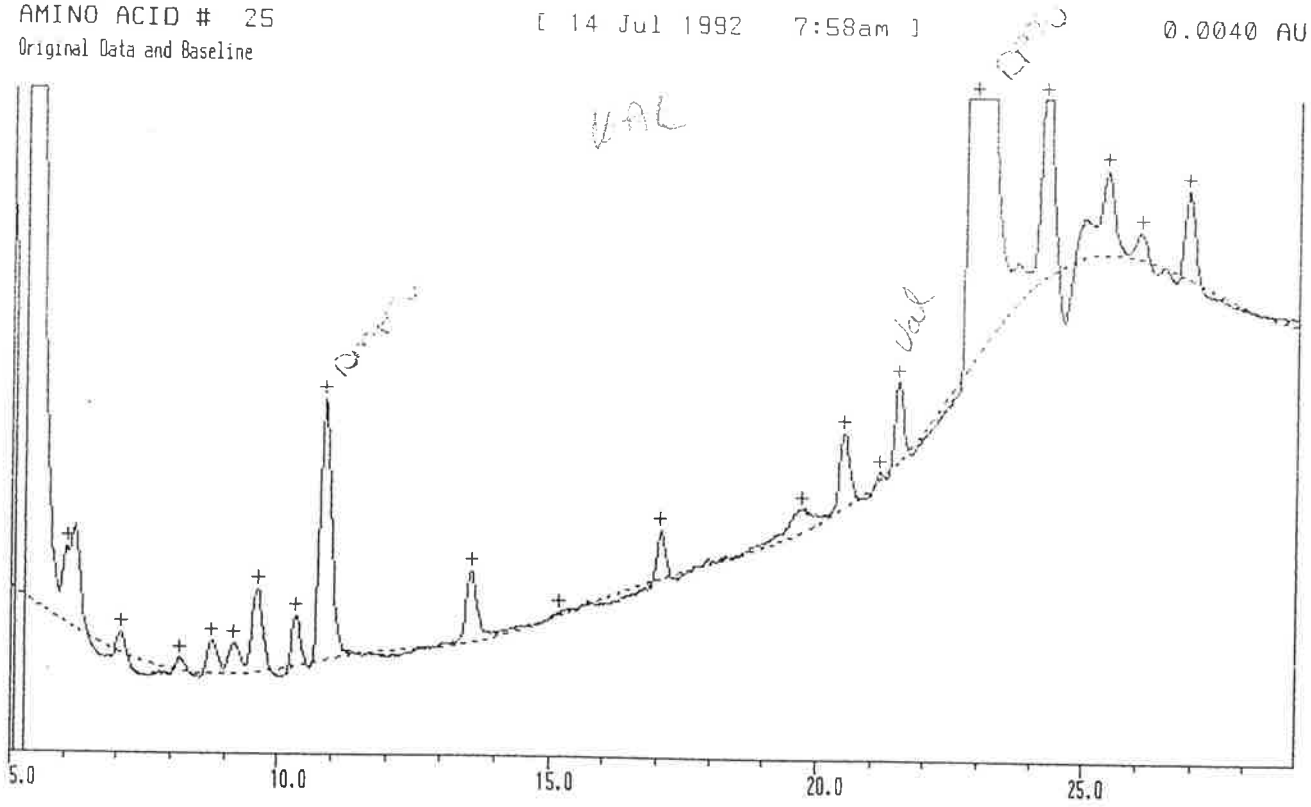
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 25 [14 Jul 1992 7:58am] 0.0040 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.40		55372		MET	21.15	20.93	129	0.12
ASP	6.05	6.07	1082	0.96	VAL	21.48	21.30	1154	1.11
	6.20		1471		DPT	22.92	22.72	241934	345.56
ASN	7.08	7.12	285	0.31		23.70		592	
SER	8.18	8.18	175	0.29	TRP	24.22	24.12	3652	3.01
GLN	8.78	8.77	480	0.56		24.98		549	
THR	9.18	9.20	441	0.73	PHE	25.38	25.13	1195	1.34
GLY	9.62	9.58	1185	1.70	ILE	26.00	25.72	384	0.52
GLU	10.35	10.32	736	0.77	LEU	26.88	26.62	1248	1.55
DMP	10.88	10.85	3744	18.63					
ALA	13.57	13.50	1036	1.29					
HIS	15.17	15.15	55	0.19					
TYR	17.07	16.92	703	0.69					
ARG	19.70	19.45	340	1.68					
PRO	20.47	20.30	1082	1.33					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

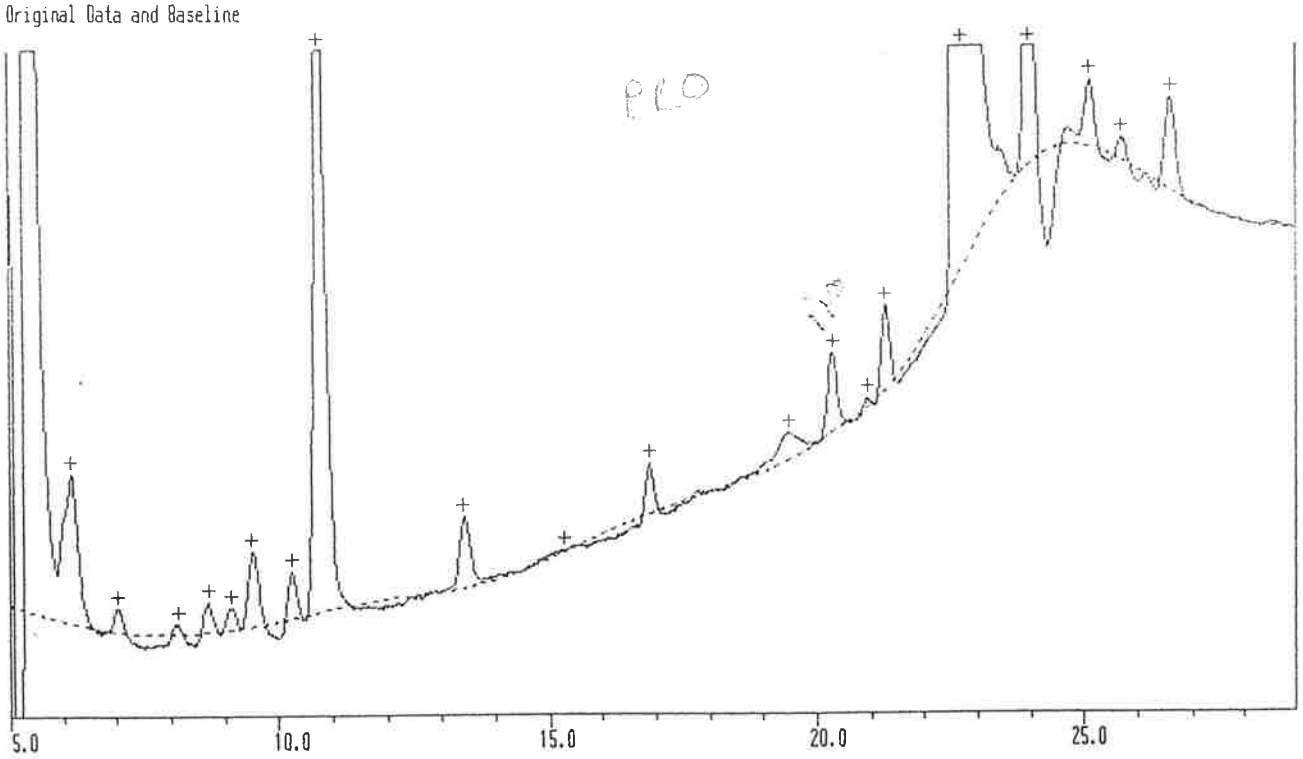
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 26 [14 Jul 1992 8:50am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.38		53282		VAL	21.28	21.30	1221	1.18
ASP	6.18	6.07	2162	1.92	DPT	22.72	22.72	363650	519.41
ASN	7.05	7.12	352	0.39	TRP	23.98	24.12	4200	3.46
SER	8.15	8.18	141	0.24	PHE	25.13	25.13	957	1.07
GLN	8.72	8.77	436	0.51	ILE	25.73	25.72	314	0.43
THR	9.15	9.20	324	0.53	LEU	26.60	26.62	1317	1.63
GLY	9.53	9.58	1082	1.56					
GLU	10.27	10.32	693	0.73					
DMP	10.80	10.85	9736	48.46					
ALA	13.43	13.50	1036	1.29					
HIS	15.30	15.15	24	0.08					
TYR	16.90	16.92	712	0.70					
ARG	19.52	19.45	393	1.94					
PRO	20.30	20.30	1135	1.40					
MET	20.95	20.93	134	0.13					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

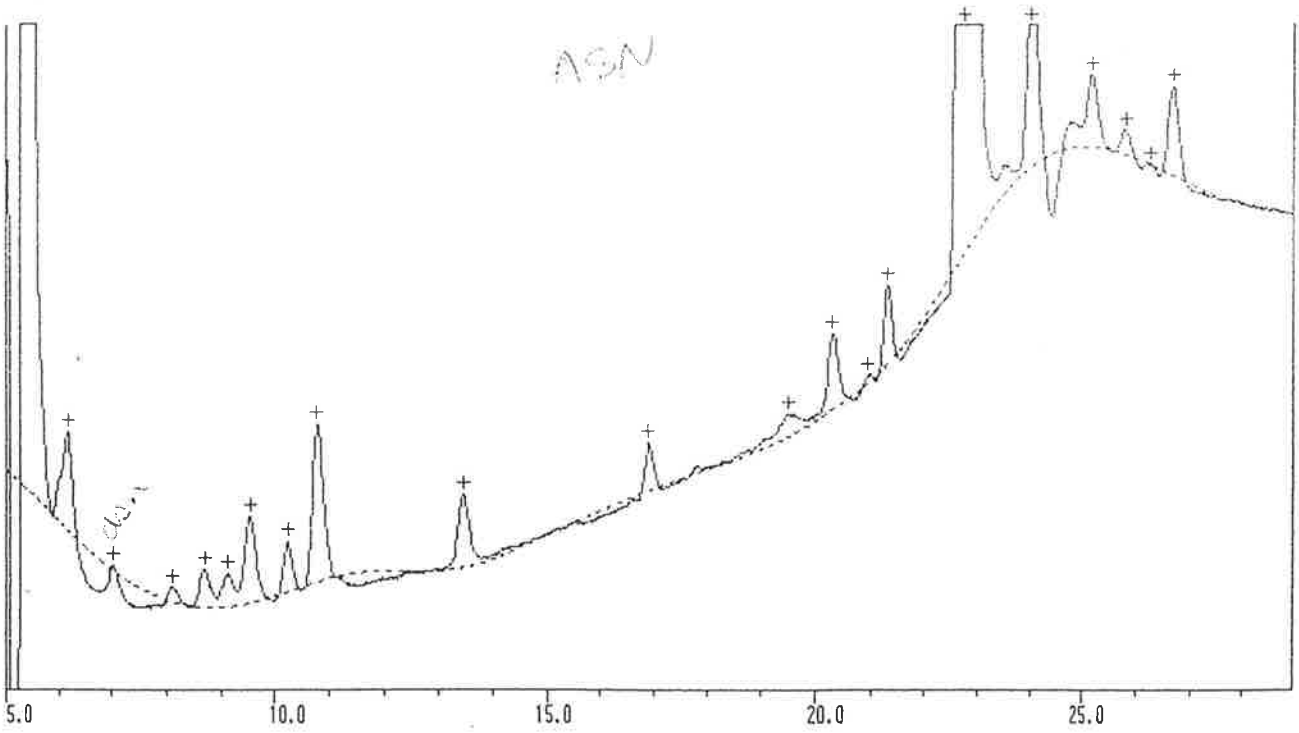
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 27 [14 Jul 1992 9:42am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.38		54016		DPT	22.75	22.72	166281	237.50
ASP	6.20	6.07	1413	1.25	TRP	24.03	24.12	2935	2.42
ASN	7.03	7.12	74	0.08	PHE	25.18	25.13	1041	1.17
SER	8.13	8.18	213	0.36	ILE	25.82	25.72	357	0.48
GLN	8.72	8.77	544	0.64	LYS	26.28	26.18	21	0.02
THR	9.13	9.20	482	0.80	LEU	26.68	26.62	1298	1.61
GLY	9.55	9.58	1255	1.80					
GLU	10.25	10.32	727	0.76					
DMP	10.80	10.85	2246	11.18					
ALA	13.47	13.50	1044	1.30					
TYR	16.92	16.92	686	0.68					
ARG	19.52	19.45	326	1.61					
PRO	20.33	20.30	1092	1.34					
MET	20.97	20.93	120	0.11					
VAL	21.33	21.30	1123	1.08					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

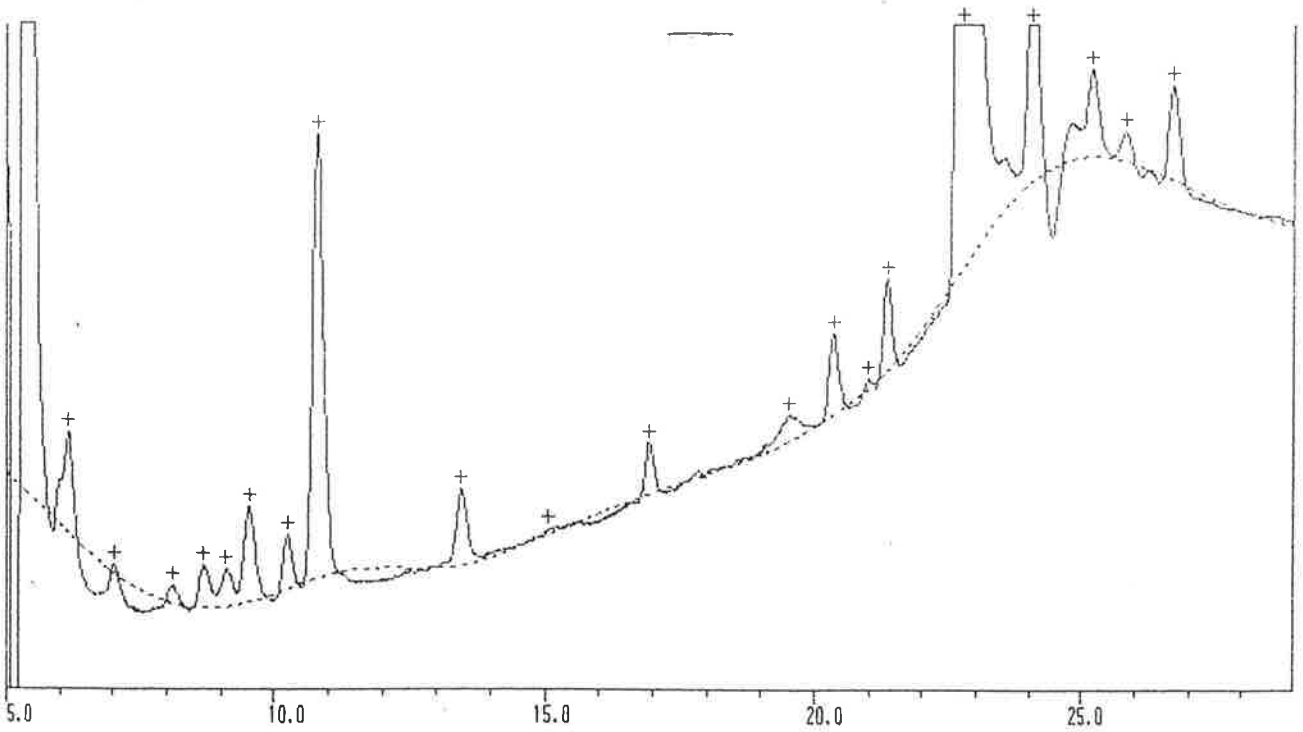
SAMPLE : JAMES STORER PAO PSE 201
[Initiated 13 Jul 1992 9:25am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 28 [14 Jul 1992 10:32am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.37		54009		VAL	21.35	21.30	1317	1.27
ASP	6.18	6.07	1447	1.28	DPT	22.77	22.72	271128	387.25
ASN	7.05	7.12	117	0.13	TRP	24.05	24.12	3888	3.20
SER	8.13	8.18	252	0.42		24.80		525	
GLN	8.70	8.77	602	0.71	PHE	25.18	25.13	1262	1.42
THR	9.12	9.20	537	0.89	ILE	25.83	25.72	422	0.57
GLY	9.55	9.58	1363	1.96	LEU	26.68	26.62	1356	1.68
GLU	10.27	10.32	784	0.82					
DMP	10.82	10.85	6384	31.77					
ALA	13.47	13.50	1089	1.35					
HIS	15.08	15.15	84	0.28					
TYR	16.93	16.92	746	0.74					
ARG	19.52	19.45	386	1.91					
PRO	20.35	20.30	1178	1.45					
MET	21.00	20.93	139	0.13					

Tabulation threshold : 500 uAU

D.5 PAO (2) Ref: PSE 204 *Date: 21/7/92*

Table D.5 *N*-Terminal Amino Acid Sequence of PSE 204 [a]

aa no.	*1° Signal*	*2°*	*3°*	*4°*	*5°*	*6°*	*7°*	*8°*	*9°*
1	Thr	Pro/Glu/Asn			Ala				
2		Val							
3		Gln							
4		Leu							
5		Val			Glu				
6		Glu							
7		Ser							
8		Gly							
9		-							
10		Gly/Glu							
11		Leu							
12		Val			Asp/Gln/Phe/Ile				
13		Gln							
14		Pro							
15		Gly							
16		Gly			Asn/Ser/Gln/Thr/Glu			Tyr/Pro/Ala	
17		Ala			Leu/Ser				
18		Leu			Val				
19		Glu			Val/Gly/Arg				
20		-							
21		Ser							
22		Gly?							
23		Val							
24		Gly			Glu/Asp?/Ala				
25		Val			Arg?				
26		-							

a. Interpretation of the chromatograms

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

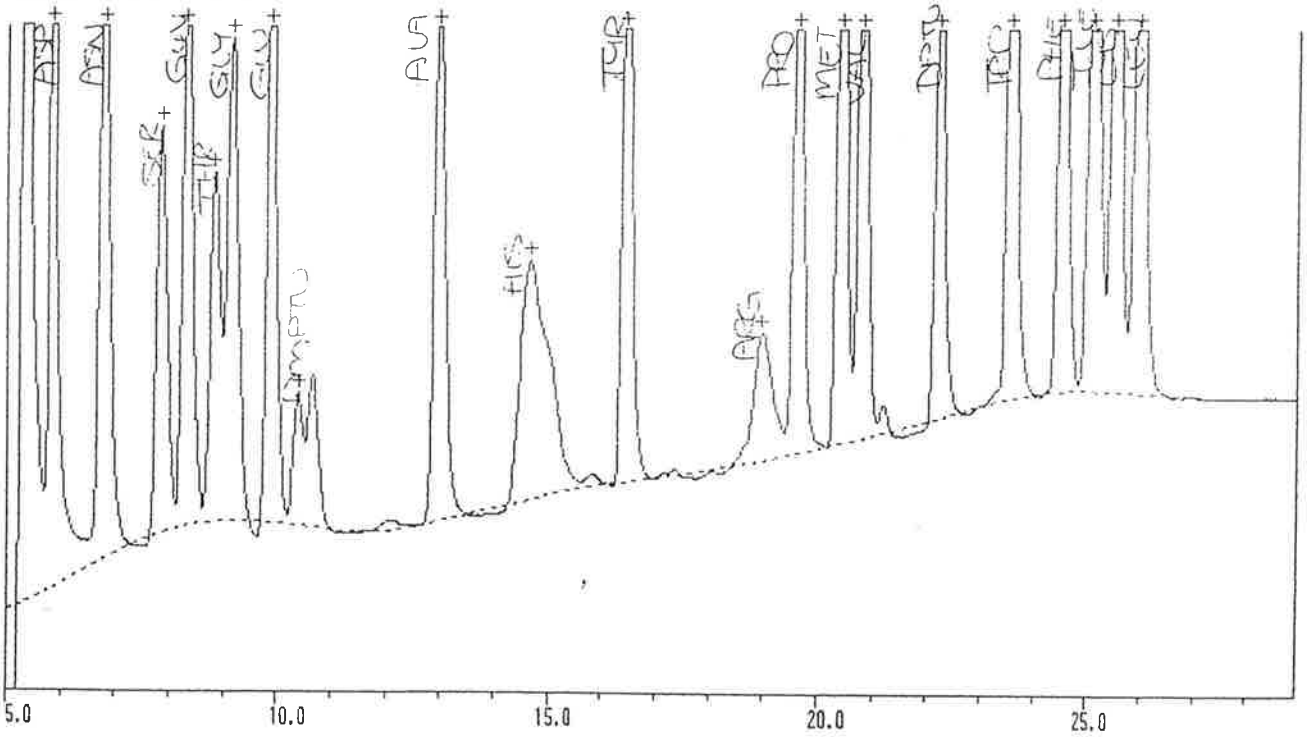
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : BGN470-1
Conversion cycle : BGN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

CALIBRATION # 1 [21 Jul 1992 11:17am] 0.0100 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.35		49387		MET	20.42	20.42	26200	25.00
ASP	5.85	5.85	28999	25.00	VAL	20.80	20.80	25828	25.00
ASN	6.80	6.80	23702	25.00		21.20		1039	
SER	7.85	7.85	14623	25.00	DPT	22.22	22.22	17973	25.00
GLN	8.33	8.33	20668	25.00	TRP	23.57	23.57	30192	25.00
THR	8.85	8.85	12532	25.00	PHE	24.52	24.52	22444	25.00
GLY	9.17	9.17	17378	25.00	ILE	25.12	25.12	19250	25.00
GLU	9.90	9.90	21789	25.00	LYS	25.52	25.52	25310	25.00
DMP	10.40	10.40	4788	25.00	LEU	25.97	25.97	20882	25.00
	10.67		5498						
ALA	12.97	12.97	20200	25.00					
HIS	14.68	14.68	8558	25.00					
TYR	16.47	16.47	24290	25.00					
ARG	18.98	18.98	4572	25.00					
PRO	19.63	19.63	20241	25.00					

Tabulation threshold : 500 uAU

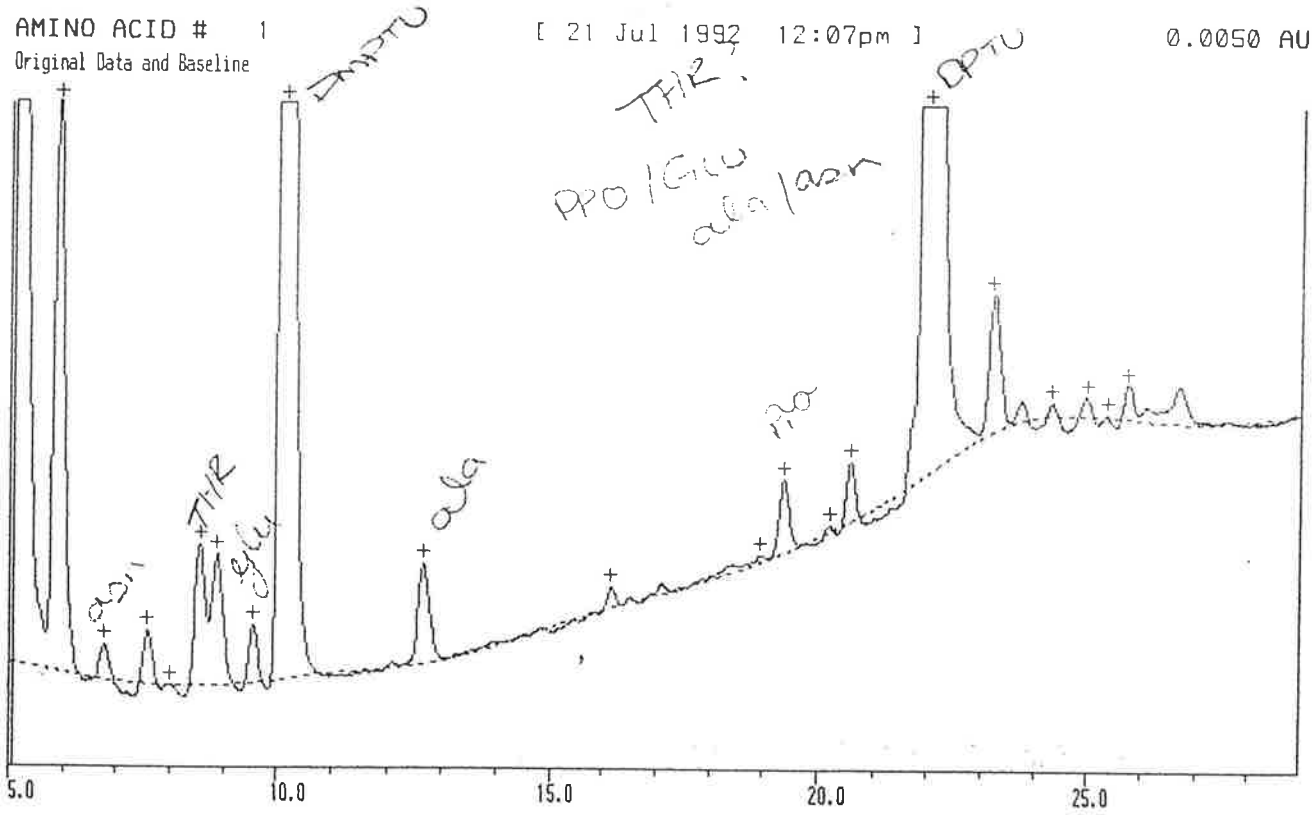
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 1 [21 Jul 1992 12:07pm] 0.0050 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		71906		DPT	22.00	22.22	180434	250.97
ASP	5.90	5.85	11203	9.66	TRP	23.22	23.57	2503	2.07
ASN	6.77	6.80	621	0.66	PHE	24.30	24.52	244	0.27
SER	7.58	7.85	948	1.62	ILE	24.95	25.12	364	0.47
GLN	8.02	8.33	24	0.03	LYS	25.33	25.52	36	0.04
THR	8.57	8.85	2556	5.10	LEU	25.73	25.97	631	0.76
GLY	8.88	9.17	2354	3.39		26.68		684	
GLU	9.57	9.90	1027	1.18					
DMP	10.15	10.40	37869	197.73					
ALA	12.67	12.97	1780	2.20					
TYR	16.15	16.47	384	0.40					
ARG	18.93	18.98	132	0.72					
PRO	19.37	19.63	1336	1.65					
MET	20.22	20.42	91	0.09					
VAL	20.57	20.80	1084	1.05					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

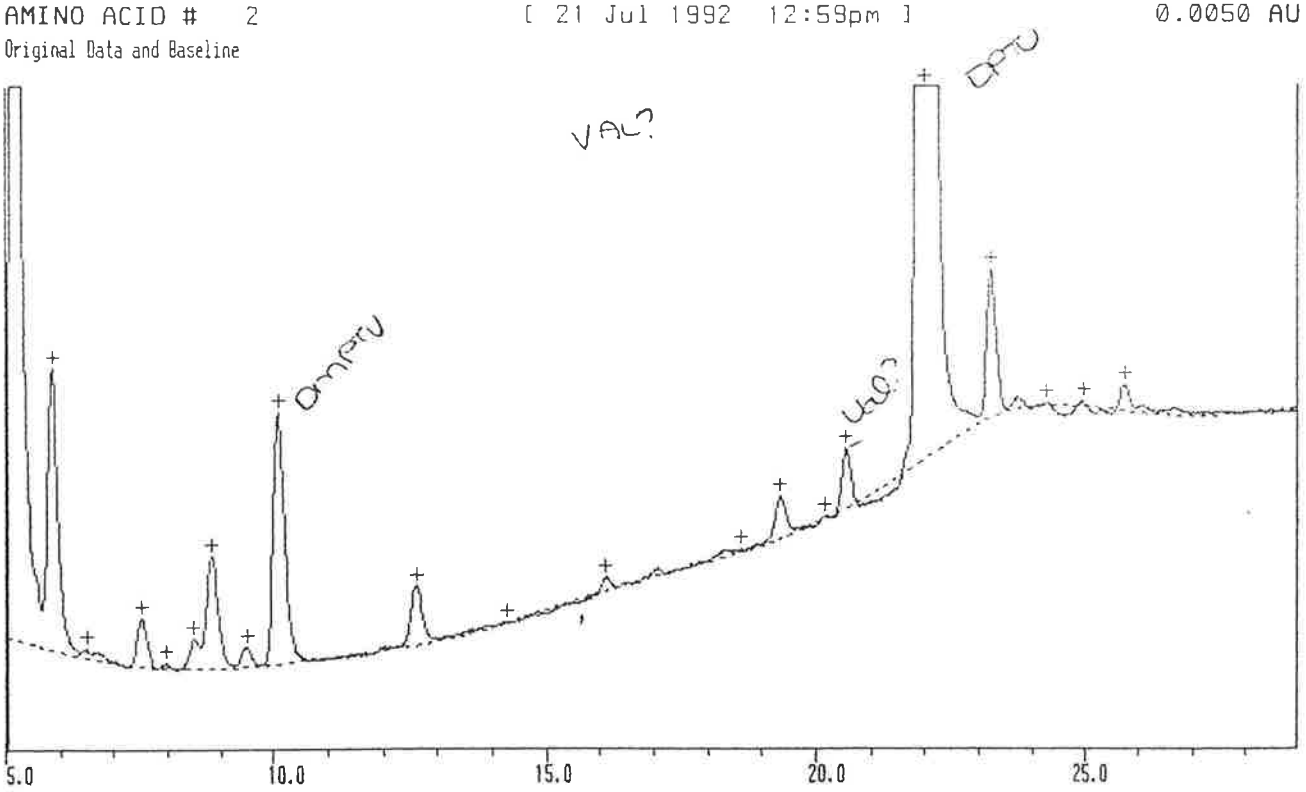
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 2 [21 Jul 1992 12:59pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.15		68721		VAL	20.55	20.80	1089	1.05
ASP	5.87	5.85	5114	4.41	DPT	22.00	22.22	186482	259.38
ASN	6.50	6.80	163	0.17	TRP	23.23	23.57	2692	2.23
SER	7.53	7.85	854	1.46	PHE	24.30	24.52	36	0.04
GLN	7.97	8.33	96	0.12	ILE	24.95	25.12	105	0.14
THR	8.52	8.85	544	1.09	LEU	25.75	25.97	468	0.56
GLY	8.83	9.17	2049	2.95					
GLU	9.50	9.90	369	0.42					
DMP	10.10	10.40	4560	23.81					
ALA	12.62	12.97	1070	1.32					
HIS	14.27	14.68	9	0.03					
TYR	16.13	16.47	240	0.25					
ARG	18.62	18.98	62	0.34					
PRO	19.35	19.63	758	0.94					
MET	20.18	20.42	60	0.06					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

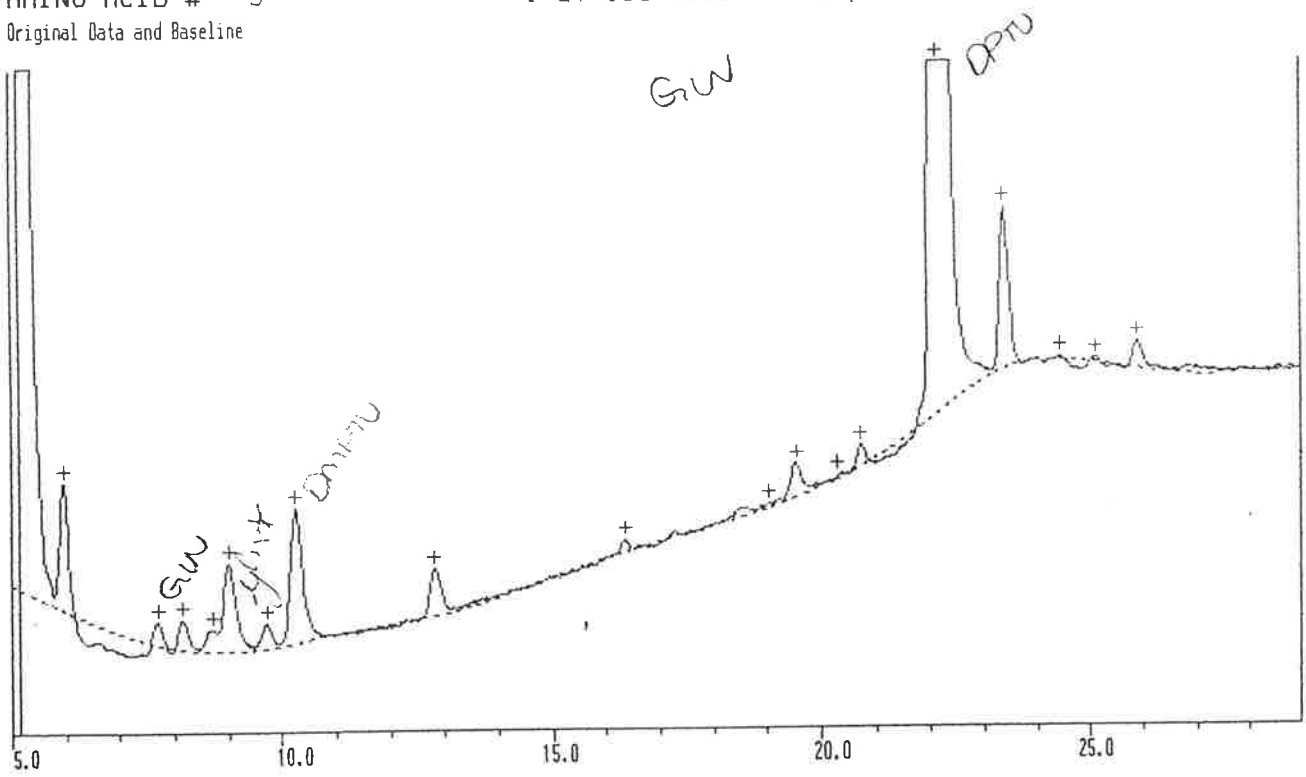
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 3 [21 Jul 1992 1:50pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.25		65277		TRP	23.40	23.57	2884	2.39
ASP	5.98	5.85	2275	1.96	PHE	24.45	24.52	64	0.07
SER	7.72	7.85	417	0.71	ILE	25.13	25.12	74	0.10
GLN	8.17	8.33	552	0.67	LEU	25.92	25.97	472	0.57
THR	8.72	8.85	410	0.82					
GLY	9.02	9.17	1584	2.28					
GLU	9.73	9.90	446	0.51					
DMP	10.27	10.40	2438	12.73					
ALA	12.83	12.97	842	1.04					
TYR	16.37	16.47	208	0.21					
ARG	19.05	18.98	76	0.42					
PRO	19.55	19.63	616	0.76					
MET	20.33	20.42	72	0.07					
VAL	20.73	20.80	393	0.38					
DPT	22.17	22.22	159180	221.41					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

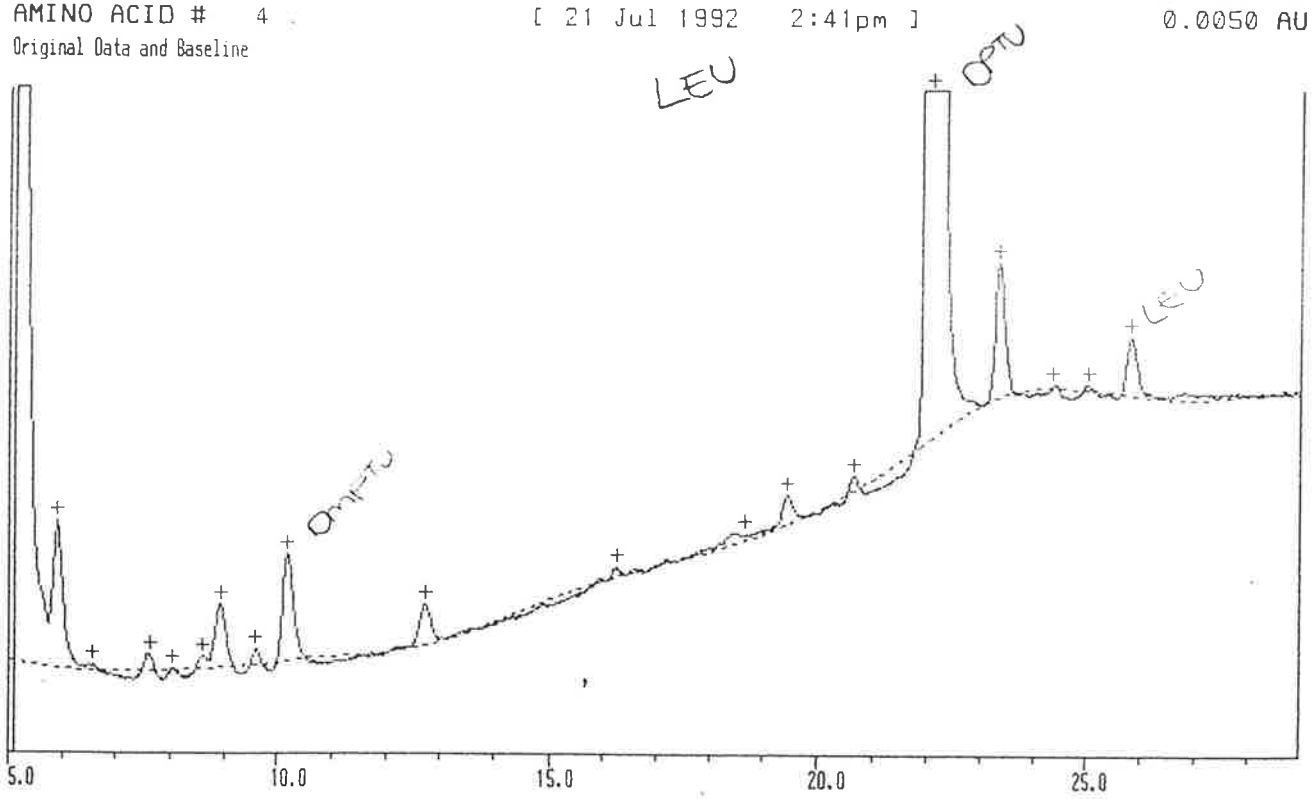
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 4 [21 Jul 1992 2:41pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		64953		TRP	23.35	23.57	2428	2.01
ASP	5.92	5.85	2623	2.26	PHE	24.38	24.52	86	0.10
ASN	6.60	6.80	93	0.10	ILE	25.03	25.12	117	0.15
SER	7.63	7.85	278	0.48	LEU	25.82	25.97	1077	1.29
GLN	8.07	8.33	28	0.03					
THR	8.62	8.85	220	0.44					
GLY	8.95	9.17	1144	1.65					
GLU	9.60	9.90	278	0.32					
DMP	10.20	10.40	1896	9.90					
ALA	12.75	12.97	727	0.90					
TYR	16.25	16.47	165	0.17					
ARG	18.68	18.98	108	0.59					
PRO	19.45	19.63	523	0.65					
VAL	20.65	20.80	278	0.27					
DPT	22.08	22.22	135784	188.87					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

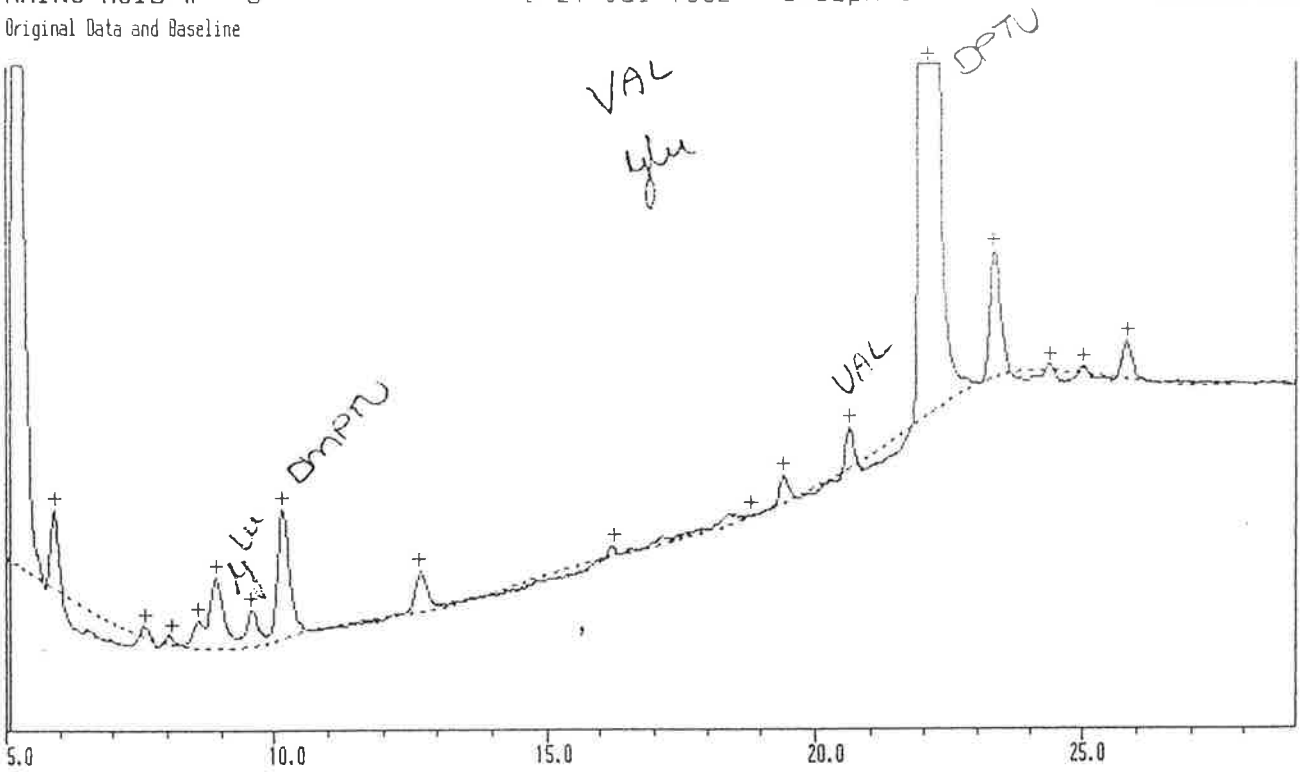
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 5 [21 Jul 1992 3:32pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		63016		PHE	24.35	24.52	108	0.12
ASP	5.92	5.85	1420	1.22	ILE	25.02	25.12	112	0.15
SER	7.60	7.85	196	0.34	LEU	25.82	25.97	693	0.83
GLN	8.08	8.33	136	0.17					
THR	8.58	8.85	504	1.01					
GLY	8.90	9.17	1293	1.86					
GLU	9.57	9.90	657	0.75					
DMP	10.17	10.40	2308	12.06					
ALA	12.68	12.97	712	0.88					
TYR	16.25	16.47	163	0.17					
ARG	18.83	18.98	36	0.20					
PRO	19.42	19.63	480	0.59					
VAL	20.62	20.80	732	0.71					
DPT	22.07	22.22	141662	197.04					
TRP	23.33	23.57	2299	1.90					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

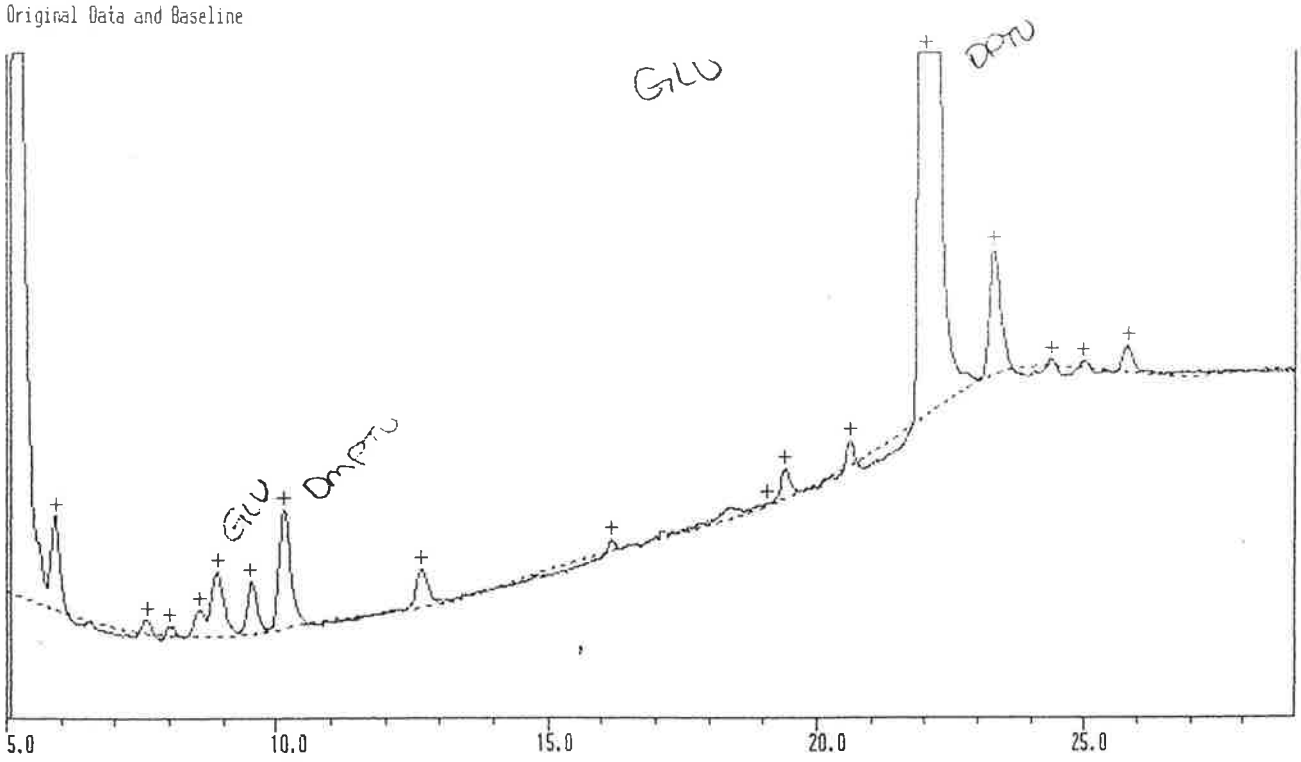
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 6 [21 Jul 1992 4:23pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		61118		PHE	24.38	24.52	129	0.14
ASP	5.90	5.85	1684	1.45	ILE	24.98	25.12	132	0.17
SER	7.60	7.85	240	0.41	LEU	25.82	25.97	496	0.59
GLN	8.03	8.33	180	0.22					
THR	8.58	8.85	484	0.97					
GLY	8.90	9.17	1154	1.66					
GLU	9.53	9.90	936	1.07					
DMP	10.15	10.40	2145	11.20					
ALA	12.67	12.97	703	0.87					
TYR	16.18	16.47	196	0.20					
ARG	19.08	18.98	52	0.29					
PRO	19.43	19.63	511	0.63					
VAL	20.60	20.80	458	0.44					
DPT	22.05	22.22	157884	219.61					
TRP	23.32	23.57	2268	1.88					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

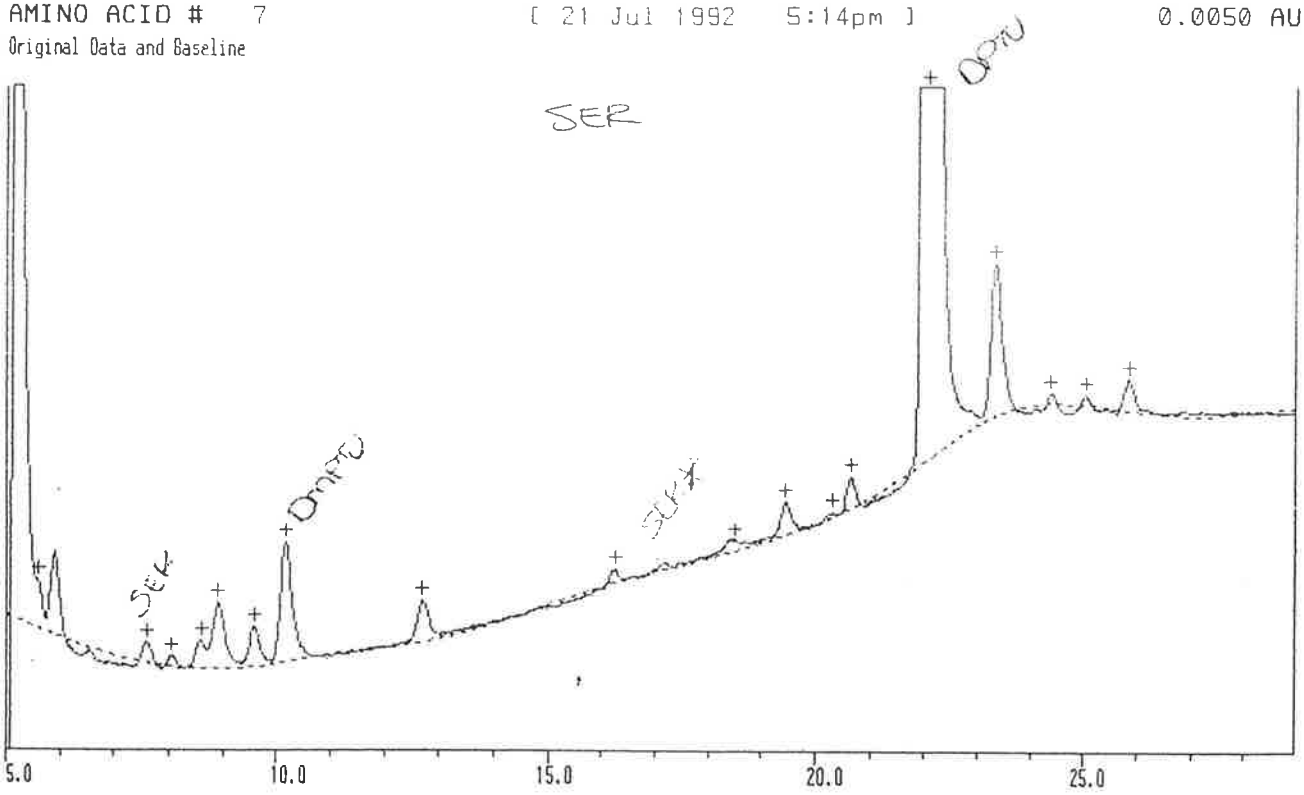
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 7 [21 Jul 1992 5:14pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		61526		DPT	22.08	22.22	184298	256.35
ASP	5.60	5.85	876	0.76	TRP	23.35	23.57	2716	2.25
	5.92		1492		PHE	24.38	24.52	189	0.21
SER	7.62	7.85	362	0.62	ILE	25.03	25.12	168	0.22
GLN	8.05	8.33	189	0.23	LEU	25.85	25.97	556	0.67
THR	8.60	8.85	525	1.05					
GLY	8.93	9.17	1185	1.71					
GLU	9.58	9.90	727	0.83					
DMP	10.18	10.40	2152	11.24					
ALA	12.72	12.97	729	0.90					
TYR	16.25	16.47	247	0.25					
ARG	18.53	18.98	187	1.02					
PRO	19.47	19.63	614	0.76					
MET	20.32	20.42	76	0.07					
VAL	20.65	20.80	576	0.56					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

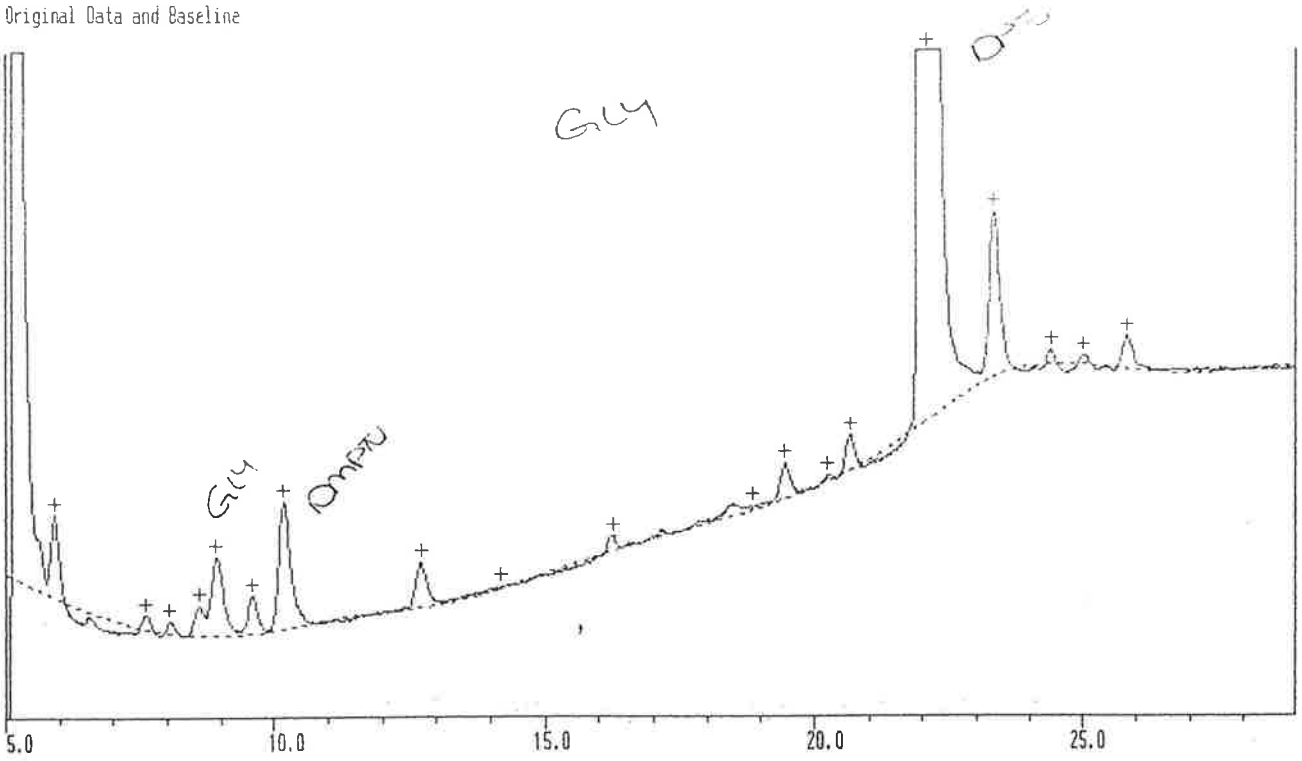
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 8 [21 Jul 1992 6:05pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		60854		DPT	22.08	22.22	192892	268.30
ASP	5.92	5.85	1456	1.26	TRP	23.35	23.57	2976	2.46
SER	7.62	7.85	254	0.43	PHE	24.40	24.52	240	0.27
GLN	8.07	8.33	218	0.26	ILE	25.02	25.12	172	0.22
THR	8.63	8.85	535	1.07	LEU	25.83	25.97	600	0.72
GLY	8.93	9.17	1430	2.06					
GLU	9.60	9.90	686	0.79					
DMP	10.20	10.40	2253	11.77					
ALA	12.73	12.97	811	1.00					
HIS	14.20	14.68	43	0.13					
TYR	16.25	16.47	285	0.29					
ARG	18.87	18.98	60	0.33					
PRO	19.47	19.63	640	0.79					
MET	20.25	20.42	88	0.08					
VAL	20.65	20.80	640	0.62					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

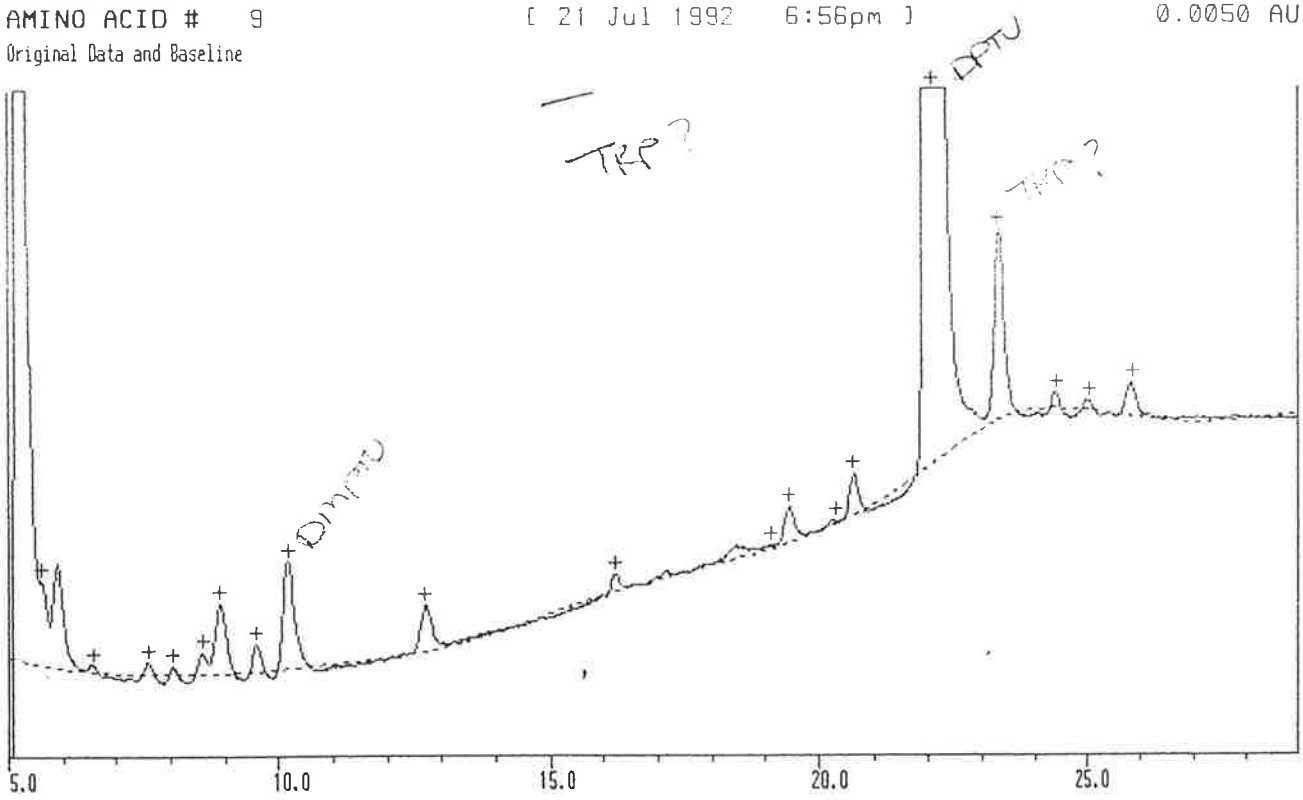
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 9 [21 Jul 1992 6:56pm] 0.0050 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		63480		VAL	20.63	20.80	729	0.71
ASP	5.63	5.85	1490	1.28	DPT	22.08	22.22	211120	293.65
	5.92		1884		TRP	23.33	23.57	3417	2.83
ASN	6.60	6.80	122	0.13	PHE	24.40	24.52	266	0.30
SER	7.60	7.85	220	0.38	ILE	25.03	25.12	175	0.23
GLN	8.07	8.33	139	0.17	LEU	25.85	25.97	595	0.71
THR	8.62	8.85	384	0.77					
GLY	8.93	9.17	1252	1.80					
GLU	9.60	9.90	487	0.56					
DMP	10.18	10.40	1946	10.16					
ALA	12.72	12.97	828	1.02					
TYR	16.22	16.47	312	0.32					
ARG	19.12	18.98	69	0.38					
PRO	19.47	19.63	628	0.78					
MET	20.30	20.42	67	0.06					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

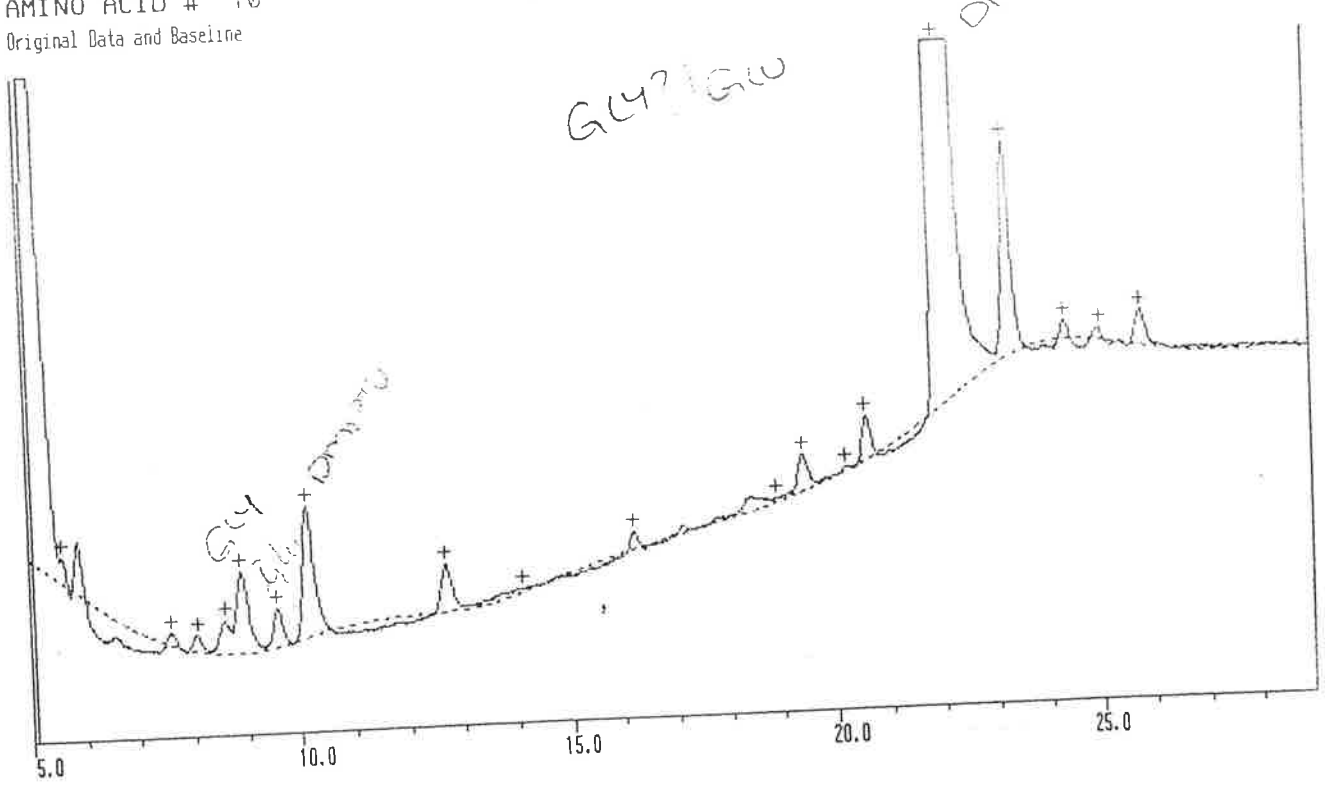
CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 10
Original Data and Baseline

[21 Jul 1992 7:47pm]

0.0050 AU



Retention Time: Minutes

Calibration : CAL470-1

PEAK TABULATION : (100% injection)

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		61341	
ASP	5.63	5.85	465	0.40
	5.92		952	
SER	7.60	7.85	235	0.40
GLN	8.10	8.33	300	0.36
THR	8.62	8.85	609	1.22
GLY	8.93	9.17	1478	2.13
GLU	9.60	9.90	693	0.80
DMP	10.18	10.40	2352	12.28
ALA	12.72	12.97	871	1.08
HIS	14.12	14.68	79	0.23
TYR	16.23	16.47	302	0.31
ARG	18.93	18.98	93	0.51
PRO	19.45	19.63	669	0.83
MET	20.23	20.42	98	0.09

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
VAL	20.63	20.80	792	0.77
DPT	22.07	22.22	240343	334.30
TRP	23.33	23.57	3837	3.18
PHE	24.38	24.52	312	0.35
ILE	25.03	25.12	196	0.26
LEU	25.82	25.97	631	0.76

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

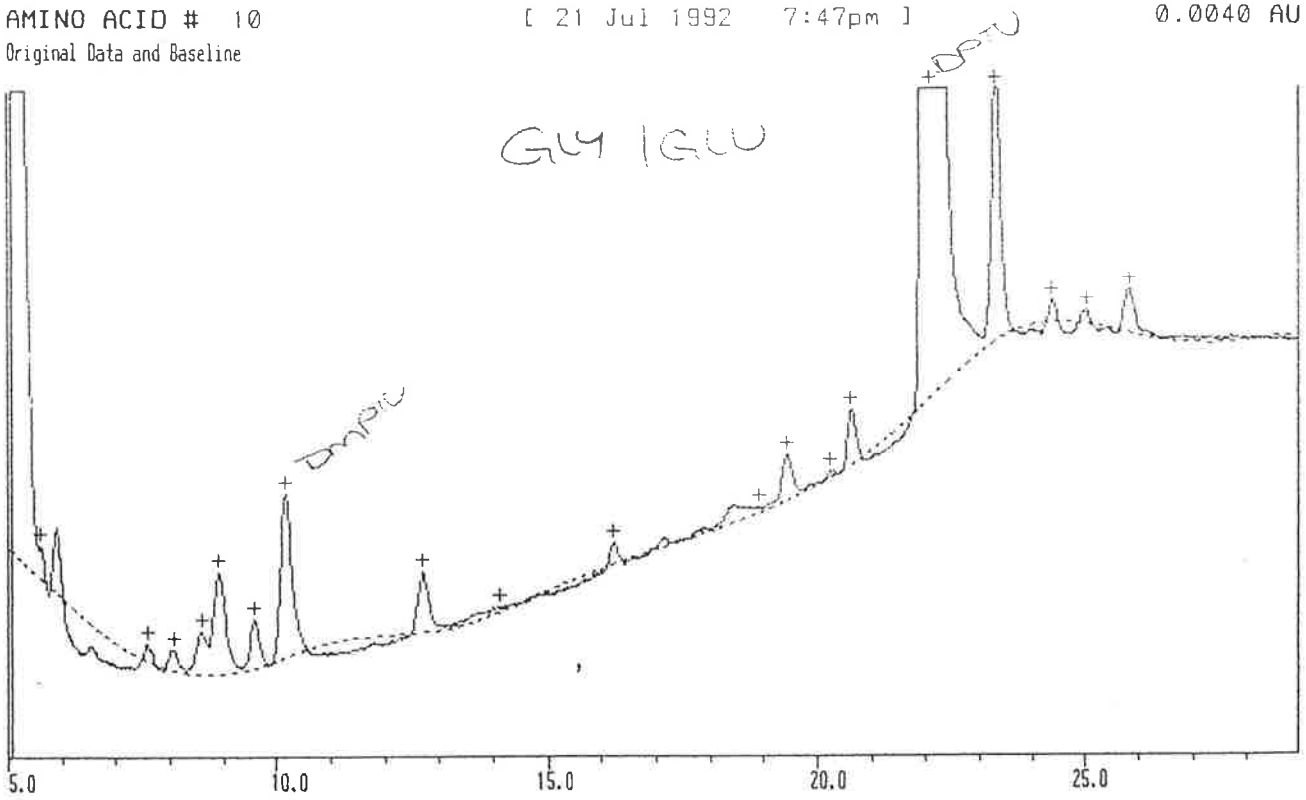
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 10 [21 Jul 1992 7:47pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		61341		VAL	20.63	20.80	792	0.77
ASP	5.63	5.85	465	0.40	DPT	22.07	22.22	240343	334.30
	5.92		952		TRP	23.33	23.57	3837	3.18
SER	7.60	7.85	235	0.40	PHE	24.38	24.52	312	0.35
GLN	8.10	8.33	300	0.36	ILE	25.03	25.12	196	0.26
THR	8.62	8.85	609	1.22	LEU	25.82	25.97	631	0.76
GLY	8.93	9.17	1478	2.13					
GLU	9.60	9.90	693	0.80					
DMP	10.18	10.40	2352	12.28					
ALA	12.72	12.97	871	1.08					
HIS	14.12	14.68	79	0.23					
TYR	16.23	16.47	302	0.31					
ARG	18.93	18.98	93	0.51					
PRO	19.45	19.63	669	0.83					
MET	20.23	20.42	98	0.09					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

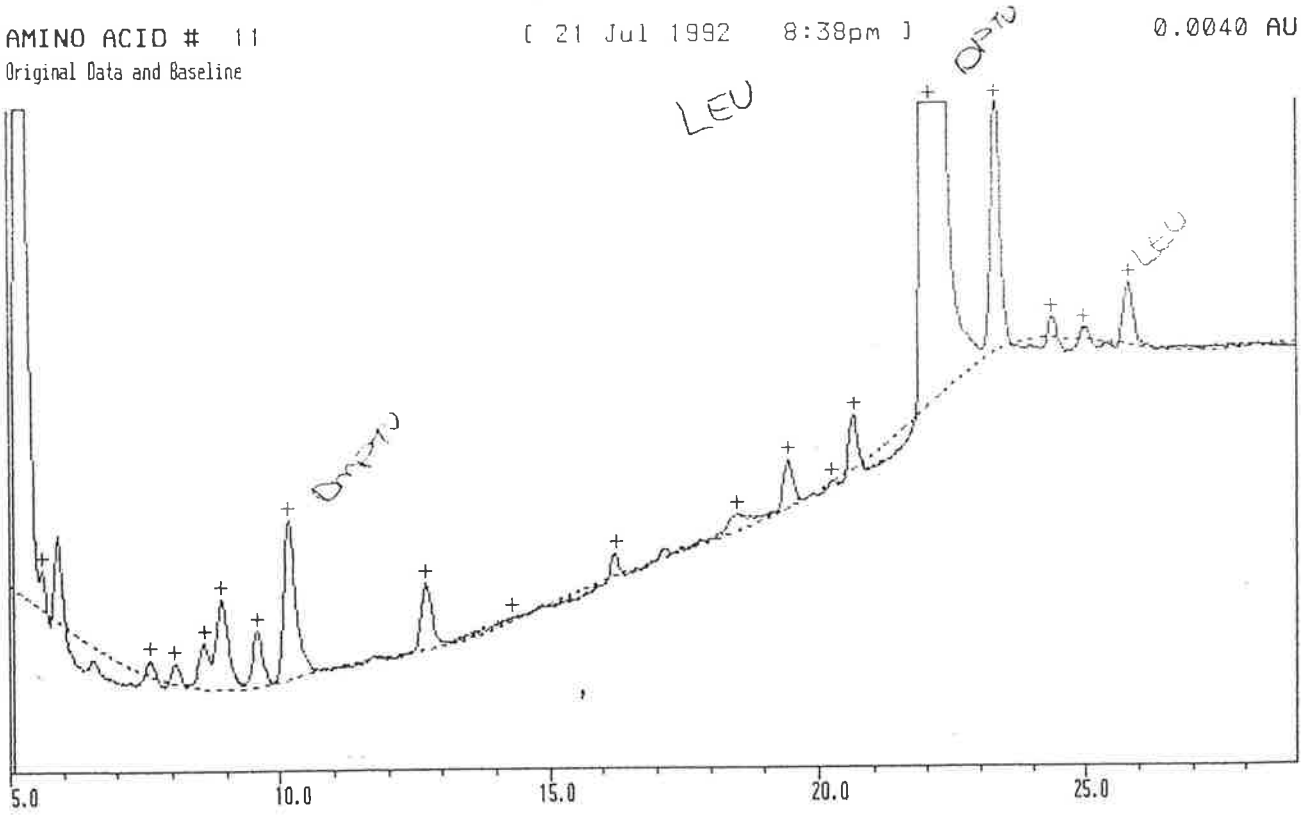
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 11 [21 Jul 1992 8:38pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		60316		VAL	20.65	20.80	777	0.75
ASP	5.62	5.85	564	0.49	DPT	22.08	22.22	251906	350.38
	5.92		1228		TRP	23.32	23.57	3760	3.11
SER	7.60	7.85	232	0.40	PHE	24.38	24.52	304	0.34
GLN	8.07	8.33	309	0.37	ILE	25.02	25.12	158	0.21
THR	8.60	8.85	655	1.31	LEU	25.83	25.97	892	1.07
GLY	8.93	9.17	1300	1.87					
GLU	9.60	9.90	811	0.93					
DMP	10.18	10.40	2282	11.92					
ALA	12.70	12.97	960	1.19					
HIS	14.33	14.68	57	0.17					
TYR	16.25	16.47	312	0.32					
ARG	18.52	18.98	247	1.35					
PRO	19.45	19.63	691	0.85					
MET	20.25	20.42	21	0.02					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

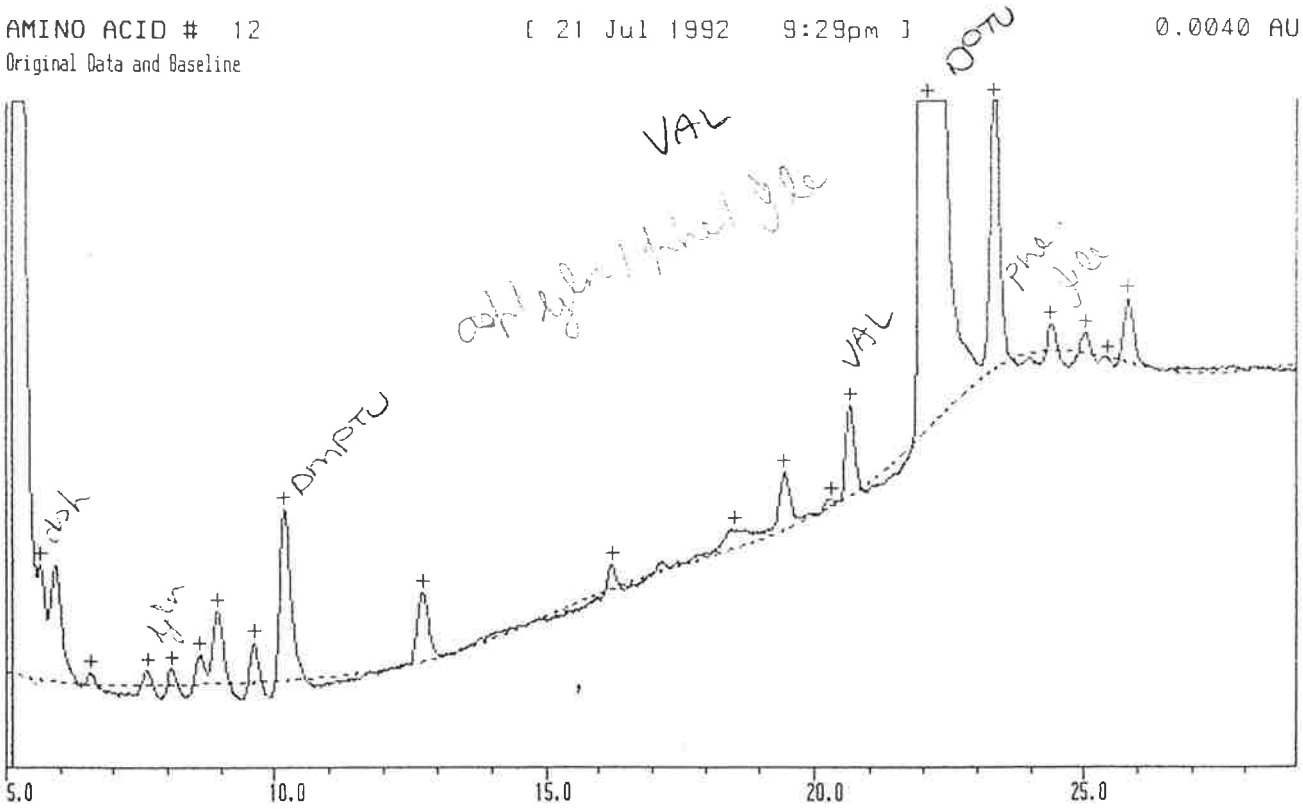
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 12 [21 Jul 1992 9:29pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		59407		VAL	20.65	20.80	1308	1.27
ASP	5.65	5.85	1629	1.40	DPT	22.08	22.22	263548	366.58
	5.92		1672		TRP	23.33	23.57	4077	3.38
ASN	6.58	6.80	177	0.19	PHE	24.38	24.52	384	0.43
SER	7.63	7.85	206	0.35	ILE	25.03	25.12	297	0.39
GLN	8.10	8.33	230	0.28	LYS	25.47	25.52	-9	*****
THR	8.63	8.85	415	0.83	LEU	25.83	25.97	912	1.09
GLY	8.95	9.17	1032	1.48					
GLU	9.62	9.90	556	0.64					
DMP	10.20	10.40	2436	12.72					
ALA	12.75	12.97	972	1.20					
TYR	16.25	16.47	345	0.36					
ARG	18.57	18.98	264	1.44					
PRO	19.47	19.63	849	1.05					
MET	20.30	20.42	112	0.11					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

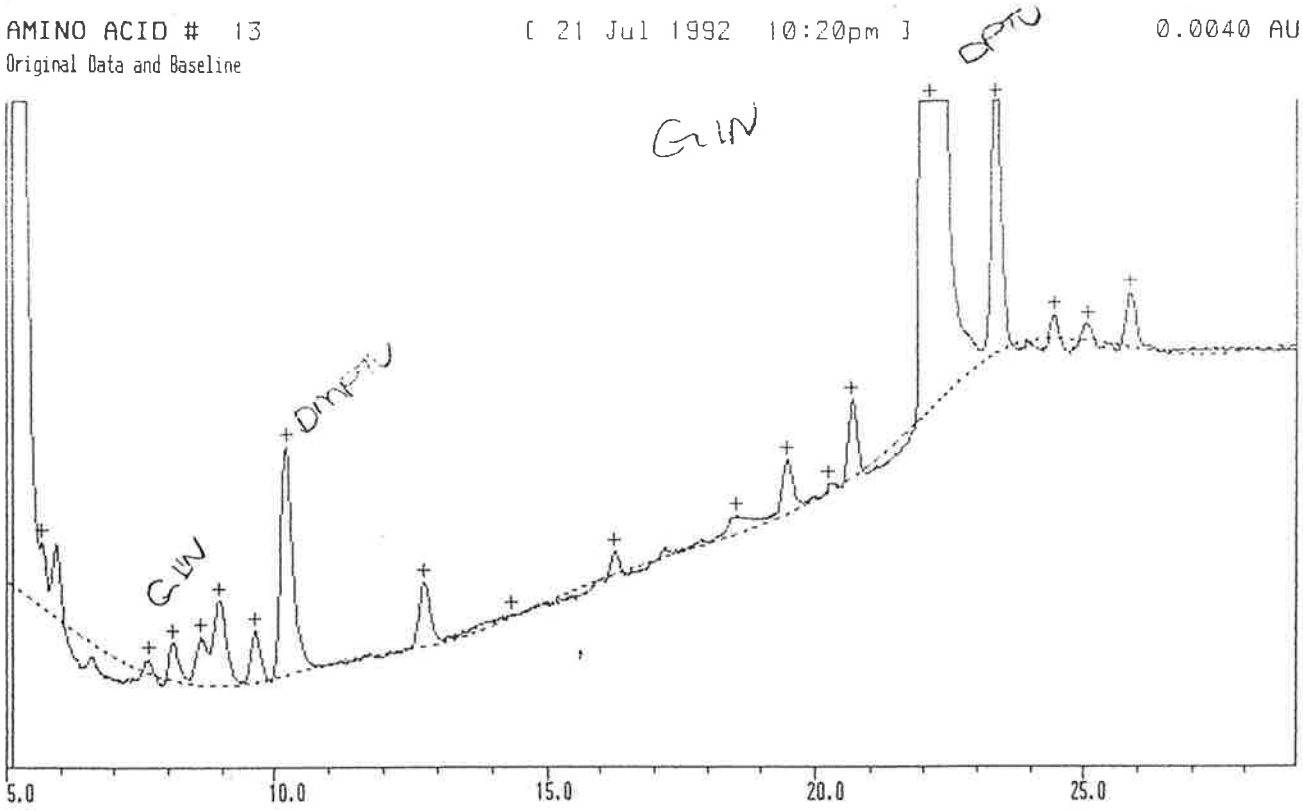
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 13 [21 Jul 1992 10:20pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.22		59258		VAL	20.67	20.80	1120	1.08
ASP	5.67	5.85	921	0.79	DPT	22.12	22.22	260265	362.01
	5.93		1048		TRP	23.35	23.57	4063	3.36
SER	7.63	7.85	184	0.32	PHE	24.43	24.52	338	0.38
GLN	8.10	8.33	540	0.65	ILE	25.07	25.12	235	0.31
THR	8.63	8.85	681	1.36	LEU	25.85	25.97	787	0.94
GLY	8.97	9.17	1226	1.76					
GLU	9.63	9.90	734	0.84					
DMP	10.22	10.40	3285	17.16					
ALA	12.75	12.97	936	1.16					
HIS	14.35	14.68	48	0.14					
TYR	16.27	16.47	336	0.35					
ARG	18.55	18.98	254	1.39					
PRO	19.48	19.63	801	0.99					
MET	20.25	20.42	117	0.11					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

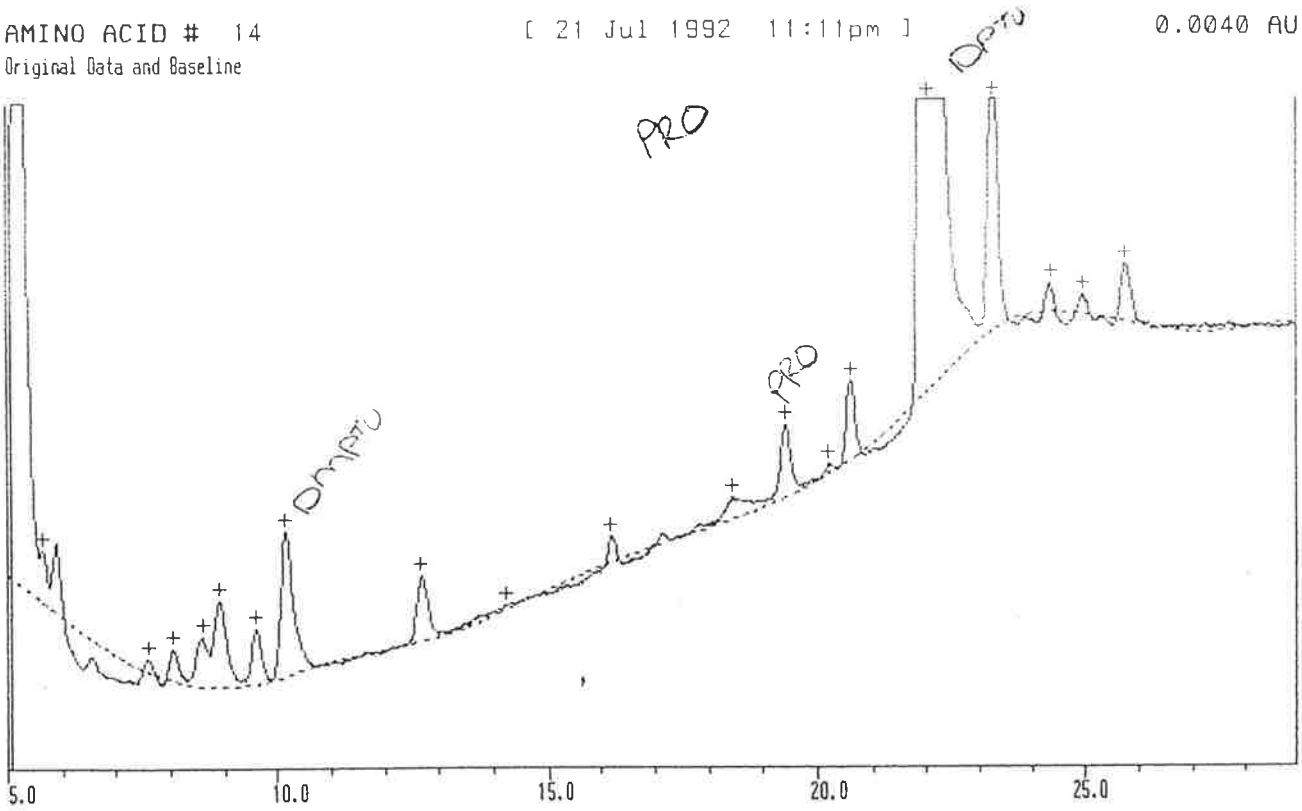
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 14 [21 Jul 1992 11:11pm] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		56467		VAL	20.60	20.80	1144	1.11
ASP	5.67	5.85	732	0.63	DPT	22.03	22.22	272472	378.99
	5.90		993		TRP	23.28	23.57	4315	3.57
SER	7.62	7.85	189	0.32	PHE	24.35	24.52	405	0.45
GLN	8.05	8.33	456	0.55	ILE	24.97	25.12	283	0.37
THR	8.60	8.85	720	1.44	LEU	25.77	25.97	828	0.99
GLY	8.93	9.17	1264	1.82					
GLU	9.60	9.90	789	0.91					
DMP	10.17	10.40	2085	10.89					
ALA	12.68	12.97	943	1.17					
HIS	14.23	14.68	74	0.22					
TYR	16.20	16.47	355	0.37					
ARG	18.45	18.98	307	1.68					
PRO	19.43	19.63	1036	1.28					
MET	20.20	20.42	151	0.14					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

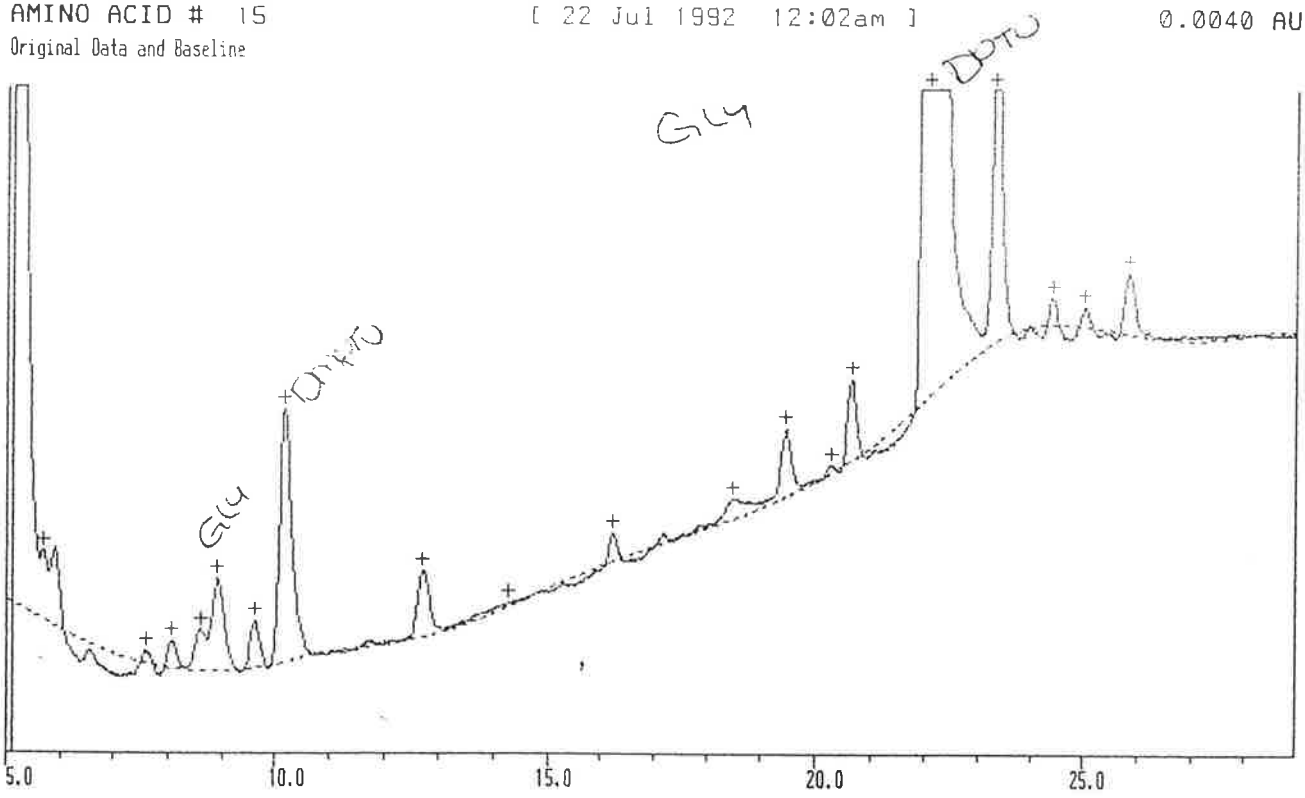
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 15 [22 Jul 1992 12:02am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		48376		VAL	20.65	20.80	1161	1.12
ASP	5.70	5.85	991	0.85	DPT	22.08	22.22	299114	416.05
	5.92		1140		TRP	23.33	23.57	4728	3.91
SER	7.60	7.85	177	0.30	PHE	24.40	24.52	386	0.43
GLN	8.10	8.33	391	0.47	ILE	25.02	25.12	271	0.35
THR	8.63	8.85	595	1.19	LEU	25.83	25.97	880	1.05
GLY	8.93	9.17	1332	1.92					
GLU	9.63	9.90	674	0.77					
DMP	10.18	10.40	3636	18.98					
ALA	12.72	12.97	962	1.19					
HIS	14.28	14.68	67	0.20					
TYR	16.23	16.47	398	0.41					
ARG	18.48	18.98	292	1.60					
PRO	19.47	19.63	964	1.19					
MET	20.27	20.42	117	0.11					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

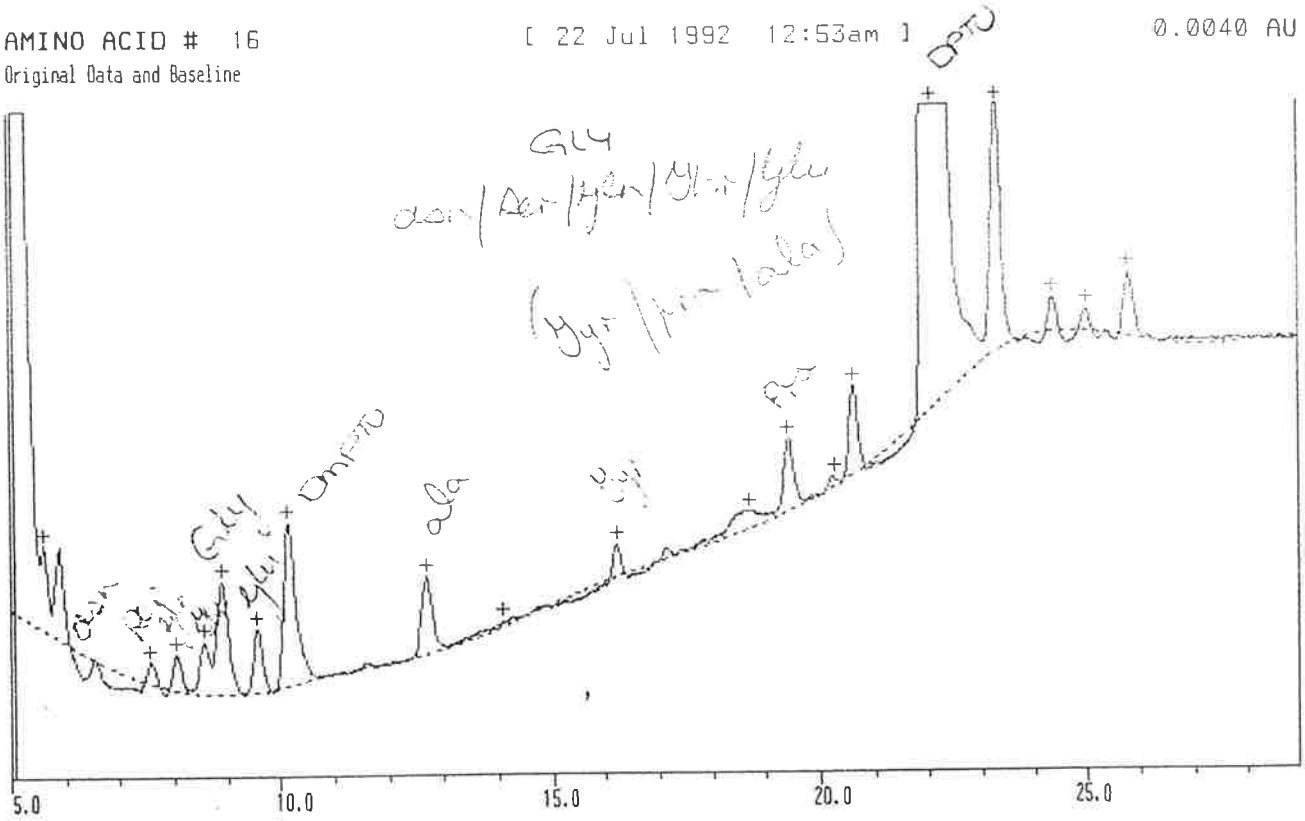
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 16 [22 Jul 1992 12:53am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.18		60643	
ASP	5.63	5.85	1231	1.06
	5.90		1334	
SER	7.58	7.85	314	0.54
GLN	8.05	8.33	511	0.62
THR	8.58	8.85	741	1.48
GLY	8.92	9.17	1629	2.34
GLU	9.57	9.90	902	1.04
DMP	10.17	10.40	2354	12.29
ALA	12.70	12.97	1130	1.40
HIS	14.08	14.68	79	0.23
TYR	16.22	16.47	475	0.49
ARG	18.72	18.98	259	1.42
PRO	19.43	19.63	1046	1.29
MET	20.27	20.42	148	0.14
VAL	20.62	20.80	1267	1.23
DPT	22.07	22.22	272858	379.53
TRP	23.30	23.57	3832	3.17
PHE	24.37	24.52	472	0.53
ILE	25.00	25.12	304	0.40
LEU	25.80	25.97	928	1.11

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

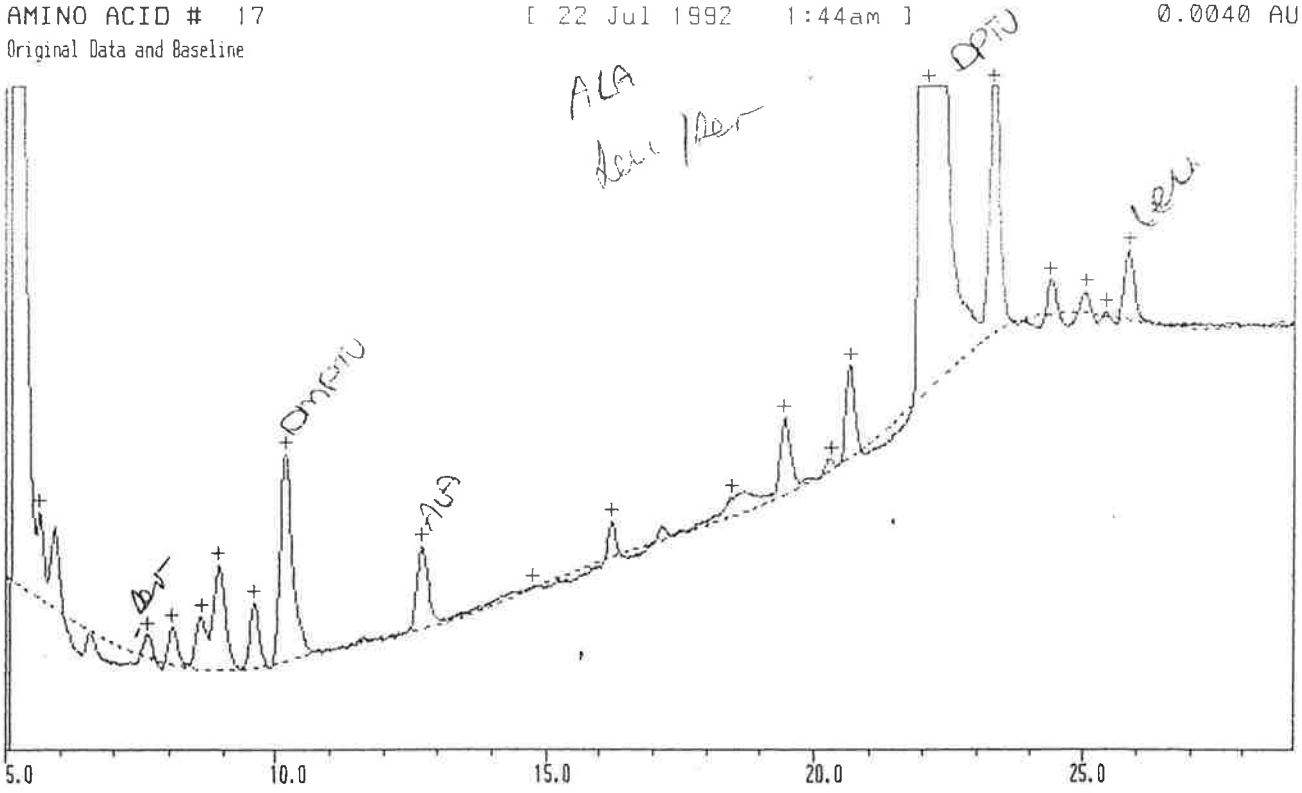
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 17 [22 Jul 1992 1:44am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		55845		VAL	20.65	20.80	1329	1.29
ASP	5.63	5.85	1236	1.07	DPT	22.08	22.22	286927	399.10
	5.90		1180		TRP	23.33	23.57	3888	3.22
SER	7.62	7.85	328	0.56	PHE	24.38	24.52	487	0.54
GLN	8.07	8.33	540	0.65	ILE	25.03	25.12	290	0.38
THR	8.60	8.85	746	1.49	LYS	25.43	25.52	52	0.05
GLY	8.93	9.17	1516	2.18	LEU	25.85	25.97	986	1.18
GLU	9.60	9.90	921	1.06					
DMP	10.18	10.40	2988	15.60					
ALA	12.70	12.97	1197	1.48					
HIS	14.75	14.68	24	0.07					
TYR	16.23	16.47	492	0.51					
ARG	18.47	18.98	280	1.54					
PRO	19.47	19.63	1104	1.36					
MET	20.30	20.42	158	0.15					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

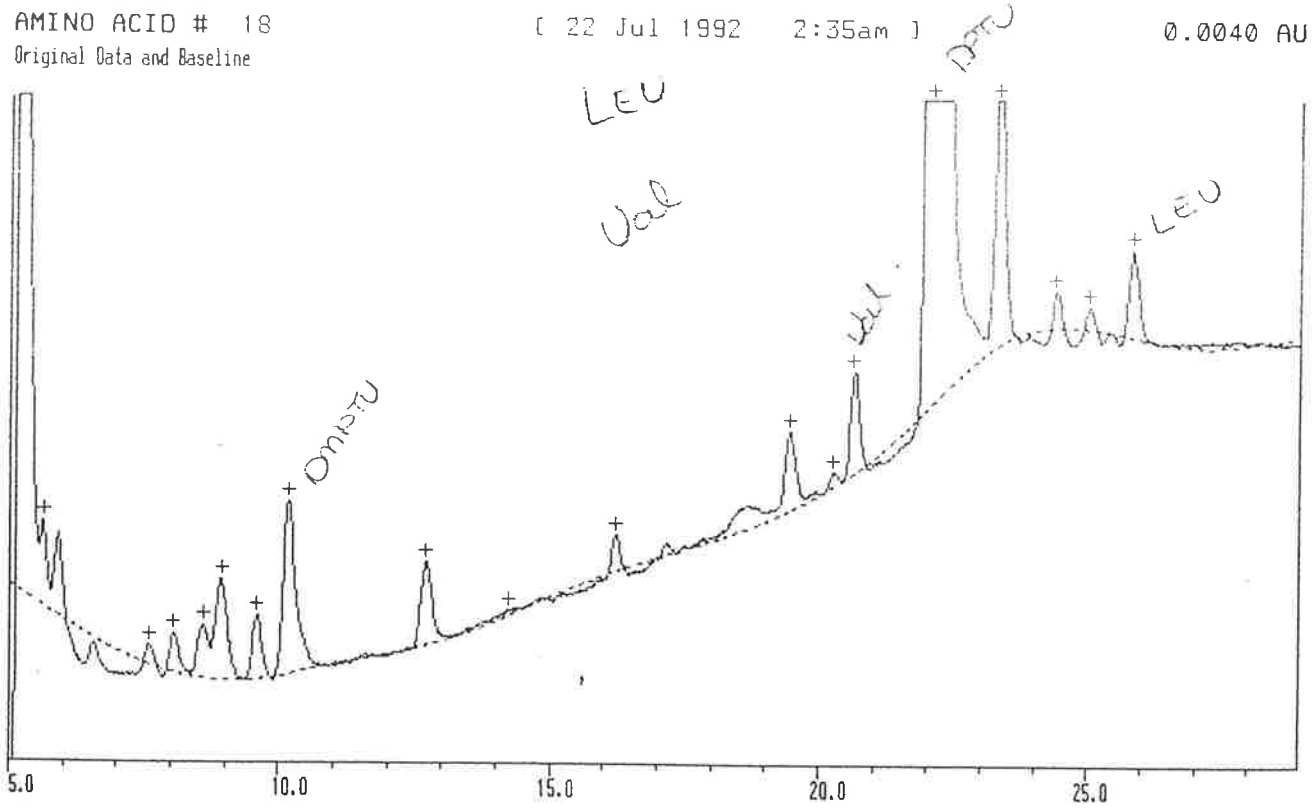
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 18 [22 Jul 1992 2:35am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		59205		DPT	22.08	22.22	293997	408.93
ASP	5.65	5.85	1209	1.04	TRP	23.32	23.57	4039	3.34
	5.92		1171		PHE	24.38	24.52	530	0.59
SER	7.60	7.85	292	0.50	ILE	25.02	25.12	324	0.42
GLN	8.07	8.33	559	0.68	LEU	25.83	25.97	1262	1.51
THR	8.62	8.85	763	1.52					
GLY	8.95	9.17	1461	2.10					
GLU	9.60	9.90	916	1.05					
DMP	10.20	10.40	2488	12.99					
ALA	12.70	12.97	1214	1.50					
HIS	14.23	14.68	79	0.23					
TYR	16.23	16.47	523	0.54					
PRO	19.45	19.63	1149	1.42					
MET	20.23	20.42	220	0.21					
VAL	20.63	20.80	1456	1.41					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

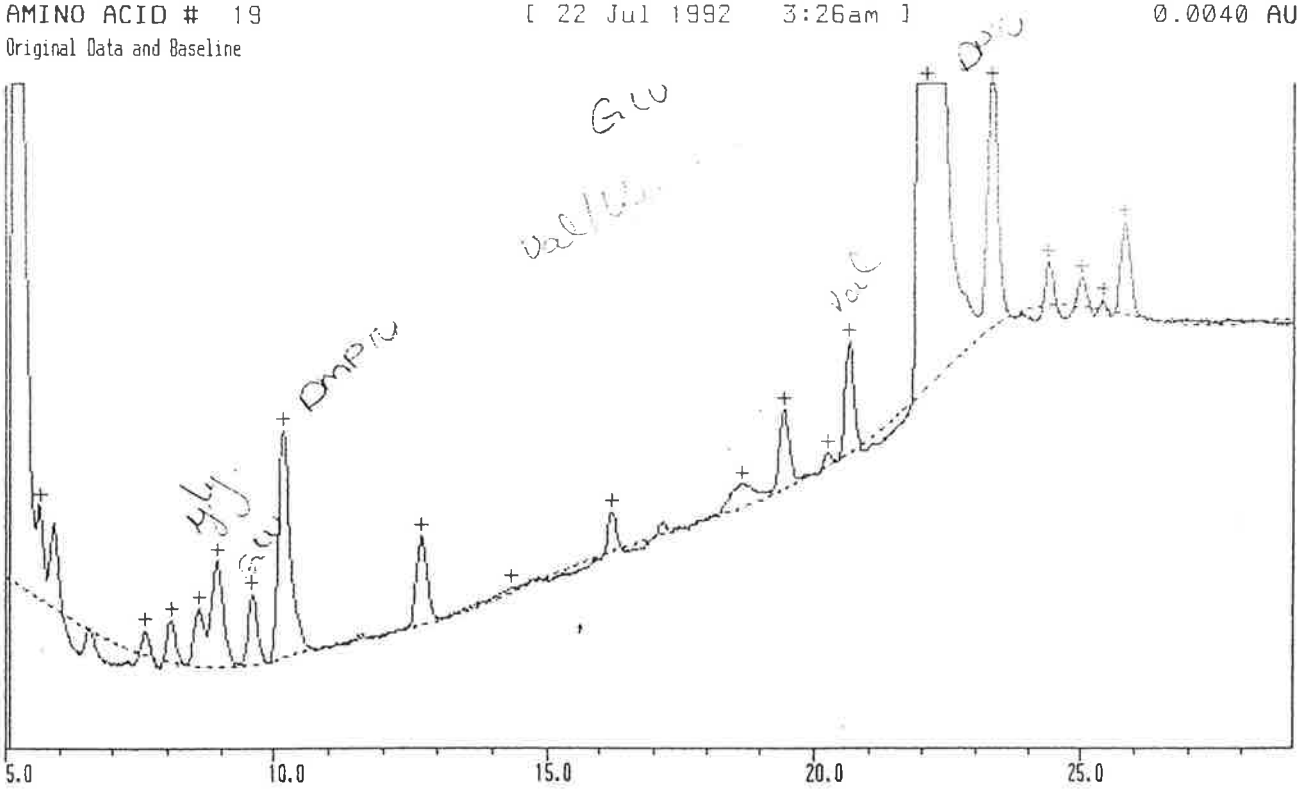
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 19 [22 Jul 1992 3:26am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		59613		VAL	20.63	20.80	1593	1.54
ASP	5.65	5.85	1351	1.16	DPT	22.07	22.22	294052	409.01
	5.90		1228		TRP	23.32	23.57	3902	3.23
SER	7.60	7.85	338	0.58	PHE	24.37	24.52	616	0.69
GLN	8.08	8.33	588	0.71	ILE	25.02	25.12	400	0.52
THR	8.60	8.85	832	1.66	LYS	25.40	25.52	122	0.12
GLY	8.95	9.17	1531	2.20	LEU	25.82	25.97	1320	1.58
GLU	9.60	9.90	1000	1.15					
DMP	10.18	10.40	3283	17.14					
ALA	12.70	12.97	1288	1.59					
HIS	14.35	14.68	72	0.21					
TYR	16.23	16.47	583	0.60					
ARG	18.67	18.98	340	1.86					
PRO	19.45	19.63	1152	1.42					
MET	20.23	20.42	192	0.18					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

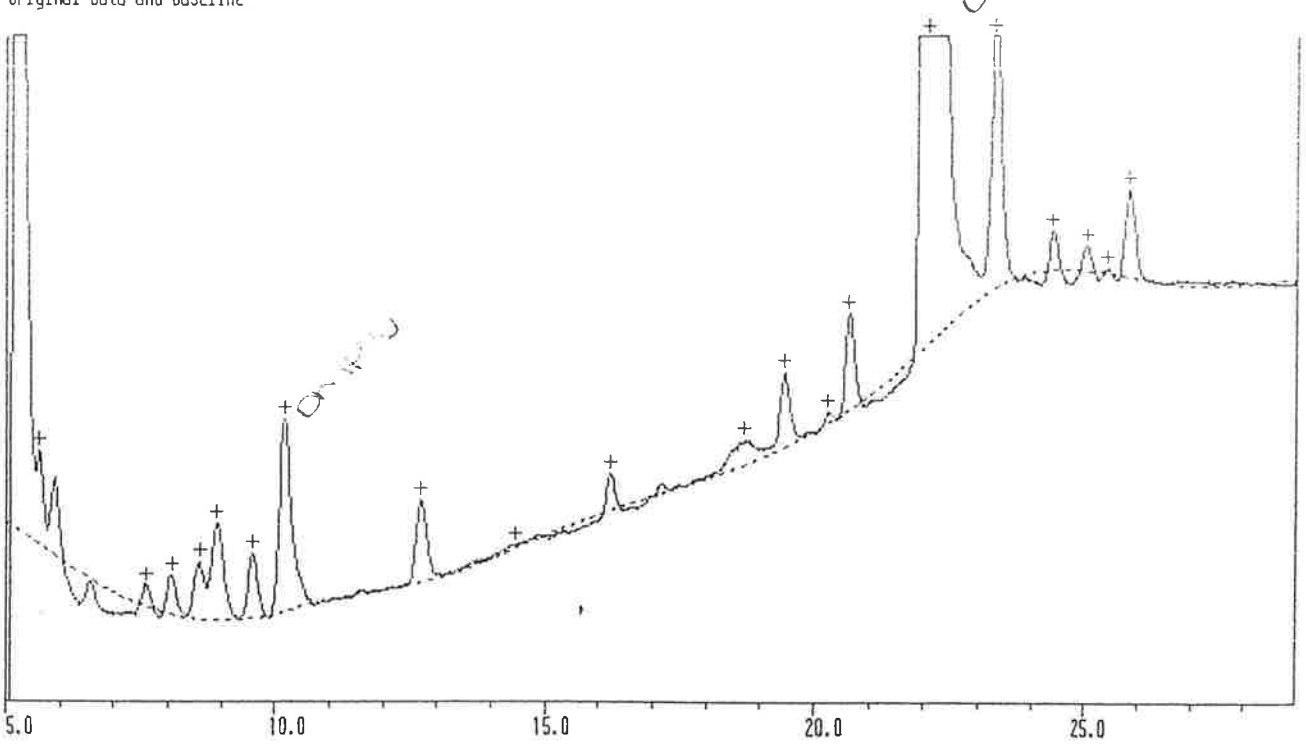
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 20 [22 Jul 1992 4:17am] 0.0040 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		58161		VAL	20.63	20.80	1396	1.35
ASP	5.63	5.85	1353	1.17	DPT	22.08	22.22	299208	416.18
	5.92		1120		TRP	23.33	23.57	3979	3.29
SER	7.62	7.85	316	0.54	PHE	24.38	24.52	568	0.63
GLN	8.08	8.33	568	0.69	ILE	25.03	25.12	352	0.46
THR	8.60	8.85	830	1.66	LYS	25.43	25.52	72	0.07
GLY	8.93	9.17	1408	2.03	LEU	25.83	25.97	1269	1.52
GLU	9.60	9.90	919	1.05					
DMP	10.18	10.40	2784	14.54					
ALA	12.72	12.97	1197	1.48					
HIS	14.45	14.68	55	0.16					
TYR	16.23	16.47	513	0.53					
ARG	18.72	18.98	343	1.88					
PRO	19.47	19.63	1070	1.32					
MET	20.25	20.42	158	0.15					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

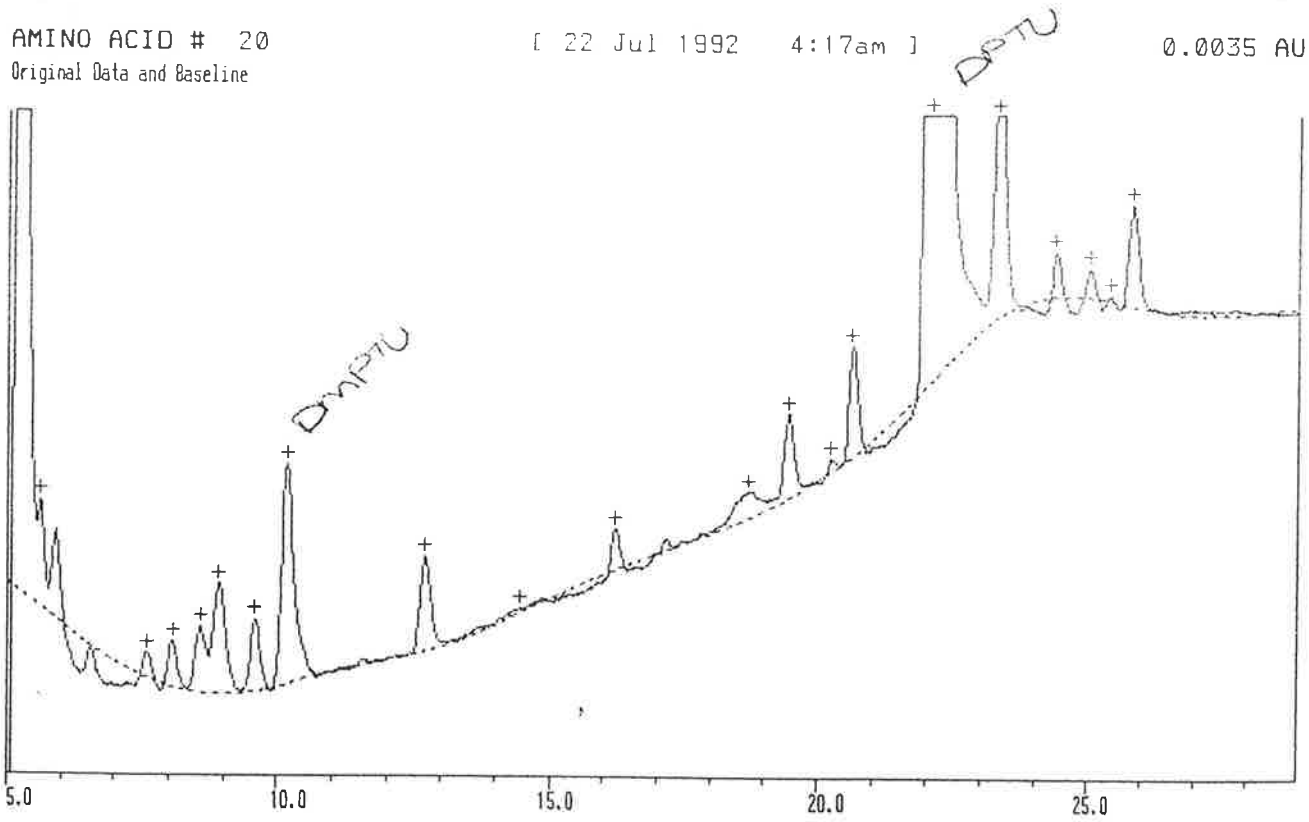
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 20 [22 Jul 1992 4:17am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

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	5.20		58161		VAL	20.63	20.80	1396	1.35
ASP	5.63	5.85	1353	1.17	DPT	22.08	22.22	299208	416.18
	5.92		1120		TRP	23.33	23.57	3979	3.29
SER	7.62	7.85	316	0.54	PHE	24.38	24.52	568	0.63
GLN	8.08	8.33	568	0.69	ILE	25.03	25.12	352	0.46
THR	8.60	8.85	830	1.66	LYS	25.43	25.52	72	0.07
GLY	8.93	9.17	1408	2.03	LEU	25.83	25.97	1269	1.52
GLU	9.60	9.90	919	1.05					
DMP	10.18	10.40	2784	14.54					
ALA	12.72	12.97	1197	1.48					
HIS	14.45	14.68	55	0.16					
TYR	16.23	16.47	513	0.53					
ARG	18.72	18.98	343	1.88					
PRO	19.47	19.63	1070	1.32					
MET	20.25	20.42	158	0.15					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

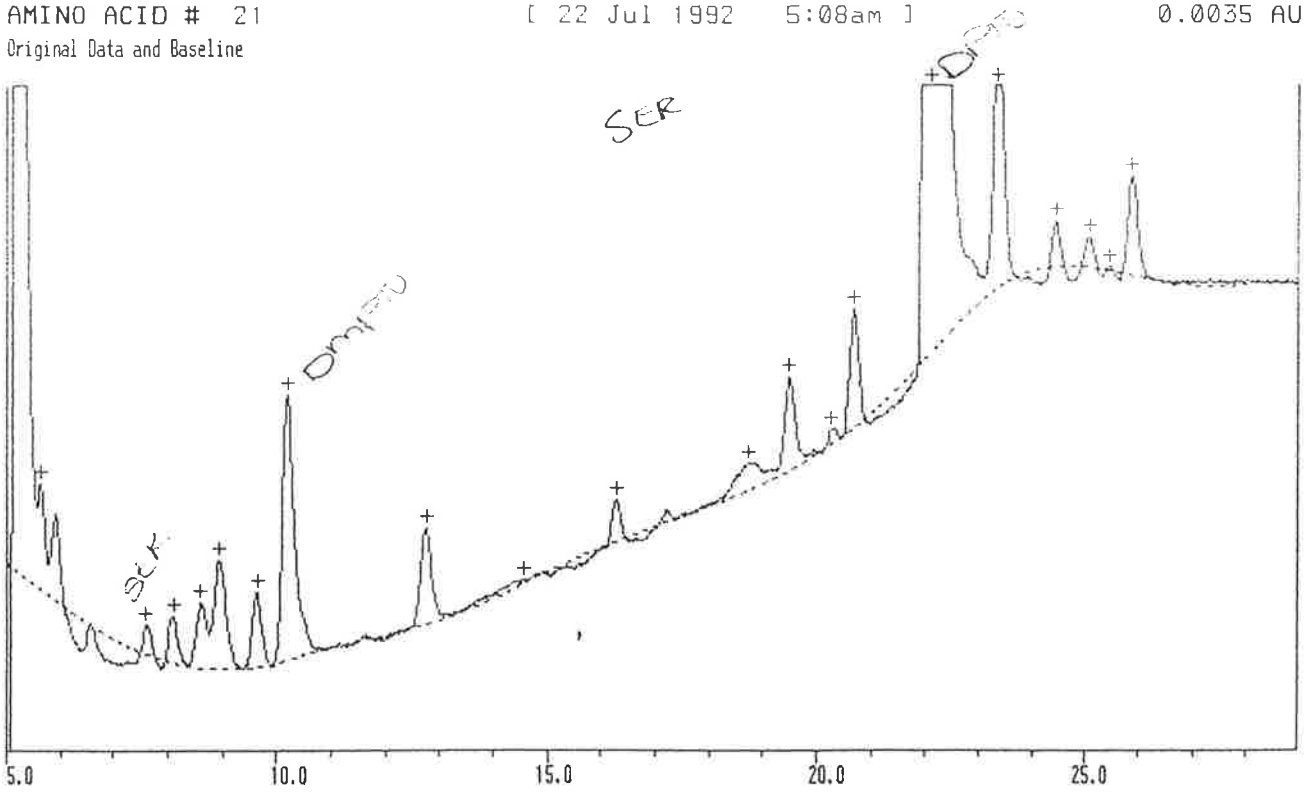
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 21 [22 Jul 1992 5:08am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

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	5.20		62872		VAL	20.68	20.80	1495	1.45
ASP	5.65	5.85	1332	1.15	DPT	22.12	22.22	264494	367.89
	5.92		1087		TRP	23.37	23.57	3475	2.88
SER	7.62	7.85	376	0.64	PHE	24.43	24.52	597	0.67
GLN	8.12	8.33	592	0.72	ILE	25.07	25.12	393	0.51
THR	8.63	8.85	816	1.63	LYS	25.45	25.52	60	0.06
GLY	8.97	9.17	1377	1.98	LEU	25.88	25.97	1250	1.50
GLU	9.65	9.90	936	1.07					
DMP	10.22	10.40	3338	17.43					
ALA	12.77	12.97	1209	1.50					
HIS	14.57	14.68	62	0.18					
TYR	16.30	16.47	528	0.54					
ARG	18.75	18.98	343	1.88					
PRO	19.50	19.63	1180	1.46					
MET	20.28	20.42	182	0.17					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

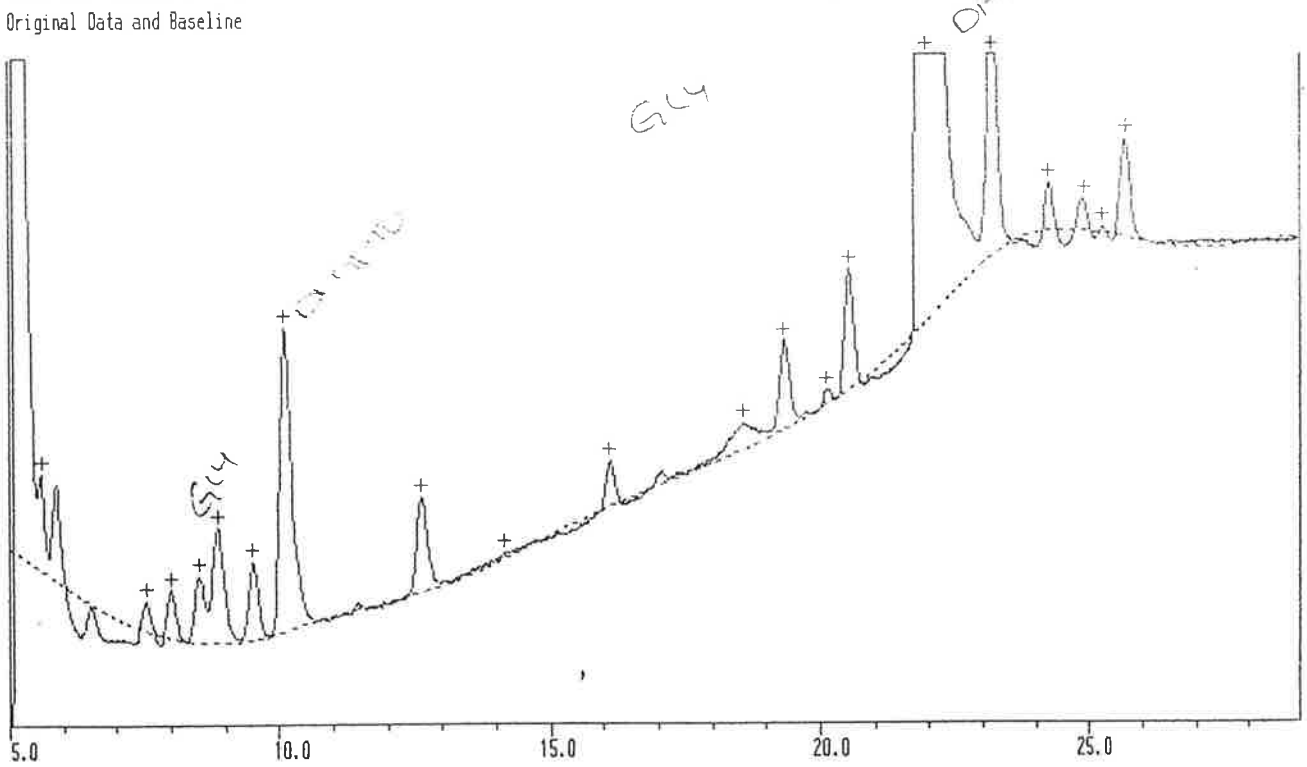
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 22 [22 Jul 1992 5:59am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

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	5.17		61557		VAL	20.53	20.80	1524	1.48
ASP	5.60	5.85	1216	1.05	DPT	21.97	22.22	275544	383.26
	5.88		1209		TRP	23.20	23.57	3669	3.04
SER	7.57	7.85	381	0.65	PHE	24.27	24.52	583	0.65
GLN	8.02	8.33	614	0.74	ILE	24.92	25.12	386	0.50
THR	8.53	8.85	825	1.65	LYS	25.27	25.52	88	0.09
GLY	8.88	9.17	1447	2.08	LEU	25.70	25.97	1216	1.46
GLU	9.53	9.90	984	1.13					
DMP	10.13	10.40	3825	19.97					
ALA	12.63	12.97	1200	1.49					
HIS	14.18	14.68	69	0.20					
TYR	16.13	16.47	573	0.59					
ARG	18.60	18.98	333	1.82					
PRO	19.35	19.63	1132	1.40					
MET	20.13	20.42	172	0.16					

Tabulation threshold : 500 uAU

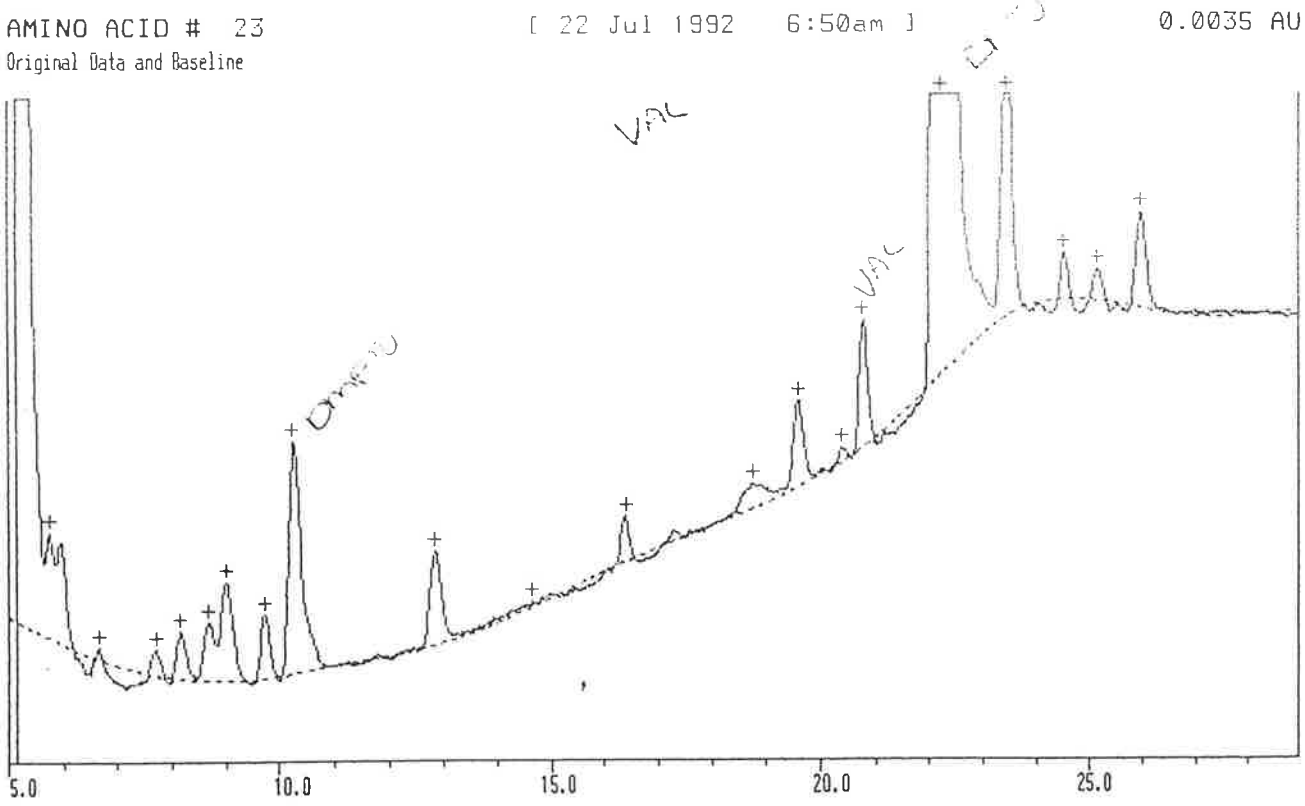
– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 23 [22 Jul 1992 6:50am] 0.0035 AU
Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

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	5.25		56709		MET	20.40	20.42	175	0.17
ASP	5.75	5.85	1334	1.15	VAL	20.78	20.80	1591	1.54
	5.97		1276		DPT	22.22	22.22	256456	356.71
ASN	6.65	6.80	151	0.16	TRP	23.47	23.57	3780	3.13
SER	7.70	7.85	321	0.55	PHE	24.52	24.52	595	0.66
GLN	8.18	8.33	616	0.75	ILE	25.17	25.12	400	0.52
THR	8.70	8.85	748	1.49	LEU	25.98	25.97	1202	1.44
GLY	9.03	9.17	1252	1.80					
GLU	9.75	9.90	808	0.93					
DMP	10.28	10.40	2930	15.30					
ALA	12.85	12.97	1183	1.46					
HIS	14.67	14.68	67	0.20					
TYR	16.40	16.47	578	0.60					
ARG	18.77	18.98	321	1.76					
PRO	19.60	19.63	1089	1.35					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

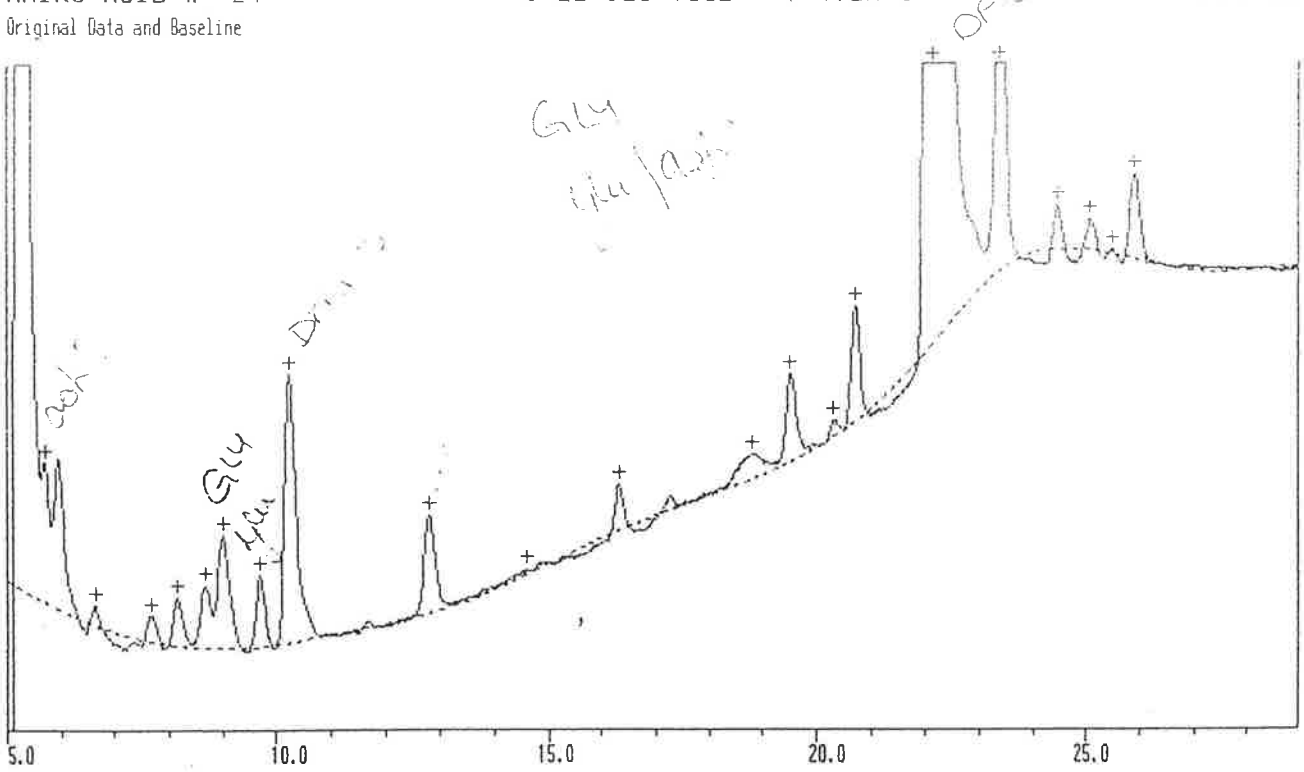
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 24 [22 Jul 1992 7:41am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

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	5.25		64416		MET	20.32	20.42	211	0.20
ASP	5.72	5.85	1766	1.52	VAL	20.73	20.80	1452	1.41
	5.97		1884		DPT	22.17	22.22	343236	477.42
ASN	6.65	6.80	261	0.28	TRP	23.40	23.57	4195	3.47
SER	7.67	7.85	338	0.58	PHE	24.47	24.52	547	0.61
GLN	8.17	8.33	607	0.73	ILE	25.08	25.12	374	0.49
THR	8.68	8.85	784	1.57	LYS	25.50	25.52	55	0.05
GLY	9.02	9.17	1416	2.04	LEU	25.92	25.97	1070	1.28
GLU	9.70	9.90	888	1.02					
DMP	10.27	10.40	3379	17.64					
ALA	12.82	12.97	1233	1.53					
HIS	14.62	14.68	69	0.20					
TYR	16.33	16.47	568	0.59					
ARG	18.82	18.98	324	1.77					
PRO	19.53	19.63	1118	1.38					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

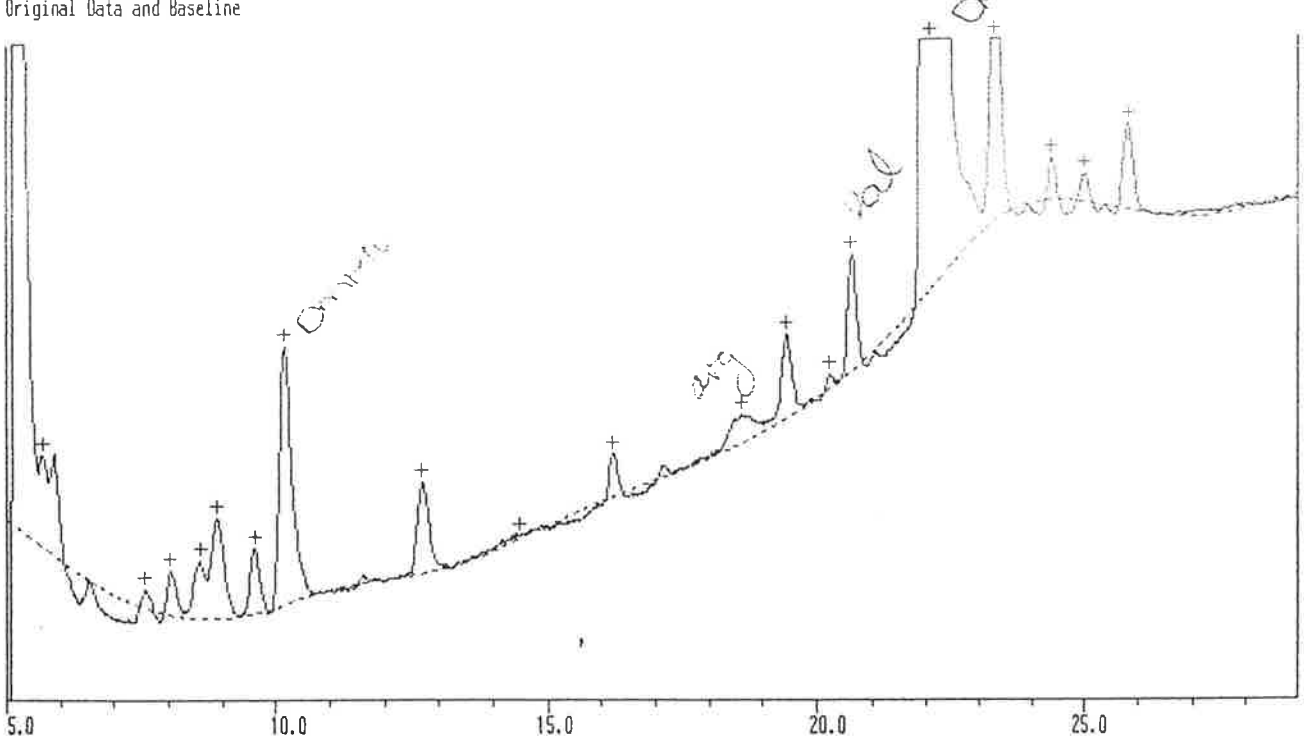
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :
Reaction cycle : RUN470-1
Conversion cycle : RUN470-1
Gradient : RUN470-1

Data collect time : 0.0 to 30.0 min
Data interval : 1.0 sec
Inject volume : 50 of 120 uL

AMINO ACID # 25 [22 Jul 1992 8:32am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

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	5.20		48182		VAL	20.63	20.80	1478	1.43
ASP	5.68	5.85	1144	0.99	DPT	22.07	22.22	306763	426.69
	5.90		1279		TRP	23.32	23.57	4416	3.66
SER	7.58	7.85	242	0.41	PHE	24.37	24.52	523	0.58
GLN	8.05	8.33	566	0.69	ILE	25.02	25.12	340	0.44
THR	8.60	8.85	729	1.46	LEU	25.82	25.97	1094	1.31
GLY	8.92	9.17	1276	1.84					
GLU	9.62	9.90	832	0.96					
DMP	10.18	10.40	3256	17.01					
ALA	12.72	12.97	1142	1.41					
HIS	14.50	14.68	38	0.11					
TYR	16.23	16.47	554	0.57					
ARG	18.62	18.98	374	2.05					
PRO	19.45	19.63	1056	1.30					
MET	20.25	20.42	163	0.16					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

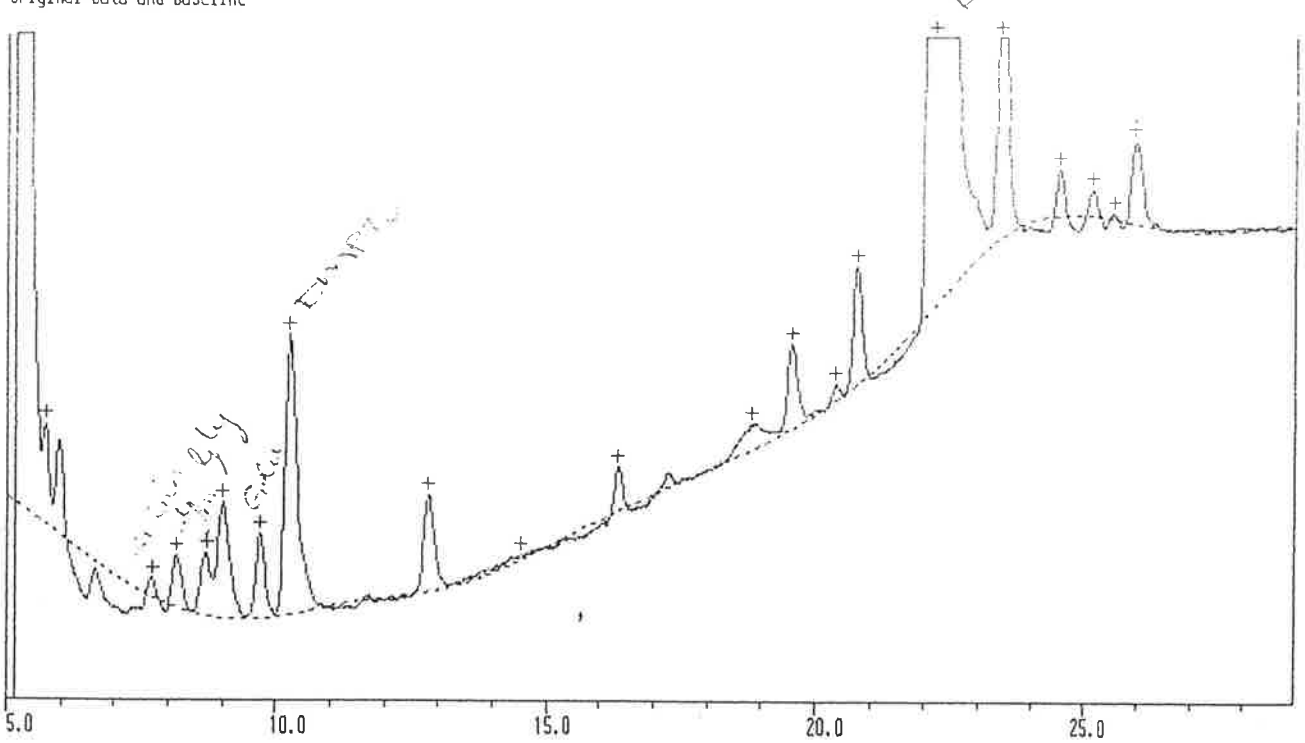
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 26 [22 Jul 1992 9:24am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.25		64440		VAL	20.75	20.80	1509	1.46
ASP	5.72	5.85	1264	1.09	DPT	22.18	22.22	302570	420.85
	5.98		1185		TRP	23.43	23.57	3696	3.06
SER	7.72	7.85	230	0.39	PHE	24.52	24.52	585	0.65
GLN	8.17	8.33	660	0.80	ILE	25.15	25.12	336	0.44
THR	8.72	8.85	768	1.53	LYS	25.57	25.52	81	0.08
GLY	9.02	9.17	1452	2.09	LEU	25.95	25.97	1116	1.34
GLU	9.72	9.90	1075	1.23					
DMP	10.27	10.40	3544	18.51					
ALA	12.82	12.97	1216	1.51					
HIS	14.55	14.68	57	0.17					
TYR	16.35	16.47	547	0.56					
ARG	18.82	18.98	333	1.82					
PRO	19.57	19.63	1068	1.32					
MET	20.35	20.42	204	0.19					

Tabulation threshold : 500 uAU

– Applied Biosystems 475A Protein Sequencer Chromatogram Report –

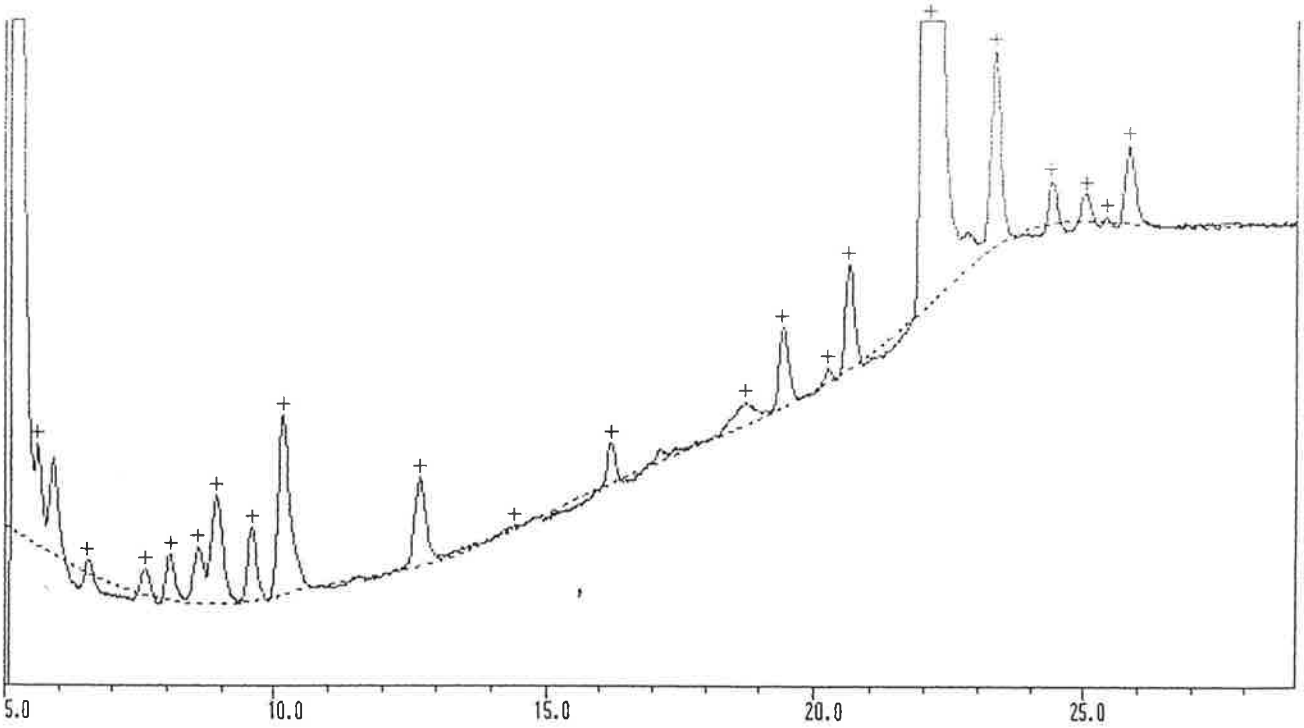
SAMPLE : JAMES STORER PAO PSE 204
[Initiated 21 Jul 1992 10:00am]

CYCLE SUMMARY :

Reaction cycle	: RUN470-1	Data collect time	: 0.0 to 30.0 min
Conversion cycle	: RUN470-1	Data interval	: 1.0 sec
Gradient	: RUN470-1	Inject volume	: 50 of 120 uL

AMINO ACID # 27 [22 Jul 1992 10:15am] 0.0035 AU

Original Data and Baseline



Retention Time: Minutes

PEAK TABULATION : (100% injection) Calibration : CAL470-1

Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol	Peak ID	R.Time (min)	C.Time (min)	Height (uAU)	Pmol
	5.20		59102		MET	20.23	20.42	168	0.16
ASP	5.63	5.85	1284	1.11	VAL	20.63	20.80	1298	1.26
	5.92		1204		DPT	22.07	22.22	109552	152.38
ASN	6.57	6.80	170	0.18	TRP	23.32	23.57	2443	2.02
SER	7.60	7.85	331	0.57	PHE	24.37	24.52	530	0.59
GLN	8.08	8.33	573	0.69	ILE	25.05	25.12	328	0.43
THR	8.58	8.85	703	1.40	LYS	25.42	25.52	57	0.06
GLY	8.92	9.17	1360	1.96	LEU	25.83	25.97	964	1.16
GLU	9.58	9.90	940	1.08					
DMP	10.18	10.40	2253	11.77					
ALA	12.70	12.97	1128	1.40					
HIS	14.43	14.68	55	0.16					
TYR	16.22	16.47	494	0.51					
ARG	18.75	18.98	288	1.57					
PRO	19.43	19.63	991	1.22					

Tabulation threshold : 500 uAU

Appendix E. CLUSTAL W Multiple Sequence Alignment

Figure E.1 CLUSTAL W Amine Oxidase Sequence Alignment

'*' is used to indicate identical residues, while '.' is used to show positions where all the sequences are 'similar' [1923]. ABP_HUMN.s, human (placental) diamine oxidase [757] [EMBL 78212]; ABP_HUKI.s, human kidney ABP/DAO [781] [M55602]; ABP_RATC.s, rat colon/lung ABP/DAO [982] [X73911]; AO_BOVIN.s, bovine serum/liver copper amine oxidase [411] [S69583]; AMO_ECOL.s, *E. coli* amine oxidase (maoA) [529] [L47571]; AMO_HANS.s, *Hansenula polymorpha* AO [566] [X15111]; AO_ANTHR.s, *Anthrobactr* methylamine oxidase (maoxII) [516] [L12990]; AO_KAERO.s, *K. aerogenes* AO [1092] [D10208]; AO_PISUM.s, pea seedling AO [649] [L39931]; AO_LENSC.s, lentil seedling AO [617] [X64201].

The CLUSTAL W (version 1.5, April 1995) multiple sequence alignment program [1922,1923], used for the sequence comparison, was obtained over the Internet by anonymous ftp from <ftp.ebi.ac.uk/pub/software/dos/clustal$.exe>. This program also provides the facility for the construction of New Hampshire phylogenetic tree formats (nested parentheses) compatible with DRAWTREE program of the PHYLIP (phylogeny inference package) program suite by J. Felstenstein. Sequences were converted from text to FASTA format files [1919] using the BBSeqF (version 1.2) MS-Windows program available from the SimTel mirror @ <archie.au/micros/pc/SimTel/win3/biology> by anonymous ftp. FASTA files were concatenated for entry into the CLUSTAL multiple sequence alignment program using the multiple sequence alignment editor of the SeqPup program (version 0.4, July 1995 release) for MS-Windows written by D. Gilbert and available by anonymous ftp from <ftp://iubio.bio.indiana.edu/molbio/seqpup/>. Swiss-Protein entries were obtained at http://www.ebi.ac.uk/Swissfetch? or http://expasy.hcuge.ch; EMBL entries were from http://www.ebi.ac.uk/emblfetch? or mirror sites.

```
ABP_HUMN.s     1 ------------------------MPALGWAVAAILMLQTAMAEP--SPGTLPRKAGVFS
ABP_HUKI.s       ------------------------MPALGWAVAAILMLQTAMAEP--SPGTLPRKAGVFS
ABP_RATC.s       ------------------------MCLAFGWAAVILVLQTVDTAS--AVRTPYDKARVFA
AO_BOVIN.s       MFIFIFLSLWTLLVMGREEGGVGSEEGVGKQCHPSLPPRCPSRSPSDQPWTHPDQSQLFA
AMO_ECOL.s       -------------MGSPSLYSARKTTLALAVALSFAWQAPVFAHG-GEAHMVPMDKTLK
AMO_HANS.S       ------------------------------------------------------------
AO_ANTHR.s       ------------------------------------------------MERLRQIAS
AO_KAERO.s       -------------MANGLKFSPRKTALALAVAVVCAWQSPVFAHG-SEAHMVPLDKTLQ
AO_PISUM.s       ------------------------------------------------MASTTTMRLA
AO_LENSC.s       ------------------------------------------------------KFA

ABP_HUMN.s    35 DLS----------NQELKAVHSFLWSKKE-------------------------------
ABP_HUKI.s       DLS----------NQELKAVHSFLWSKKE-------------------------------
ABP_RATC.s       DLS----------PQEIKAVHSFLMNREE-------------------------------
AO_BOVIN.s       DLS----------REELTTVMSFLTQQLG-------------------------------
AMO_ECOL.s       EFGADVQWDDYAQLFTLIKDGAYVKVKPGAQTAIVNGQPLALQVPVVMKDNKAWVSDTFI
AMO_HANS.S       QAT----------------AASAAPARP-------------------------------
AO_ANTHR.s       GVS------------------------------------------------------------
AO_KAERO.s       EFGADVQWDDYAQMFTLIKDGAYVKVKPGAKTAIVNGKSLDLPVPVVMKEGKAWVSDTFI
AO_PISUM.s       LFS----------VLTLLSFHAVVSVTP-------------------------------
AO_LENSC.s       LFS----------VLTLLSFHAVFSFTP-------------------------------

ABP_HUMN.s    54 -----------LRLQ---PSSTTTMAKN---TVFLIEMLLPKKYHVLRFLDKGERHPVRE
ABP_HUKI.s       -----------LRLQ---PSSTTTMAKN---TVFLIEMLLPKKYHVLRFLDKGERHPVRE
ABP_RATC.s       -----------LGLQ---PSKEPTLAKN---SVFLIEMLLPKKKHVLKFLDEGRKGPNRE
AO_BOVIN.s       ----------------PDLVDAAQARPSDNCVFSVELQLPPKAAALAHLDRGSPPPARE
AMO_ECOL.s       NDVFQSGLDQTFQVEKRPHPLNALTADEIKQAVEIVKASADFKPN-TRFTEISLLPP--D
AMO_HANS.S       ----------------AHPLDPLSTAEIKAATNTVKS--YFAGKKISFNTVTLREP--A
AO_ANTHR.s       ----------------HPLDPLSRVEIA-RAVAILKEGPAAAESFRFISVELREP---
AO_KAERO.s       NDVFQSGLDQTFQVEKRPHPLNSLSAAEISKAVTIVKAAPEFQPN-TRFTEISLHEP--D
AO_PISUM.s       -----------LHVQ---HPLDPLTKEEFLAVQTIVQNKYPISNNRLAFHYIGLDDP--E
AO_LENSC.s       -----------LHTQ---HPLDPITKEEFLAVQTIVQNKYPISNNKLAFHYIGVDDP--E
                                                          .                *

ABP_HUMN.s    97 ARAVIFFGDQEHPNVTEFAVGPLPGPCYMRALS-PRPGYQSSWASRPISTAEYALLYHTL
ABP_HUKI.s       ARAVIFFGDQEHPNVTEFAVGPLPGPCYMRALS-PRPGYQSSWASRPISTAEYALLYHTL
ABP_RATC.s       ARAVIFFGAQDYPNVTEFAVGPLPRPYYIRALS-PRPGHHLSWSSRPISTAEYDLLYHTL
AO_BOVIN.s       ALAIVFFGGQPQPNVTELVVGPLPQPSYMRDVTVERHGGPLPYYRRPVLLREYLDIDQMI
AMO_ECOL.s       KEAVWAFALE--N--KPVDQ---PRKADVIMLD-GKHIIEAVVDLQNNKLLSWQPIKDAH
AMO_HANS.S       RKAYIQWKEQGGP----LP----PRLAYYVILEAGKPGVKEGLVDLASLSVIETRALETV
AO_ANTHR.s       SKDDLRAGVA-------VAR---EADAVLVDRA-QARSFEAVVDLEAGTVDSWKLLAENI
AO_KAERO.s       KAAVWAFALQ--G--TPVDA---PRTADVVMLD-GKHVIEAVVDLQNKKILSWTPIKGAH
AO_PISUM.s       KDHVLRYETH--P--TLVSI---PRKIFVVAII-NSQTHEILINLRIRSIVSDNIHNGYG
AO_LENSC.s       KDLVLKYETS--P--TLISI---PRKIFVVAII-NSQTHEILIDLTIKSIVSDNIHNGYG
```

```
ABP_HUMN.s   156 QEATKPLHQFFLNTTGFSFQDCHDRCLAFTDVAPRGVASGQR--RSWLIIQRYVEGYFLH
ABP_HUKI.s       QEATKPLHQFFLNTTGFSFQDCHDRCLAFTDVAPRGVASGQR--RSWLIIQRYVEGYFLH
ABP_RATC.s       KRATMPLHQFFLDTTGFSFLGCDDRCLTFTDVAPRGVASGQR--RSWFIVQRYVEGYFLH
AO_BOVIN.s       FNRELPQAAGVLHHCCSYKQGGQK--LLTMNSAPRGVQSGDRSTWFGIYYNITKGGPYLH
AMO_ECOL.s       GMVLLDDFASVQNIINNSEE-------FAAAVKKRGITD-----AKKVITTPLTVGYFDG
AMO_HANS.S       QPILTVEDLCSTEEVIRNDP------AVIEQCVLSGIPAN---EMHKVYCDPWTIGYD--
AO_ANTHR.s       QPPFMLDEFAECEDACRKDPE------VIAALAKRGLTN-----LDLVCFEPWSVGYF--
AO_KAERO.s       GMVLLDDFVSVQNIINTSSE-------FAEVLKKHGITD-----PGKVVTTPLTVGFFDG
AO_PISUM.s       FPILSVDEQSLAIKLPLKYPP------FIDSVKKRGLN------LSEIVCSSFTMGWF--
AO_LENSC.s       FPVLSAAEQFLAIDLPLKYPP------FIASVNKRGLN------ISEIVCSSFTMGWF--
                                                 *.                  *

ABP_HUMN.s   214 PTGLELLVDHGSTDAGHWAVEQVWYNGKFYGSPEELARKYADGEVDVVVLEDPLPGGKGH
ABP_HUKI.s       PTGLELLVDHGSTDAGHWAVEQVWYNGKFYGSPEELARKYADGEVDVVVLEDRCLGARGM
ABP_RATC.s       PTGLEILLDHGSTDVQDWRVEQLWYNGKFYNNPEELARKYAVGEVDTVVLEDPLPNG---
AO_BOVIN.s       PVGLELLVDHKALDPADWTVQKVFFQGRYYENLAQLEEQFEAGQVNVVVIPDDGTG----
AMO_ECOL.s       KDGLKQDARLLKVIS--YLDVG---DGNYWAHPIENLVAVVDLEQKKIVKIEEGP-----
AMO_HANS.S       --ERWGTGKRLQQALVYYRSDE---DDSQYSHPLDFCPIVDTEEKKVIFIDIPNRRRK--
AO_ANTHR.s       --GEDNEGRRLMRALVFVRDEA---DDSPYAHPIENFIVFYDLNAGKVVRLEDDQ-----
AO_KAERO.s       KDGLQQDARLLKVVS--YLDTG---DGNYWAHPIENLVAVVDLEAKKIIKIEEGP-----
AO_PISUM.s       --GEEKNVRTVRLDC--FMKES---TVNIYVRPITGITIVADLDLMKIVEYHDRD-----
AO_LENSC.s       --GEEKNSRTVRVDC--FMKES---TVNIYVRPITGITIVADLDLMKIVEYHDRD-----
                                                          .

ABP_HUMN.s   274 DSTEEPPLFSSHKPRGDFPSPIHVSGPRLVQ---PHGPRFRLEGNAVLYGGWSFAFRLRS
ABP_HUKI.s       TAQRSRPSSPPQAPR-DFPQPHPCERPPLGP---APRPSLQAGGQRCALRRLELCLPVRS
ABP_RATC.s       --TEKPPLFSSYKPRGEFHTPVNVAGPHVVQ---PSGPRYKLEGNTVLYGGWSFSYRLRS
AO_BOVIN.s       -----------GFWSLKSQVPPGPTPPLQFH---PQGPRFSVQGNRVASSLWTFSFGLGA
AMO_ECOL.s       --VVPVPMTARPFDGRDRVAPAVKPMQIIE----PEGKNYTITGDMIHWRNWDFHLSMNS
AMO_HANS.S       VSKHKHANFYPKHMIEKVGAMRPEAPPINVTQ--PEGVSFKMTGNVMEWSNFKFHIGFNY
AO_ANTHR.s       --AIPVPSARGNYLPKYVGEARTDLKPLNITQ--PEGASFTVTGNHVTWADWSFRVGFTP
AO_KAERO.s       --VIPVPMEPRPYDGRDRNAPAVKPLEITE----PEGKNYTITGDTIHWQNWDFHLRLNS
AO_PISUM.s       --IEAVPTAENTEYQVSKQSPPFGPKQHSLTSHQPQGPGFQINGHSVSWANWKFHIGFDV
AO_LENSC.s       --TEAVPTAENTEYQVSKQSPPFGPKQHSLTSHQPQGPGFQINGTSVSWANWKFHIGFDV
                                                          *

ABP_HUMN.s   331 SSGLQVLNVHFG--G---ER-IAYEVSVQEAVALYGGHTPAGMQTKYLDVG-WGLGSVTH
ABP_HUKI.s       SSGLQVLNVHFG--G---ER-IAYEVSVQEAVALYGGHTPAGMQTKYLDVG-WGLGSVTH
ABP_RATC.s       SSGLQIFNVLFG--G---ER-VAYEVSVQEAVALYGGHTPAGMQTKYLDVG-WGLGSVTH
AO_BOVIN.s       FSGPRVFDVRFQ--G---ER-LAYEISLQEAGAVYGGNTPAAMLTRYMDSG-FGMGYFAT
AMO_ECOL.s       RVGPMISTVTYNDNG--TKRKVMYEGSLGGMIVPYGDPDIGWYFKAYLDSGDYGMGTLTS
AMO_HANS.S       REGIVLSDVSYNDHG--NVRPIFHRISLSEMIVPYGSPEFPHQRKHALDIGEYGAGYMTN
AO_ANTHR.s       REGLVLHQLKFKDQG--VDRPVINRASLSEMVVPYGDTAPVQAKKNAFDSGEYNIGNMAN
AO_KAERO.s       RVGPILSTVTYNDNG--TKRQVMYEGSLGGMIVPYGDPDVGWYFKAYLDSGDYGMGTLTS
AO_PISUM.s       RAGIVISLASIYDLEKHKSRRVLYKGYISELFVPYQDPTEEFYFKTFFDSGEFGFGLSTV
AO_LENSC.s       RAGIVISLASIYDLEKHKSRRVLYKGYISELFVPYQDPTEEFYFKTFFDSGEFGFGLSTV
                   *  .              * .    .      *              * *  . *  .

ABP_HUMN.s   384 ELAPGIDCPETATFLDTFHYYDADDPVHYPRALCLFEMPTGVPLRRHFNSNFKGGFNFYA
ABP_HUKI.s       ELAPGIDCPETATFLDTFHYYDADDPVHYPRALCLFEMPTGVPLRRHFNSNFKGGFNFYA
ABP_RATC.s       ELAPGIDCPETATFLDAFHYYDSDGPVHYPHALCLFEMPTGVPLRRHFNSNFKGGFNFYA
AO_BOVIN.s       PLIRGVDCPYLATYMDWHFVVESQTPKTLHDAFCVFEQNKGLPLRRHHSD---FLSHYFG
AMO_ECOL.s       PIARGKDAPSNAVLLNETIADYTGVPMEIPRAIAVFER-YAGPEYKHQEM---GQPNVST
AMO_HANS.S       PLSLGCDCKGVIHYLDAHFSDRAGDPITVKNAVCIHEE-DDGLLFKHSDFR----DNFAT
AO_ANTHR.s       SLTLGCDCLGEIKYFDGHSVDSHGNPWTIENAICMHEE-DDSILWKHFDFR-EGTAETRR
AO_KAERO.s       PIVRGKDAPSNAVLLDETIADYTGKPTTIPGAVAIFER-YAGPEYKHLEM---GKPNVST
AO_PISUM.s       SLIPNRDCPPHAQFIDTYVHSANGTPILLKNAICVFEQ-YGNIMWRHTEN---GIPNESI
AO_LENSC.s       SLIPNRDCPPHAQFIDTYIHSADGTPIFLENAICVFEQ-YGNIMWRHTET---GIPNESI
                  . . *.                  *     * .. *        .*

ABP_HUMN.s   444 G--LKGQVLVLRTTSTVYNYDYIWDFIFYPNGVMEAKMHATGY--VHATFYT-------P
ABP_HUKI.s       G--LKGQVLVLRTTSTVYNYDYIWDFIFYPNGVMEAKMHATGY--VHATFYT-------P
ABP_RATC.s       G--LKGYVLVLRTTSTVYNYDYIWDFIFYSNGVMEAKMHATGY--VHATFYT-------P
AO_BOVIN.s       G--VAQTVLVFRSVSTMLNYDYVWDMVFYPNGAIEVKLHATGY--ISSAFLFG-------
AMO_ECOL.s       E--RRE--LVVRWISTVGNYDYIFDWIFHENGTIGIDAGATGIEAVKGVKAKTMHDETAK
AMO_HANS.S       SLVTRATKLVVSQIFTAANYEYCLYWVFMQDGAIRLDIRLTGILNTYILGDD-------E
AO_ANTHR.s       S---RK--LVISFIATVANYEYAFYWHLFLDGSIEFLVKATGILSTAGQLPG-------E
AO_KAERO.s       E--RRE--LVVRWISTVGNYDYIFDWVFHDNGTIGIDAGATGIEAVKGVLAKTMHDPSAK
AO_PISUM.s       EESRTEVNLIVRTIVTVGNYDNVIDWEFKASGSIKPSIALSGILEIKGTNIK--HKDEIK
AO_LENSC.s       EESRTEVDLAIRTVVTVGNYDNVLDWEFKTSGWMKPSIALSGILEIKGTNIK--HKDEIK
                           *     *  **.          * .      .*
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ABP_HUMN.s   493 EGLRHGTRLHTHLIGNIHTHLVHYRVDLDVAGTKNSFQTLQMKLENITNP-WSPRHRVVQ
ABP_HUKI.s       EGCARHSPAHPPDWQHTHS-LVHYRVDLDVAGTKNSFQTLQMKLENITNP-WSPRHRVVQ
ABP_RATC.s       EGLRHGTRLQTHLLGNIHTHLVHYRVDMDVAGTKNSFQTLTMKLENLTNP-WSPSHSLVQ
AO_BOVIN.s       AARRYGNQVGEHTLGPVHTHSAHYKVDLDVGGLENWVWAEDMAFVPTAIP-WSPEHQIQR
AMO_ECOL.s       DDTRYGTLIDHNIVGTTHQHIYNFRLDLDVDGENNSLVAMDPVVKPNTA--GGPR-TSTM
AMO_HANS.S       EAGPWGTRVYPNVNAHNHQHLFSLRIDPRIDGDGNSAAACDAKSSPYPLGSPENMYGNAF
AO_ANTHR.s       KNPYGQSLNNDGLYAPIHQHMFNVRMDFELDGVKNAVYEVDMEYPEHNP----TG--TAF
AO_KAERO.s       EDTRYGTLIDHNIVGTTHQHIYNFRLDLDVDGENNTLVAMDPEVKPNTA--GGPR-TSTM
AO_PISUM.s       EDL-HGKLVSANSIGIYHDHFYIYYLDFDIDGTHNSFEKTSLKTVRIKD--GSSKRKSYW
AO_LENSC.s       EEI-HGKLVSANSIGIYHDHFYIYYLDFDIDGTQNSFEKTSLKTVRIVD--EVQE-KSYW
                                 *       .*  . *  *

ABP_HUMN.s   552 PTLEQTQYSWERQAAFRFKRKLPKYLLFTSPQENPWG-HKRSYRLQI-HSMADQVLP---
ABP_HUKI.s       PTLEQTQYSWERQAAFRFKRRLPKYLLFTSPQENPWG-HKRSYRLQI-HSMADQVLP---
ABP_RATC.s       PTLEQTQYSQEHQAAFRFGQTLPKYLLFSSPQKNCWG-HRRSYRLQI-HSMAEQVLP---
AO_BOVIN.s       LQVTRKQLETEEQAAFPLGGASPRYLYLASKQSNKWG-HPRGYRIQTVSFAGGPMPQ---
AMO_ECOL.s       QVNQYNIGN-EQDAAQKFDPGTIRLLS-NPNKENRMG-NPVSYQIIPYAGGTHPVAKGAQ
AMO_HANS.S       YSEKTTFKTVKDSLTNYESATGRSWDIFNPNKVNPYSGKPPSYKLVS--TQCPPLLAK--
AO_ANTHR.s       MAVDRLLETEQKAIRKTNEAKHRFWKIANHESKNLVN-EPVAYRLIP-TNGIQLAAR---
AO_KAERO.s       QVNQYTIDS-EQKAAQKFDPGTIRLLS-NTSKENRMG-NPVSYQIIPYAGGTHPAATGAK
AO_PISUM.s       TTETQTAKT-ESDAKITIGLAPAELVVVNPNIKTAVG-NEVGYRLIP-AIPAHPLLT---
AO_LENSC.s       TTETQTAKT-ESDAKITIGLAPAELVVVNPNIKTAVG-NEVGYRLIP-AIPAHPLLT---
                                                 .        *..

ABP_HUMN.s   607 --PGWQEEQAITWARYPLAVTKYRESELCSSSIYHQNDPWHPPVVFEQFLHN-NENIENE
ABP_HUKI.s       --PGWQEEQAITWARYPLAVTKYRESELCSSSIYHQNDPWDPPVVFEQFLHN-NENIENE
ABP_RATC.s       --PGWQEERAVTWARYPLAVTKYRESERYSSSLYNQNDPWDPPVVFEEFLRN-NENIEDE
AO_BOVIN.s       --NSP-MERAFSWGRYQLAITQRKETEPSSSSVFNQNDPWTPTVDFSDFIN--NETIAGK
AMO_ECOL.s       FAPDEWIYHRLSFMDKQLWVTRYHPGERFPEGKYP--NRSTHDTGLGQYSKD-NESLDNT
AMO_HANS.S       --EGSLVAKRAPWASHSVNVVPYKDNRLYPSGDHVPQWSGDGVRGMREWIGDGSENIDNT
AO_ANTHR.s       --DDAYVSKRAQFARNNLWVTAYDRTERFAAGEYP-NQATGADDGLHIWTQK-DRNIVDT
AO_KAERO.s       FAPDEWIYHRLSFMDKQLWVTRYHPTERYPEGKYP--NRSAHDTGLGQYAKD-DESLTNH
AO_PISUM.s       --EDDYPQIRGAFTNYNVWVTAYNRTEKWAGGLYV--DHSRGDDTLAVWTKQ-NREIVNK
AO_LENSC.s       --EDDYPQIRGAFTNYNVWVTQIIELKNGLVDFML--I----------------------
                                 . .

ABP_HUMN.s   664 DLVAWVTVGFLHIPHSEDIPNTATPGNSVGFLLRPFNFFPEDPSLASRDTVIVWPRD---
ABP_HUKI.s       DLVAWVTVGFLHIPHSEDIPNTATPGNSVGFLLRPFNFFPEDP----------SP-----
ABP_RATC.s       DLVAWVTVGFLHIPHSEDVPNTATPGNSVGFLLRPFNFFPEDPSLASRDTVIVWPQD---
AO_BOVIN.s       DLVAWVTAGFLHIPHAEDIPNTVTVGNGVGFFLRPYNFFDQEPSMDSADSIYFREGQDAG
AMO_ECOL.s       DAVVWMTTGTTHVARAEEWP--IMPTEWVHTLLKPWNFFDETPTLGA-----LKKDK---
AMO_HANS.S       DILFFHTFGITHFPAPEDFP--LMPAEPITLMLRPRHFFTENPGLDIQPSYAMTTSEAK-
AO_ANTHR.s       DLVVWYTFGMHHVVRLEDWP--VMPRQNIGFMLEPHGFFNQNPTLN-------LPTS---
AO_KAERO.s       DDVVWITTGTTHVARAEEWP--IMPTEWALALLKPWNFFDETPTLGE-----KKK-----
AO_PISUM.s       DIVMWHVVGIHHVPAQEDFP--IMPLLSTSFELRPTNFFERNPVLKT-----LSPRD---
AO_LENSC.s       ------------------------------------------------------------


ABP_HUMN.s   721 NGPNYVQRWIPEDRDCSMPPPFSYNGTYRPV
ABP_HUKI.s       -------PWHPETL----------------
ABP_RATC.s       KGLNRVQRWIPEDRRCLVSPPFSYNGTYKPV
AO_BOVIN.s       SCEINPLACLPQAATCAPDLPVFSHGGYPEY
AMO_ECOL.s       --------------------------------
AMO_HANS.S       ----RAVHKETKDKTSRLAFEGSCCGK----
AO_ANTHR.s       ----TSTTQTGEADTCCHTDK----------
AO_KAERO.s       --------------------------------
AO_PISUM.s       ------VAWPGCSN-----------------
AO_LENSC.s       --------------------------------
```

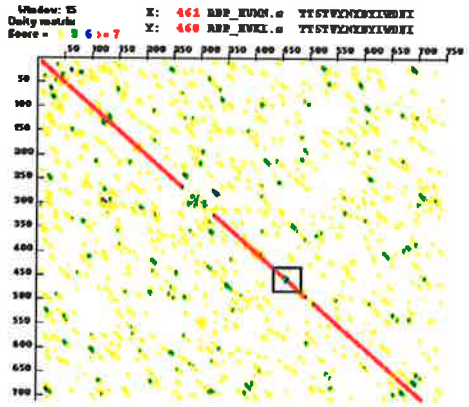
Appendix F. Amine Oxidase Sequence Comparison Dotplots

Figure F.1 Amine Oxidase Sequence Comparison Dotplots.
ABP_DAO-Human (placental) (di)amine oxidase [757] [EMBL 78212]; ABP_DAO-Human kidney [781] [M55602]; ABP_DAO-rat colon/lung [982] [X73911]; AO-bovine serum/liver copper amine oxidase [411] [S69583]; AMO-*E. coli* amine oxidase [529] [L47571]; AO-*Hansenula polymorpha* [566] [X15111]; AO-*Anthrobactr* methylamine oxidase (maoxII) [516] [L12990]; AO-*K. aerogenes* [1092] [D10208]; AO-Pea seedling [649] [L39931]; AO-Lentil seedling [617] [X64201].

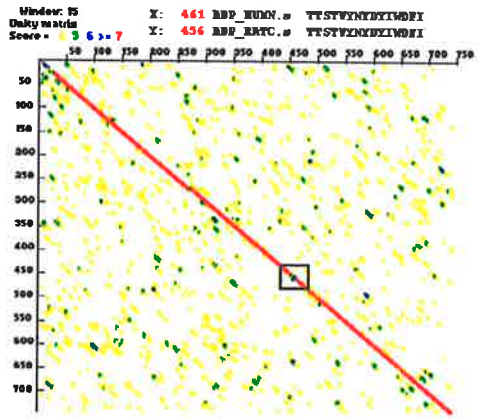
If the similarity score was greater than a predefined threshold score then a point was plotted at the middle of both segments indicating that the similarity is significant. A colour coding system was used to indicate similarities:

Yellow:	Threshold
Green:	Threshold + 1
Blue:	Threshold + 2
Red:	Threshold + 3

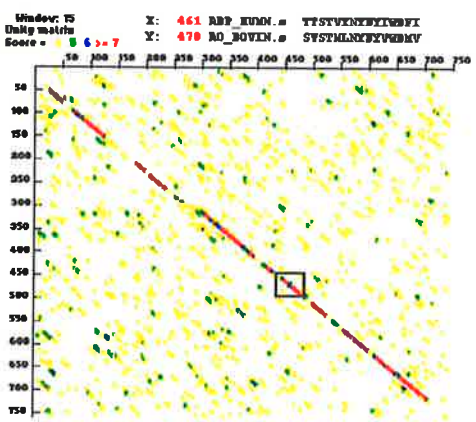
A cursor window of 15 residues was placed at the TOPA consensus site (NYD/E) and highlights the conservation of all sequences about this site. The sequence comparison dotplots were generated using ANTHEPROT software for MS-Windows, release 1.0 by G. Deleage from the Institut de Biologie et Chimie des Proteins, Lyon available from <ibcp.fr/pub/ANTHEPROT/windows/ anthepro.exe + prosite.exe + swiss.exe> by anonymous ftp.



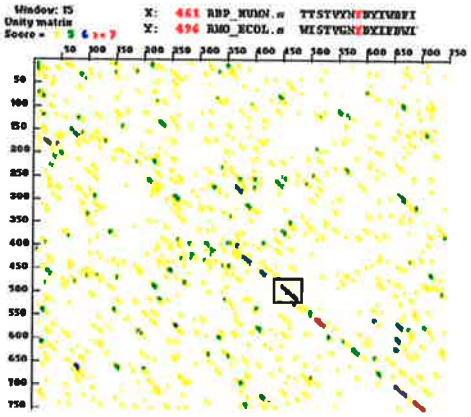
A. ABP_DAO-Human/ABP_DAO-Human kidney



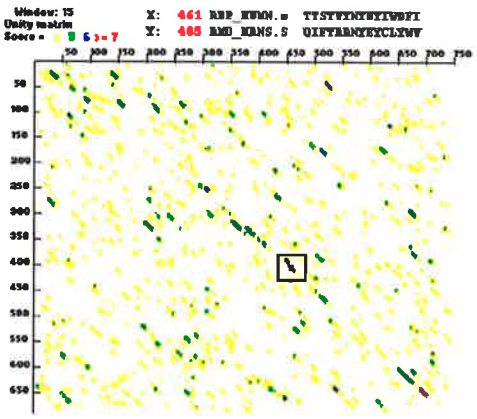
B. ABP_DAO-Human/ABP_DAO-rat colon



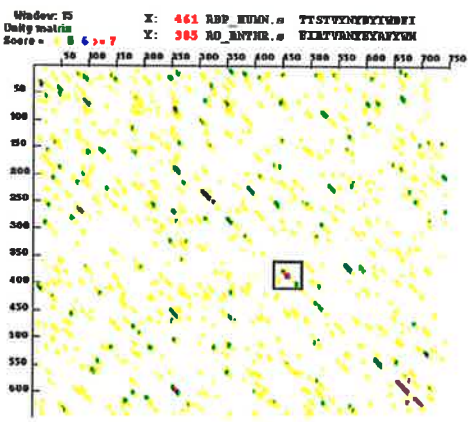
C. ABP_DAO-Human/AO-bovine serum



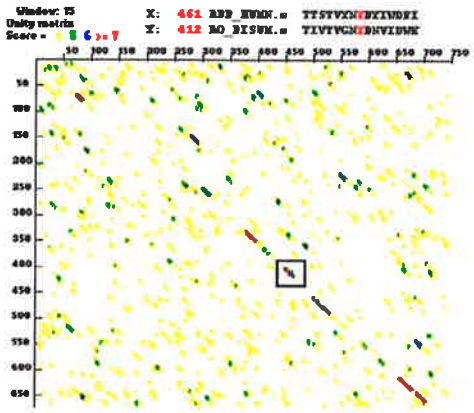
D. ABP_DAO-human/AMO-*E. coli*



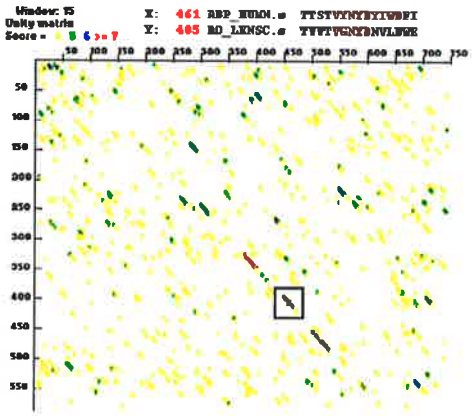
E. ABP_DAO-Human/AO-*Hansenula*



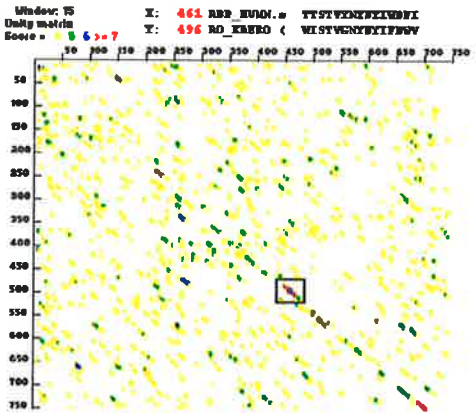
F. ABP_DAO-Human/AO-*Anthrobactr*



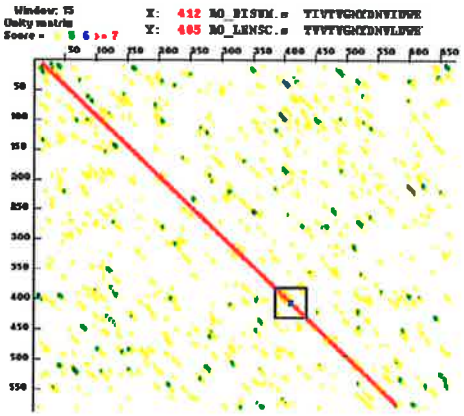
G. ABP_DAO-Human/AO-Pea seedling



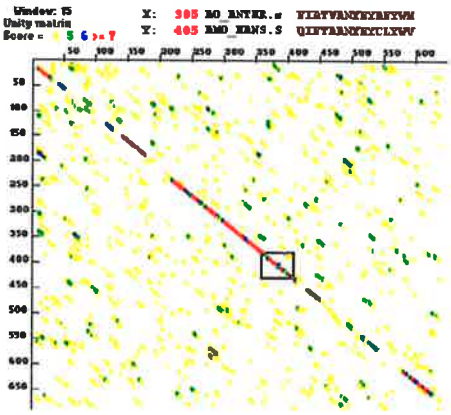
H. ABP_DAO-Human/AO-Lentil seedling



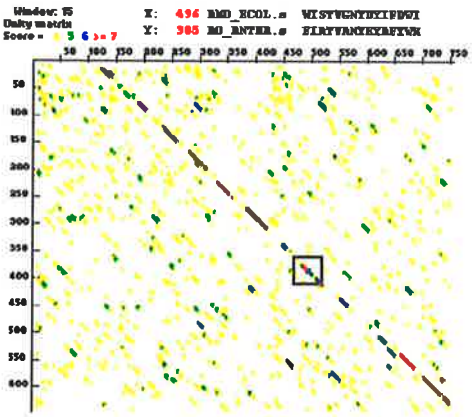
I. ABP_DAO-Human/AO-*K. aerogenes*



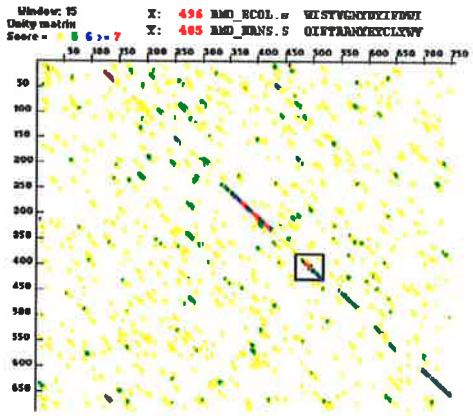
J. AO-Pea seedling/AO-Lentil seedling



K. AO-*Anthrobactr*/AO-*Hansenula*



L. AO-*E. coli*/AO-*Anthrobactr*



M. AO-*E. coli*/AO-*Hansenula*

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Human Retroplacental Serum Polyamine Oxidase: Purification and Characterization

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SYNOPSIS

Polyamine oxidases catalyse the oxidation of polyamines to aminoaldehydes. The enzymes have a wide range of physiological and pathophysiological functions. Plasma polyamine oxidase activity increases during human pregnancy and is especially high in retroplacental blood.

Limitations to our understanding of the polyamine oxidases have included a lack of their definitive characterization. Neither monoclonal antibodies nor a sequence for the human retroplacental serum polyamine oxidase have previously been available. Here we describe the preparative immunoaffinity purification of the human retroplacental serum enzyme using novel monoclonal antibodies. The immunoaffinity purified enzyme was identified as a copper-containing amine oxidase by *N*-terminal sequence analysis. Substrate selectivity of the enzyme was: histamine > putrescine > N^1-acetylspermine > spermidine > spermine.

The enzyme was sensitive to carbonyl group reagents but not monoamine oxidase inhibitors. Metabolic studies indicated that the enzyme acts by cleaving its

polyamine substrates at secondary amino groups in an EC 1.5.3 manner, suggesting a broader classification for the enzyme than the current EC 1.4.3.6 classification of copper-containing amine oxidases.

The enzyme was found to exist as two multiple forms that are composed of homodimers of M_r 108,000 glycoprotein subunits. Deglycosylation of the enzyme reduced the apparent M_r of the enzyme subunit to 86,000, consistent with the calculated molecular mass of the mature enzyme subunit polypeptide. One copper atom per subunit was indicated by atomic absorption spectroscopy. Sequence similarities with other amine oxidases suggested that the copper atom is at the active site of the enzyme along with 2,4,5-trihydroxyphenylalanine quinone.

INTRODUCTION

Polyamine oxidases catalyse the oxidative deamination of histamine, diamines, polyamines and their acetylated derivatives according to the following scheme:

$$R'CH_2NHR'' + H_2O \rightarrow R'CHO + H_2NR'' + H_2O_2$$

Oxidation of polyamines may take place at either the primary or secondary amino groups. In the first case the enzyme would be classified as an EC 1.4.3 type amine:oxygen oxidoreductase deaminating the CH—NH_2 group of donors, in the second case the enzyme would be classified as an EC 1.5.3 type amine:oxidoreductase deaminating the CH—NH group of donors [1].

Amine oxidases have attracted a wide range of interest because of their ubiquity and their role in a broad spectrum of physiological and pathophysiological functions including the regulation of polyamine levels, detoxification of amines, cancer, allergy, hepatitis, and central nervous system function [2]. Moreover, the aminoaldehyde

products of polyamine oxidation may have antimicrobial, anti-inflammatory, antiproliferative and immunomodulatory effects [3,4]. They have also been shown to cause vascular endothelial cell damage [5]. Human immunodeficiency virus type I (HIV-I) is rapidly inactivated by exposure to polyamines spermine and spermidine in the presence of polyamine oxidase and myeloperoxidase [6]. Hydrogen peroxide generated by amine oxidase may have a role in signal transduction, apoptosis and non-specific immunity [5,7-9].

Polyamine oxidases have been localized to placenta, liver, kidney, small intestine, macrophages and neutrophils [2,10]. Plasma polyamine oxidase activity is high in ruminants [2], is increased after parenteral heparin administration [11], and is elevated during pregnancy [12,13]. Retroplacental blood, which is composed mainly of intervillous blood with an admixture of placental and decidual interstitial fluid, contains a particularly high level of polyamine oxidase activity [14].

The retroplacental serum enzyme has been partially purified and characterized by Morgan [15,16]. Although it was not clear which type of amine oxidase was present in human retroplacental serum, the enzyme isolated by Morgan appeared to exist as multiple forms and resembled the FAD-containing Fe^{2+} dependant rat liver polyamine oxidase, EC 1.5.3.11 [17]. However, the enzyme also had many features in common with the copper- and TPQ- containing amine oxidases currently classified as EC 1.4.3.6 and known by their trivial names diamine oxidase and histaminase [18].

The nomenclature of the pregnancy-associated amine oxidases is confusing because of the possibility of different modes of action and their broad substrate specificity. Many reports have been made of the purification of pregnancy associated amine oxidases [15,16,19-35]. The starting materials used in these purifications

included placental homogenates and extracts, pregnancy sera and amniotic fluid, which have specific activities more than an order of magnitude less than that of retroplacental serum [14]. The placental enzyme appears to be identical to the human gene enzyme that has been characterized as copper- and TPQ- containing [35]. cDNA sequences for variant forms of the enzyme derived from the human placental cDNA library have been reported [36,37].

Here we report the purification of the polyamine oxidase from human retroplacental serum to a high degree of purity using immunoaffinity techniques based on novel monoclonal antibodies to the enzyme. The results of a detailed characterization including *N*-terminal sequencing identified the enzyme as a homodimer of glycosylated subunits with an apparent M_r 108000 existing as multiple forms in a manner similar to other copper- and TPQ- containing enzymes.

EXPERIMENTAL

Protein Assays

Protein concentrations were assayed using Peterson's [38] modification of Lowry's method, Smith's bicinchoninic acid (Pierce, Rockford, IL) based method [39], an adaptation [38] of Viet's *o*-phthalaldehyde method, or by A_{205} and/or A_{280} [40]. Where necessary proteins were first purified by precipitation with sodium deoxycholate (Aldrich, Milwaukee, WI) and trichloroacetic acid (BDH, Poole, UK), which was also useful for concentrating dilute protein solutions [38].

Amine oxidase assays

In general, amine oxidase activity was measured using a fluorometric method [41], based on that first described by Guilbault *et al.* [42] and Snyder and Hendley [43], in

which hydrogen peroxide formed in the enzymatic amine oxidation is measured by coupling it to the oxidation of homovanillic acid (Sigma Chemical Co., St Louis, MO) which dimerizes to a fluorogenic compound in the presence of horseradish peroxidase (EC 1.11.1.7) (Sigma, Type II). Hydrogen peroxide (BDH, AnalaR) standards were determined by ε_{230} 62.4 $M^{-1}.cm^{-1}$ after titration of stock solutions with ceric(IV) sulphate solution (BDH) that had been standardized with anhydrous arsenious oxide (BDH) [44]. The purest substrates commercially available were used (Sigma; Calbiochem-Novabiochem, San Diego, CA). The purity of the polyamine substrates was examined by reversed-phase HPLC of dansylated derivatives [45] and was found to conform to the manufacturer's specifications.

Where appropriate polyamine oxidase activity was measured using a radiochemical method [4] based on the oxidation the oxidation of [^{14}C]spermine (Amersham, Bucks., UK) and the separation of reaction products on Dowex 50W (Bio-Rad, Richmond, CA) by stepwise elution with hydrochloric acid (BDH, AnalaR) before liquid scintillation counting in Ready-Solv EP scintillation cocktail (Beckman, Fullerton, CA). Separation of the substrate and reaction products was confirmed by dual channel liquid scintillation counting in an LS 3801 spectrometer (Beckman) using [^{3}H]spermidine (du Pont de Nemours & Co., Boston, MA) and [^{14}C]spermine. Diamine oxidase activity was assayed radiometrically using a modification of the method first reported by Okuyama and Kobayashi [46] using [^{14}C]putrescine as a substrate [47].

Retroplacental serum

Retroplacental blood was collected from human placentae after both caesarean section and vaginal deliveries at around 38-40 weeks gestation as described by Klopper and Hughes [48]. Placental membranes were inverted over the maternal decidual surface of

the placentae and retroplacental blood, which is composed mostly of intervillous blood with admixtures of decidual and placental interstitial fluid from the placental–endometrial interface, collects at the placental margin and between the cotyledons on the placental surface. The retroplacental blood was syringed up and placed in serum collection tubes. After the blood clotted, serum was separated by centrifugation and stored at –70 °C. Placentae were usually obtained within 20 min *post partum*.

Chromatographic procedures

Low pressure chromatography. Chromatography was conducted at 4 °C using a 2120 Varioperpex II pump interfaced with a 2070 UltroRac fraction collector (LKB, Bromma, Sweden). All buffers were filtered through 0.45 μm membrane filters (Millipore, Bedford, MA) under reduced pressure. Protein concentration in column eluates was measured by A_{280} in an SP8-100 UV–visible spectrophotometer (PYE Unicam, Cambridge, UK) using matched quartz cuvettes (Starna, Essex, UK) with a 10 mm path length. Unless stated otherwise enzyme activity in column fractions was determined fluorometrically using putrescine as a substrate. Salt gradients were measured by chloridimetry or osmolality using a CMT Chloride Titrator (Radiometer, Copenhagen, Denmark) or Digital Micro-Osmometer (Roebling, Germany).

High pressure liquid chromatography. HPLC was conducted at ambient room temperature. Instrumentation consisted of a Model 344 HPLC system (Beckman Instruments) equipped with 144M pumps controlled by a 421 programmable digital computer. Column eluate was monitored at $A_{280 \text{ or } 205}$ using a Beckman 163 variable wavelength detector fitted with a standard flow cell. Fluorescence detection was with an LS-50 Luminescence Spectrophotometer (Perkin–Elmer, Beaconsfield, UK) fitted with a 1.3 mm flow cell. Size-exclusion HPLC buffer was 100 mM sodium phosphate

containing 0.3 M NaCl, pH 6.80 and was prepared from stock solutions that had been purified by sequential chromatography through AG-1-X8, Chelex 100 resin (Bio-Rad) and a C_{18} SepPak cartridge (Millipore, Bedford, MA) to remove impurities that might interfere with spectrophotometric protein detection and contaminate the enzyme preparation. The buffer was filtered through a 0.45 μm membrane (Millipore) under reduced pressure and dissolved air removed by helium sparging.

Electrophoresis

Protein samples were analysed in polyacrylamide slab gels using a method based on the SDS-PAGE technique using the discontinuous buffer system described by Laemmli [49]. Gels were assembled in Protean II apparatus (Bio-Rad) essentially as recommended by the manufacturer. Proteins were detected by staining with Coomassie Brilliant Blue R (CI 42660; Sigma) [50], by a high sensitivity Coomassie Brilliant Blue G (CI 42655; Sigma) staining procedure adapted from that of Neuhoff *et al.* [51], or by silver staining using a Bio-Rad kit based on the method of Gottlieb and Chavako [52].

Purification of polyamine oxidase for monoclonal antibody production

Retroplacental serum samples with relatively high specific activity were pooled (100 ml) and applied to a column of Sephadex G-50 (Pharmacia, Uppsala, Sweden) (95 cm × 5 cm i.d.) equilibrated with 25 mM Tris–HCl containing 30 mM NaCl, pH 8 and eluted with the same buffer. Polyamine oxidase activity eluted just after the void volume and was loaded onto a column of Blue Sepharose CL-6B (Pharmacia) (45 cm × 5 cm i.d.) that had been equilibrated in the Tris–Saline buffer containing 10 mM Ca^{2+}. The column was eluted with the Ca^{2+} containing buffer and when A_{280} indicated that most of the unbound protein had eluted, enzyme activity was desorbed with a 600 ml linear NaCl gradient (0.03 – 1 M) and concomitant reverse Ca^{2+} gradient (10 – 0 mM) in 25 mM

Tris–HCl, pH 8. Following further elution with limit buffer until the enzyme activity had been desorbed, 0.5 M NaSCN in 25 mM Tris–HCl, containing 20 mM EDTA, pH 8.0 was used to remove more tenaciously bound proteins and regenerate the column. Active fractions from the Blue Sepharose chromatography were pooled and concentrated by pressure dialysis over a YM30 membrane with 25 mM Tris–HCl, pH 8 (Amicon, Danvers, MA). Subsequently, the enzyme sample was applied to a column of DEAE Trisacryl M (Reactifs IBF, Villeneuve-la-Garenne, France). After elution with the Tris–Saline buffer a 500 ml linear NaCl gradient (0.03 – 0.3 M) desorbed the enzyme activity in two separate peaks, designated PAO I and PAO II. PAO I eluted first from the anion exchange column under the salt gradient, and PAO II, representing 90% of the activity eluted next. The two forms derived from the anion exchange chromatography were individually processed on ω-aminoalkylagarose affinity columns arranged in tandem with preceding alkylagarose columns. Pooled fractions containing PAO I were applied to a column of ω-aminobutylagarose (Sigma, 6 cm × 1.6 cm i.d.), arranged in tandem with a butylagarose column (Sigma, 1 cm × 1.6 cm i.d.). Pooled fractions containing PAO II were applied to a column of ω-aminohexylagarose (Sigma) (7 cm × 1.6 cm i.d.), arranged in tandem with a pentylagarose column (Sigma) (1 cm × 1.6 cm i.d.). The columns were eluted with 25 mM Tris–HCl containing 0.05-0.5 M NaCl, pH 8, and enzyme activity was desorbed with 1.5 M NaSCN in 50 mM Tris–HCl, pH 8. Fractions containing enzyme activity were pooled as PAO I and PAO II and concentrated over YM30 membranes (Centricon 30s, Amicon). The concentrates were injected onto TSK (4000 + 3000) sw size-exclusion columns (Bio-Rad) ((300 mm + 300 mm) × 7.5 mm i.d.)) connected in tandem, preceded by a TSK guard column (75 mm × 7.5 mm i.d.) and eluted with SE-HPLC buffer at 0.8 ml/min. Active fractions were pooled and

recycled on the TSK columns. The enzyme preparations were analysed by SDS-PAGE and used for the preparation of monoclonal antibodies.

Monoclonal antibody production

Purified retroplacental serum polyamine oxidase was used to immunize mice. Spleen cell–myeloma hybridomas were produced and screened for the production of antibodies to the enzyme. Specific antibody producing hybridomas were cloned, monoclonal antibodies were produced in tissue culture and specifically purified using anti-mouse IgG immunoaffinity columns. Purified monoclonal antibodies were used for the construction of immunoaffinity columns for the preparative scale purification of retroplacental polyamine oxidases.

Eight- to nine-week-old BALB/c mice were immunized subcutaneously with 15 μg of either PAO I or PAO II emulsified in Freund's complete adjuvant (Difco, Detroit, MI). A intraperitoneal boosting immunization of 50 μg purified PAO I or PAO II in Freund's incomplete adjuvant (Sigma). Seven days after the boosting immunization a blood sample was taken from each mouse. Sera were screened for antibodies to the two enzyme forms using a single-site direct ELISA. Sera from mice immunized with PAO I was observed to crossreact with PAO II and vice versa. Since PAO II was the most abundant form of the enzyme, a mouse with a high titre of antibodies to PAO II was selected for hybridoma production and immunized intraperitoneal injection of 86 μg purified PAO II in saline four days before spleen cell harvesting. Spleen cells were hybridised with P3-x63-Ag8.653 myeloma cells using methods based on the protocols described by Zola [53]. Hybridomas were selected in complete RPMI 1640 medium (Flow Laboratories, Irvine, UK) containing 20% FBS (Flow), hypoxanthine, aminopterin and thymidine (Sigma), cloned by limiting dilution, and screened for

production of antibodies to polyamine oxidase using a single-site direct ELISA and an enzyme capture immunosorbent assay. Clones producing desired antibodies were expanded into bulk culture and supernatants were harvested by centrifugation. Antibodies were specifically purified from culture supernatants on affinity columns prepared from affinity purified anti-mouse antibodies (Silenus, Hawthorn, Australia) and Affi-Prep 10 (Bio-Rad,). Affinity purified mAbs, pao-2.21 (>98% pure), were bound to 8 ml of Affi-Gel 10 at 9.86 mg/ml gel according to the manufacturer's protocols for subsequent use in the immunoaffinity purification of polyamine oxidase.

Single-site indirect ELISA for anti-amine oxidase mAbs

Alternate columns of Immunlon IV microtitre plate wells (Dynatech, Chantilly, VA) were coated with 250 ng purified polyamine oxidase antigen in 50 µl, 15 mM sodium carbonate/35 mM sodium bicarbonate buffer, pH 9.6, per well. Remaining columns of control wells were coated with 250 ng BSA (CSL, Melbourne, Australia) in 50 µl of the same buffer. Plates were incubated for 5–16 h at 4 °C. After washing wells with PBS–T, remaining binding sites in the wells were blocked with PBS–T containing 10% (v/v) sheep serum (blocking buffer) for 3 h at 37 °C. Wells were washed with PBS–T and undiluted hybridoma supernatants were incubated in wells coated with antigen and in the BSA coated wells (50 µl/well) for 5–16 h at 4 °C. After washing with PBS–T, bound monoclonal antibodies were detected using an immunoaffinity purified species specific, goat anti-mouse immunoglobulin.β-galactosidase linked whole antibody conjugate (Amersham) and its substrate *o*-nitrophenol-β-D-galactopyranoside (Sigma). Absorbances at λ_{405} were quantitated on a Titertek Multiskan II microplate reading spectrophotometer (Flow Laboratories).

Enzyme capture immunosorbent assay

Alternate columns of wells in Immulon IV microtitre plates were coated with 500 ng affinity purified sheep anti-mouse immunoglobulin (Silenus) in 50 µl 50 mM carbonate/bicarbonate buffer, pH 9.6. Wells in the remaining columns were coated with 500 ng BSA in 50 µl of the same buffer as controls. Plates were incubated for 5–16 h at 4 °C. Wells were washed with PBS–T and remaining sites in the wells were blocked with PBS–T containing 10% (v/v) donor sheep serum for 3 h at 37 °C. Subsequently hybridoma supernatants were added (50 µl/well). Wells were washed with PBS–T and a solution of PAO from the anion exchange purification step (see above) was added (50 µl/well). The moderate salt concentration in the ion exchange fraction (approx. 0.3M) suppresses nonspecific adsorption of the enzyme. After incubation for 10–16 h at 4 °C wells were washed with PBS–T, and *in situ* enzyme activity detected by reaction with 1 mM ABTS (Boehringer–Mannheim, Germany), 40 IU/ml horseradish peroxidase (EC 1.11.1.7) and 100 µM putrescine (spermine or spermidine) in 0.05 M Tris–HCl, pH 7.4, at 37 °C (100 µl/well). Absorbances at λ_{415} were quantified using a Titertek Multiskan II microplate reading spectrophotometer.

Immunoaffinity purification of polyamine oxidase

Polyamine oxidase was purified from pooled retroplacental serum samples. Retroplacental serum was diluted 1 : 1 in 25 mM Tris–HCl containing 150 mM NaCl, pH 8 and centrifuged at 20,000 × *g*. Supernatant was filtered through a Millipak 40/60 filter to 0.22 µm (Millipore). The filtrate was applied at to an ω-aminohexylagarose affinity column (21 cm × 2.6 cm i.d.) which was preceded by a pentylagarose column (7 cm × 2.6 cm i.d.). The columns had been equilibrated in the Tris–Saline buffer and eluted with the same buffer. PAO activity was desorbed from the ω-aminohexylagarose column with 1.5 M NaSCN in 25 mM Tris–HCl, pH 8. Fractions containing PAO

activity were pooled and concentrated over a YM 30 membrane. Thiocyanate was removed from the sample by gel filtration at 50 ml/h on Sephadex S-300 (45 cm × 2.6 cm i.d.) equilibrated in a low ionic strength Tris–saline buffer (25 mM Tris–HCl containing 30 mM NaCl, pH 8) in preparation for ion exchange chromatography. The multiple enzyme forms, PAO I and PAO II, were resolved by ion exchange chromatography on DEAE-Trisacryl M equilibrated in the low ionic strength Tris–saline buffer. The column was eluted with the same buffer to remove unbound protein followed by a linear NaCl gradient, resolving the enzyme activity into two separate peaks (Figure 1). Fractions from the DEAE column containing PAO I were pooled and applied to the mAb pao-2.21—Affi-Gel 10 column arranged in tandem with a bovine γ-globulin—Affi-Gel 10 column which protects the immunoaffinity column and adsorbs nonspecifically binding proteins. PAO activity completely adsorbed to the immunoaffinity column which was then eluted with 25 mM Tris–HCl containing 250 mM NaCl, pH 8, followed by a more rigorous wash with 25 mM Tris–HCl containing 1 M NaCl, pH 8, to remove nonspecifically adsorbed protein. PAO I activity was desorbed from the immunoaffinity column with 1.5 M NaSCN (Figure 2A) and further purified by SE-HPLC on BioSil SEC 400 (Bio-Rad) (Figure 3A). PAO II was similarly purified (Figures. 2B, 3C and D). SE-HPLC fractions were analysed by SDS-PAGE under reducing conditions.

Deglycosylation

An immunoaffinity purified preparation of polyamine oxidase (20 μg, forms I and II combined) was denatured at 100 °C (10 min) in the presence of 0.5% (w/v) SDS and 1% (v/v) 2-mercaptoethanol. The denatured protein was treated with 2000 units (0.16 μg) of recombinant peptide:*N*-glycosidase F (EC 3.5.1.52; PNGase F, New England Biolabs,)

for one hour at 37 °C in 0.05 mM sodium phosphate buffer, pH 7.5 containing 1% NP-40 (v/v).

HPLC separation of polyamine oxidase reaction products

Products of enzymatic [^{14}C]spermine cleavage were identified by HPLC in a study of the retroplacental polyamine oxidase reaction. Reactions were in 0.05 M Tris–HCl, pH 7.4 [at 37 °C] with 50 μM [^{14}C]spermine (*N,N'*-bis-(3-aminopropyl)[1,4-^{14}C]-tetramethylene-1,4-diamine; specific activity 1.85 Gbq/mmol) as the substrate in a total volume of 1 ml. Reactions were initiated by adding approx 0.9 mU enzyme (determined using [^{14}C]putrescine as the substrate as described above) purified by affinity chromatography on ω-aminohexylagarose. Reaction mixtures were incubated at 37 °C and reactions terminated by adding 100 μl 72% (w/v) TCA. Precipitated protein was sedimented by centrifugation at 10,000 × *g* for 5 min (Eppendorf microcentrifuge). [^{14}C]spermine and its reaction products were separated by reversed phase, ion pairing HPLC on a C_{18} column (Sperisorb ODS-2, 150 mm × 4.6 mm i.d.; Phase Separations, Deeside, UK) with a gradient of methanol:water in 20 mM potassium hydrogen orthophosphate containing 20 mM sodium octane-1-sulphonate, pH 3. The gradient comprised: solvent B (methanol:water; 70:40) 75% to 100% over 10 min, held for 10 min at 100% B, returned to starting conditions over 10 min and equilibrated for at least 15 min. Solvent A was methanol:water; 40:60. Solvents were delivered at 1 ml/min. Aliquots of the reaction mixtures were diluted 1:4 in solvent A before injection (100 μl). The [^{14}C]polyamine standards mixture included 46 kBq [^{14}C]spermine, 27 kBq [^{14}C]spermidine and 1.9 kBq [^{14}C]putrescine per ml. Eluate was collected as 0.2 ml fractions, 4 ml Optiphase 'Hisafe' 3 scintillation fluid was added to each fraction and

the radioactivity determined by scintillation counting in an LS-3801 spectrometer (Beckman Instruments).

_N_-terminal amino acid sequencing

The *N*-(amino)terminal amino acid sequence of purified proteins was examined using a 475A automated protein sequencer (Applied Biosystems Instruments, Foster City, CA). Applied Biosystems operating procedures and programs for both the sequencer and the on-line PTH- amino acid analyser were used. FASTA [54] searches of the Swiss-Prot protein data bank [55] were made through fasta@ebi.ac.uk.

Sample preparation. Sample preparation was based on electrophoretic transfer of proteins to a chemically inert support. Proteins were purified by SDS-PAGE as described by Laemmli [49], with modifications as suggested by Moos *et al.* [56] to avoid *N*-terminal amino acid modification, alteration of amino acid side chains, cleavage of peptide chains and introduction of contaminants. Western blotting to Immobilon–P^{SQ} (or P) membranes (Millipore,) was performed according to a protocol modified from that of Towbin *et al.* [57] using Trans-Blott Cell apparatus (Bio-Rad) and 10 mM CAPS (Calbiochem, Ultrol) in 10% methanol, pH 11.0 as the transfer buffer. Transfers were conducted at 150 mA for 40 min followed by 200 mA for 2.5 h with cooling to 4 °C. The proteins were stained with Coomassie Brilliant Blue R [58]. Stained bands for sequencing were excised from the PVDF membrane and stored at –20 °C until sequencing.

Copper analysis

Copper content of proteins was determined by analysis in a 5100PC Zeeman thermal atomic absorption spectrophotometer (Perkin–Elmer) against a plasma trace elements

control (Utak Laboratories, Valencia, CA). Samples were filtered through a Chelex disk (Bio-Rad) to remove any loosely bound adventitious copper.

RESULTS AND DISCUSSION

The volumes of retroplacental serum obtained showed wide variation, 36 ± 22 ml per placenta, n = 292 (mean ± SD). Polyamine oxidase activity also showed wide variation 1.683 ± 0.868 U/l (n = 50) when assayed radiometrically using spermine as the substrate [47] and 12.48 ± 5.62 U/l (n = 250) when assayed fluorometrically using putrescine as the substrate [41]. These levels are in agreement with those found previously [14,16] and parallel the wide individual variation seen in pregnancy serum [12,13].

Purification

Purification of human RPS PAO yielded two forms of PAO activity. PAO II, the higher molecular form with higher affinity for DEAE chromatographic media at pH 8, had a relative abundance of approx. 90%. SDS-PAGE under reducing conditions indicated that the PAO II preparations contained a protein which ran as a diffuse band with a M_r 108000 as its major constituent. The PAO I preparations also contained the M_r 108000 band, however they also contained other protein bands with different relative abundances (not shown).

The resolution of retroplacental serum polyamine oxidase into multiple forms is in agreement with earlier findings [16] and studies of pregnancy-associated amine oxidases from other sources. Lin and Kirley first reported the resolution of human placental histaminase (diamine oxidase) activity into two components by chromatography on DEAE cellulose [25]. Tufvesson reported the separation of amniotic fluid diamine oxidase into two forms by chromatography on DEAE-Sephadex A50,

corresponding to multiple forms composed of M_r 110000 subunits [28] in agreement with the results presented here for the retroplacental enzyme. Multiple forms of the bovine plasma enzyme which can be separated by DEAE chromatography have also been observed [59].

Starting from 4.4 g protein with a specific activity of 0.96 mU/mg purifications of 770- and 916- fold were achieved in 4.5% and 42% yield for PAO I and PAO II respectively by the end of the ω-aminoalkylagarose affinity step. The final specific activities attained were higher than any previously reported for pregnancy-associated amine oxidases, although the degree of purification of purification was not as high as that reported by some groups. Since the degree of purification of an enzyme to a particular specific activity is dependent on the specific activity of the starting material, higher degrees of purification are obtained with starting materials of lower specific activity. It has been noted that greater purity and specific activity of pregnancy-associated amine oxidase preparations were obtained using a starting material with relatively high specific activity [30].

Preparative scale immunoaffinity purification of two polyamine oxidase forms from human retroplacental serum was achieved using monoclonal antibodies specifically purified from cell culture supernatants. Immunoaffinity purification of PAO I and PAO II using mAb pao-2.21—Affi-Gel 10 is summarized in Table 1. The resolution of two forms of polyamine oxidase by anion exchange chromatography is shown in Figure 1. Both forms of PAO adsorbed completely to the immunoaffinity column and were desorbed with the chaotrophic ion, thiocyanate (Figure 2), before further purification and analysis by SE-HPLC. From 20.8 g protein with a specific activity of 2 mU/mg purifications of 689- and 1412- fold were achieved in 1% and 18%

yield for PAO I and PAO II respectively by the end of the immunoaffinity step. SDS-PAGE indicated one major band with an apparent M_r of approx. 108000 in each preparation (Figure 3).

Proteins from the purification steps were examined by SDS-PAGE under reducing conditions (Figure 4). Enzyme activity correlated with a diffuse band with an apparent M_r 108215 ± 2844 (n = 11; ± SD) in gels containing 7.5% acrylamide. The *N*-terminal amino acid sequence of protein associated with this band in a PAO II preparation (Figures 3E and 4 *lanes 4,5,6*) was identical to that of human TPQ- and copper- containing amine oxidase [35] (EC 1.3.4.6; formerly known as amiloride binding protein; Swiss-Prot protein sequence data bank entry no. P19801) for at least 27 amino acids (PAO I preparation (Figures 3B and 4 *lane 7*) for at least 16 amino acids). The relative abundance of protein in the M_r 108000 band was enriched after immunoaffinity purification (Figure 4 *lane 2 cf. 6 and 8 cf. 7*) and depleted after immunoadsorbtion of enzyme activity (Figure 4 *lane 2 cf. 3 and 8 cf. 9*). Deglycosylation caused a shift in apparent M_r from 108000 to 86000 with a concomitant sharpening of the band (Figure 5). Glycosylation of the retroplacental enzyme is consistent with the glycosylation observed for other amine oxidases [2]. The diffuse nature of the M_r 108000 band correlated with enzyme activity appears to be related to its glycosylation. The relative molecular mass of the deglycosylated enzyme polypeptide subunit, M_r 86000, is in general agreement with the minimum values reported for the human pregnancy-associated amine oxidase subunit (see below) and in close agreement with the calculated molecular mass of the mature peptide subunit of the human gene diamine oxidase (EMBL entry no. X78212; 83,415 Da) [35,60]. Slight variations cDNA sequence have observed for the human pregnancy-associated enzyme subunit [36,37].

The relative molecular mass of the enzyme subunit falls within the range of expected molecular weight values for copper-containing amine oxidases (60000 – 110000). In contrast, the FAD-containing polyamine oxidases, such as the rat liver enzyme and the polyamine oxidases of the Gramineae (e.g. maize and oats) are monomeric and have a molecular weight range 53000 – 85000.

Glycosylation is well known to cause anomalous behaviour of glycoproteins on SDS-PAGE in gels of different concentrations [61]. Indeed, Lin *et al.* observed a decrease in the apparent M_r of the placental enzyme from 110000 to 91000 with increasing acrylamide concentration [30]. The anomalously high M_r observed and the variation in the reported values for the pregnancy-associated subunit molecular weight can be attributed to the change in the subunit's apparent relative molecular mass in gel of different acrylamide concentrations. The assignment of slightly different M_rs to marker proteins contributes to the variation in reported subunit molecular weight.

Other bands, with M_rs 218000, 79000, 74000, 68000, 64000 and 53000 were also observed in our preparations. The M_r 218000 band (219824 ± 12845 (n = 17; ± SD)) vanished after deglycosylation (Figure 5) and remained under nonreducing conditions (Figure 6). Proteins associated with this band had an *N*-terminal amino acid sequence that was identical to that of the M_r 108000 band for at least 10 residues. This suggests that the M_rs 218000 species is a homodimer of the M_r 108000 subunit held together by disulphide bonds. The faint band at M_r 53000 was a trace of mouse IgG heavy chain, identified by an *N*-terminal stretch of 25 amino acids. The band at M_r 79000 in PAO II preparations (Figure 4 *lanes 4,5,6*) was also seen in immunoaffinity column eluates from which all enzyme activity had been adsorbed (Figure 4 *lane 3*). The faint bands at M_r 74000, 68000, and 64000 were not present or appeared in variable

amounts in other immunoaffinity preparations (not shown). Similar bands have been observed in preparations of the pig kidney amine oxidase (ABP); tryptic digests indicated that they were likely to be degradation products of the enzyme subunit, indeed their relative abundance was observed to increase on storage [62]. On the other hand, the faint protein bands seen in the retroplacental serum enzyme preparations may have been proteins strongly adherent to the polyamine oxidase, nonenzyme proteins bearing a determinant recognized by the mAb, or proteins with a strong nonspecific binding to the immunoaffinity column. Under nonreducing conditions a high molecular weight species is observed in addition to the M_r 218000 band (Figure 6); this species is possibly a tetrameric form of the M_r 108000 subunit.

Immunoaffinity purified PAO I activity eluted from the SE-HPLC columns with an apparent M_r 336000 (Figure 3A). The protein in the fractions correlating with enzyme activity ran as a diffuse band M_r 108000 after SDS-PAGE (Figure 3B). PAO I may represent the homodimer. This is consistent with the existence of most copper- and TPQ- containing amine oxidases as homodimers [2]. PAO II activity eluted with an apparent M_r 644000 as a shoulder on the major protein peak (Figure 3C). The peak of protein concentration (fraction 10) from the first pass through the BioSil SEC 400 column (Figure 3C) had almost identical to the SDS-PAGE profile (Figure 3E *lane [10]*) of the fractions containing the highest activity on the second pass suggesting that the major protein peak with relatively large apparent molecular weight was comprised of inactive aggregates of forms containing the M_r 108000 subunit. Protein in fractions correlating with enzyme activity ran as a diffuse band M_r 108000. PAO II may represent a tetrameric form of the enzyme subunit. The anomalously high apparent relative molecular masses of the PAO forms indicated by size exclusion HPLC is probably due,

in part, to their large hydrodynamic radii as a consequence of the increased hydration of the glycoprotein carbohydrate chains compared to the smaller hydrodynamic radii of similarly sized polypeptide chains alone [63]. Moreover, if the enzyme shape is not spherical, as suggested by X-ray crystallographic studies of other amine oxidases [64,65], this would also contribute to the appearance of anomalously high molecular weight on size exclusion HPLC analysis.

The purification process inactivates a proportion of the enzyme resulting in a reduced active site purity. The activity losses are indicated in Table 1. Formation of protein aggregates is suggested by the SDS-PAGE data. Protein aggregates were observed at the interface of the stacking and resolving gels (Figures 3,4 and 5), even under reducing conditions which decreased their relative abundance (Figure 6 *lane a cf. c*). The formation of enzyme aggregates is in agreement with the observation of aggregate formation by other amine oxidases. In particular, the bovine plasma enzyme appeared to self-associate and this was favoured by time and high protein concentration [66]; conditions such as would be encountered during the purification of the retroplacental serum enzymes. The porcine kidney diamine oxidase was also found to be subject to association, particularly in anoxic media (such as encountered here during chromatography and electrophoresis), and a tetrameric form has been observed [67]. In SDS-PAGE studies of the porcine kidney enzyme (ABP), aggregation to larger forms, especially on storage, was noted [68], and protein aggregates were observed at the interface of stacking and resolving gels [62]. The aggregates may be equivalent to the large form of human placental diamine oxidase observed by electron microscopy [69].

The high apparent molecular weights of the retroplacental enzyme observed in this study after size-exclusion chromatography are in contrast to an earlier report of a

narrow peak of retroplacental polyamine oxidase activity corresponding to M_r 67000 after gel filtration [15]. On the other hand, a previous report indicated the association of retroplacental polyamine oxidase activity with molecules with a relative molecular mass > 150000 after gel filtration. The apparent appearance of a M_r 67000 species in earlier gel filtration experiments [15] is curious and may be a result of including a reducing agent in the chromatography buffer causing the enzyme to dissociate into its subunits, and thus coelute with serum albumin (see below). Serum albumin was probably the major protein (reported as M_r 67000; 80%) seen after SDS-PAGE analysis, the amine oxidase may have been represented in one of the three minor bands observed. Estimation of M_r by gel filtration is notoriously imprecise and probably led to the assumption of the enzyme being associated with the M_r 67000 band. An amine oxidase subunit with an apparent molecular weight of around 90000 – 110000 would elute in approximately the same volume on Sephacryl S-200 media. The high molecular weight forms of placental amine oxidase have been reported by Paolucci *et al.* who observed four active molecular forms of the enzyme after chromatography on BioGel-A5m. These appeared to be multiples of a 125000 ± 5000 molecular weight subunit [20]. Smith too, observed that histaminase from human placentae eluted in the first peak (void volume) from a Sephadex G-200 column suggesting a relative molecular mass greater than 200000, though the molecular weight was not characterised [19]. This was also observed by Baylin and Margolis [23]. Lin and Kirley reported a M_r 195000 species after examining oligomeric placental histaminase using PAGE, and a subunit M_r 95000 after SDS-PAGE under reducing conditions [25]. Similarly, Bardsley *et al.* demonstrated a M_r 235000 species of placental diamine oxidase by native sedimentation–equilibrium ultracentrifugation [24], which appeared to be comprised of M_r 90000 subunits when

examined by SDS-PAGE [22]. The M_r 70000 protein they observed was most likely a contaminant, probably albumin, which has been observed by others in their preparations [15,23,27,28]. Under dissociating conditions they observed an 82000 molecular weight subunit by sedimentation–equilibrium ultracentrifugation, which is closer to the molecular weight reported here for retroplacental polyamine oxidases (M_r 108000) and that reported by others for pregnancy-associated amine oxidases using SDS-PAGE under reducing conditions, viz. 90000 [23,34], 100000 [27], 110000 [28], 90000–110000 [30], 84,000 [33], 95000 [25] and 105000 [35]. Tufvesson observed multiple high molecular weight forms in amniotic fluid [28], 'DAO A' eluting first from a DEAE column with a M_r 245000 and a major form eluting later from a DEAE column with a M_r 485000. These findings are very similar to the findings presented here for the H2O serum enzymes. It has been suggested that high molecular weight multiple forms are a result of concentration-dependent aggregation [24]. The molecular weights of the porcine kidney and bovine serum amine oxidases remained controversial for some time because of their tendency to show association–dissociation phenomena [66,67,70]. The molecular weight of the proteins associated with the enzyme activity did not correspond to that of ceruloplasmin [71] or lysyl oxidase [72], with which their amine oxidase activity might be confused [73-75].

UV-visible absorption spectrum

Concentrated immunoaffinity purified enzyme preparations were a pink-orange colour. The absorption spectrum showed the characteristic absorption peak of proteins at 280 nm and a broad less intense peak around 450–500 nm. The purified retroplacental amine oxidase lacked the distinctive intense sky-blue colour that is observed when ceruloplasmin is purified [71], nor were preparations yellow like those of typical

flavoproteins [76]. Like the rat liver polyamine oxidase, which is thought to contain flavin adenine dinucleotide as a cofactor [17], the purified retroplacental serum polyamine oxidase did not show the typical three-banded spectrum for flavoproteins [76]. Purified preparations of copper- and TPQ- containing amine oxidases are a pink colour because of a broad absorption maximum around 470 – 480 nm in the visible spectrum. TPQ, rather than copper, is the chromophore that gives rise to this absorption band [2].

Copper content

Thermal atomic absorption spectroscopy indicated 2.09 copper atoms per M_r 218000. The finding of one copper atom per subunit is consistent with data for other copper-containing amine oxidases [2]. The UV–visible spectral data suggest that the retroplacental enzyme is a type-II copper protein[1]. Conserved histidine residues in the primary sequence of copper-containing amine oxidases act as ligands to copper in other amine oxidases [64,65]. X-ray crystallographic analyses have revealed the presence of copper at the active sites of the *E. coli* [64] and pea seeding [65] amine oxidases, where it is proposed to have a bifunctional role in the generation of the functional quinone cofactor and in the catalytic mechanism. The presence of copper in pregnancy-associated enzymes has been suggested by earlier studies. Crabbe *et al.* identified copper by EPR spectroscopy in a partially purified placental diamine oxidase preparation. Copper stoichiometry was suggested to be 0.7 g-atom per M_r 70000 subunit by EPR and was found to be 1.0 g-atom per M_r 70000 by atomic absorption spectroscopy [24]. Retroplacental serum polyamine oxidase has been reported to contain 0.09 g-atoms of copper per M_r 67000, however this preparation was likely to be less than 10% pure [15]. EPR spectroscopy of partially purified enzyme preparations by Crabbe *et al.* revealed

the presence of iron which was thought to arise from haem-containing protein contaminants [24]. These contaminants may have also been the source of iron previously identified in retroplacental serum polyamine oxidase preparations [15]. Consistent with findings by Paolucci [20] and Crabbe *et al.* [24] that the placental enzyme contains Mn^{II} in addition to Cu^{II}, there is evidence that the human pregnancy-associated amine oxidase, in which three aspartic acid residues whose carboxylate groups coordinate the second metal binding site in pea-seedling amine oxidase [65] are conserved, contains Mn^{II}.

Steady-state kinetic parameters and enzyme inhibition

Preliminary experiments indicated that enzyme activity spermine (1), N^1-acetylspermine, spermidine (3), and putrescine (5) could not be resolved. Both molecular weight forms of the enzyme were active with each substrate, and the relative rates of oxidation by each form (indicated in parentheses) were in approximately the same ratio. The relative rates of oxidation were in general agreement with earlier reports [15,16]. Both molecular forms were insensitive to 0.1 mM pargyline at pH 7.4, but were completely inhibited by 0.1 mM semicarbazide at pH 7.4 using both spermine and putrescine as substrates. The insensitivity of both enzyme forms to pargyline is in agreement with an earlier report of inhibitor sensitivity for the retroplacental serum polyamine oxidase sensitivity. Carbonyl group reagent sensitivity indicates the possible involvement of TPQ at the active site and is similar to the aminoguanidine sensitivity previously reported for the retroplacental enzyme [15]. These observations are not only similar to those found for pregnancy-associated amine oxidases, but also similar to those found for other amine oxidases from different species [2].

Steady-state parameters were determined for each enzyme form and are shown in Table . The substrate affinities (reflected by K_m values) and k_{cat} values for the two enzyme forms, PAO I and PAO II, appear to be almost identical. These findings are similar to those of Tufvesson who reported that the two amine oxidase forms observed in amniotic fluid exhibited similar substrate affinities [28]. In contrast, the two amine oxidases isolated from human placenta by Bardsley *et al.* were reported to have different K_m values [22]; one of these enzymes may have been serum or mitochondrial monoamine oxidase. The selectivity of the retroplacental enzyme based on the k_{eff} derivative is: histamine > putrescine > N^1-acetylspermine > spermidine > spermine > N^1-acetylspermidine. These findings indicate that histamine and putrescine are good substrates for the enzyme and that acetylated spermine is a better substrate than unconjugated spermine. The substrate specificity pattern is similar to that seen for amniotic fluid diamine oxidase, pregnancy serum diamine oxidase and post-heparin serum diamine oxidase [28,77]. Human pregnancy-associated enzymes have been shown to be active with histamine and diamines, such as putrescine, it is now accepted that the two activities are a result of the same enzyme [78]. A broad substrate specificity has been found for the human pregnancy-associated amine oxidases [22], namely, acetylpolyamines [79], histamine [19], putrescine [30,33], spermine [13-16,80,81] and spermidine [15,16,31]. *Ad hoc* trivial nomenclature of amine oxidases appears to have arisen because the substrate used for activity determinations has provided what might be termed 'epithetical nomenclature' for example, histaminase, spermine oxidase, spermidine oxidase, putrescine oxidase. Multisubstrate specificity is reflected in nomenclature such as monoamine oxidase, diamine oxidase and polyamine oxidase. However, the human pregnancy-associated amine oxidases, in particular the

retroplacental enzyme as presented here have been found to have an even broader substrate specificity encompassing all of these categories.

Retroplacental polyamine oxidase was strongly inhibited by quinicrine, aminoguanidine and MGBG. The monoamine oxidase inhibitors, pargyline and clorgyline were not inhibitory at 1.0 mM (Table). The retroplacental serum polyamine oxidase was inhibited in a similar way to the rat liver enzyme: it is inhibited by quinacrine, a well known inhibitor of flavoproteins, and by the carbonyl reagents aminoguanidine and semicarbazide. It therefore belongs to the group of enzymes known as semicarbazide-sensitive amine oxidases [5]. Since the rat polyamine oxidase is thought to be an FAD-containing enzyme [17] and the retroplacental serum enzyme has TPQ at its active site, the carbonyl group reagents and quinicrine are not particularly discriminating. Substrates at high concentrations caused substrate inhibition, with putrescine (K_i = 61 μM) and histamine (K_i = 26 μM) the most potent inhibitors. This finding is similar to that for the human amniotic fluid enzyme [28] and pronounced substrate inhibition by histamine was also observed for amine oxidases from other sources [82-84].

Enzymatic reaction products

Enzymatic production of spermidine from spermine, and putrescine from spermidine, was demonstrated by the use of a [^{14}C]spermine substrate, radiolabelled at its internal putrescine moiety, clearly indicating that the retroplacental polyamine oxidase cleaves polyamines at their secondary amino groups, thus acting as an EC 1.5.3 class enzyme (Figure 7). These results confirm the proposed site of polyamine cleavage by the retroplacental enzyme [15], indicate a mode of action similar to that of the rat liver enzyme [17] and more recently demonstrated with the bovine serum enzyme [85]. The

progressive formation of spermidine and then putrescine suggests a feed-forward competitive inhibition of putrescine oxidation by spermidine as previously associated with extracellular adenine nucleotide metabolism by endothelial cells where ADP and/or ATP exert a feed-forward inhibition on AMP hydrolysis [86].

The human retroplacental serum polyamine oxidase may be classified as an EC 1.4.3 or EC 1.5.3 enzyme depending on the substrate being used to assess its action. It may also be classified as a semicarbazide-sensitive amine oxidase because of its inhibitor sensitivity. The trivial nomenclature is even more confusing. In view of the difficulty in the unambiguous classification of the enzyme based on phenotypic criteria, the classification of amine oxidases according to their intrinsic physicochemical properties has been suggested [87,88]. The retroplacental enzyme belongs to that heterogenous group of copper- and TPQ- containing amine oxidases currently classified as EC 1.4.3.6. Structural and functional analogies presented here suggest that the human retroplacental serum polyamine oxidase has a strong identity with pregnancy-associated amine oxidases found in placenta, amniotic fluid, and pregnancy serum.

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Table 1. Purification of polyamine oxidase using mAb-pao 2.21—Affi-Prep 10

Purification step	Volume	Activity	Protein	Specific activity	Purification	Yield
	ml	*Units*[b]	*mg*	*mU/mg*	*-fold*	*%*
1. Retroplacental serum[a]	500	41.7	20780	2	1	100
2. Pentyl-/ω-aminohexylagarose	115	27.5	164	168	84	66
3. Sephacryl S-300	116	37.9	162	234	116	91
4. DEAE-Trisacryl						
(PAO I)	47	2.3	3.0	78	39	6
(PAO II)	260	24.4	79	308	153	59
5. mAb-pao-2.21—Affi-Gel 10						
(PAO I)	37	0.41	0.3	1385	689	1
(PAO II)	50	7.52	2.7	2838	1412	18

Notes

a. from 10 placentae b. One μmole/min H_2O_2 production with 10 μM putrescine as the substrate.

Table 2. Steady-state parameters for polyamine oxidase

A	K_{mA} (μM)[a]		k_{cat} (s^{-1})[b]	
Substrate	I	II	I	II
putrescine	1.3	1.1	43	44
spermidine	5.5	5.5	43	40
spermine	1.86	2.11	6.97	6.65
N^1-acetylspermidine	4.9	5.0	15	14
N^1-acetylspermine	0.16	0.14	2.3	2.5
histamine	0.30	0.27	26	24

Table 3. Polyamine oxidase inhibition

Inhibitor	*mM*	*%inhibition*[a]
quinicrine	1.00	99.7
	0.10	97.6
	0.01	91.5
isoniazid	1.00	22.1
	0.01	3.0
pargyline	1.00	–3.4
	0.10	–9
semicarbazide	1.00	93.8
	0.10	49.3
	0.01	2.3
aminoguanidine	1.00	99.8
	0.10	99.7
	0.01	96.0
chlorgyline	1.00	–2.4
	0.10	–4.1
MGBG	1.00	99.7
	0.10	98.8

Table 2. Polyamine oxidation was measured at pH 7.43 ± 0.05, 37.0 ± 0.1 °C in 0.05 M Tris–HCl using the fluorometric assay system described above. Sufficient HRPO (40 U/ml) was included in the assay to accurately reflect the true initial rate of polyamine oxidations. Reaction mixtures were pre-equilibrated at 37 °C and the reactions initiated by addition of enzyme.

a. Initial rate velocities were determined for substrate concentrations in the range 0.01 – 1000 μM at oxygen concentrations from atmospheric equilibration and Michaelis parameters were calculated for substrate concentrations in the range 0.2 – 50 K_{mA}. Initial velocities were determined by analysis of the slopes of the initial, linear portion of the progress curves of the coupled enzyme reaction by a least squares linear regression of the progress curve between a nominal lag time of 20 s and t = 30 – 70 s. During this time, substrate depletion was generally less than 5%, except at the lowest substrate concentrations where depletion did not exceed 10%. When the assay mixture was saturated with pure oxygen, no increase in reaction velocity was observed. Michaelis constants, K_{mA}, for the various polyamines and histamine were calculated by fitting the Michaelis–Menton equation, modified to compensate for the observed substrate inhibition [89], to untransformed data sets of at least 10 initial rates at varying substrate concentrations by non-linear regression using 'Enzfitter' software (Biosoft, Cambridge, UK).

b. $k_{cat} = V_{max}/[E]_0$, where $[E]_0$, the stoichiometric concentration of active catalytic centres, was based on calculations of the active site concentrations in the anion exchange fractions used in the kinetics assays. Fractions with relatively high specific activities were used. Calculation of active site concentrations was based on the activity losses suggested by the data in Table 1, enzyme purity based on electrophoretic data, the

specific activity of the PAO I and PAO II preparations (505 mU/mg and 217 mU/mg respectively), assuming a single catalytic site per M_r 108000 subunit.

Table 3. Inhibition was measured in 0.05 M Tris–HCl, pH 7.4 using a procedure based on the method of Okuyama and Kobayashi [47]. Inhibitors were included in the assay and the reactions were initiated by the addition of approx. 0.45 mU of enzyme that had been affinity purified using ω-aminohexylagarose.

Figure 1. Resolution of PAO multiple forms by anion exchange chromatography. Active fractions from S-300 size exclusion chromatography were pooled and applied to a DEAE-Trisacryl M column (94 cm × 2.6 cm i.d.) equilibrated in 25 mM Tris–HCl containing 30 mM NaCl, pH 8. The column was eluted with the Tris–saline buffer followed by a 500 ml linear NaCl gradient (0.03 – 0.3 M NaCl in 25 mM Tris–HCl, pH 8), resolving the enzyme activity into two separate peaks, designated PAO I and PAO II. Fraction pools containing the multiple forms PAO I (**I**)and PAO II (**II**) are indicted by the bars. Elution was continued with limit buffer followed by 25 mM Tris–HCl containing 0.5 M NaCl, pH 8 to remove tightly bound protein.

Figure 2. Immunoaffinity purification of polyamine oxidase on mAb pao 2.21—Affi-Gel 10. Pooled DEAE column fractions containing PAO I were applied to an immunoaffinity column, prepared from anti-PAO mAbs and Affi-Gel 10 at 9.86 mg/ml gel (4 cm × 1.6 cm i.d.), preceded by a bovine γ-globulin—Affi-Gel 10 column (5 cm × 1.6 cm i.d.). PAO activity completely adsorbed to the immunoaffinity column which was eluted with 250 mM NaCl in 25 mM Tris–HCl, pH 8, followed by 1.0 M NaCl in 25 mM Tris–HCl, pH 8 (arrow). PAO I activity was desorbed from the immunoaffinity column with 1.5 M NaSCN in 25 mM Tris–HCl, pH 8 (bent arrow, panel A). PAO II in

the second anion exchange column fraction pool was similarly purified (panel B). Pooled immunoaffinity column fractions are indicated by the bars.

Figure 3. SE-HPLC of multiple PAO forms and analysis of fractions by SDS-PAGE. Pooled fractions from the immunoaffinity purification of PAO were concentrated using a 25,000 MWCO Micro-ProDiCon ultrafiltration system (Spectrum Industries, Los Angeles, CA) and applied to a BioSil SEC 400 column set ((80 mm + 300 mm) × 7.8 mm i.d.). Columns were calibrated using Bio-Rad size-exclusion chromatography standards and eluted at 1 ml/min with SE-HPLC buffer. *Panel A*, PAO I; *Panels C and D*, PAO II. *Panel B*, SDS-PAGE of PAO I SE-HPLC fractions (panel A) under reducing conditions. The proteins were silver stained using the method described by Gottleib and Chavako [52]. Panel E, SDS-PAGE of PAO II SE-HPLC fractions; panel C fraction 10, *lane [10]* and panel D fractions (without square brackets) in the other lanes. Proteins were stained using the highly sensitive CBB G (CI 42655) based method described by Neuhoff *et al.* [51]. The concentration of polyacrylamide in the resolving gels was 7.5%, *o* = origin and BPB = bromophenol blue tracker dye.

Figure 4. SDS-PAGE analysis of immunoaffinity (mAb pao-2.21) polyamine oxidase purification. SDS-PAGE was conducted under reducing conditions in 7.5% polyacrylamide resolving gels using the discontinuous buffer system of Laemmli [49]. Protein bands were stained with Coomassie Blue R. *Lane 1*, DEAE-Trisacryl eluate polyamine oxidase type II (PAO II) activity peak fraction; *lane 2,* DEAE-Trisacryl PAO II fraction pool; *lane 3,* inactive immunoaffinity column eluate from PAO II purification (Figure 2, panel B) fraction 5 (40 – 50 ml); *lanes 4,5 and 6* immunoaffinity purified PAO II (11 μg, 6 μg and 3 μg respectively). *Lane 7,* immunoaffinity purified polyamine oxidase type I (PAO I); *lane 8,* DEAE-Trisacryl eluate PAO I fraction pool; *lane 11,*

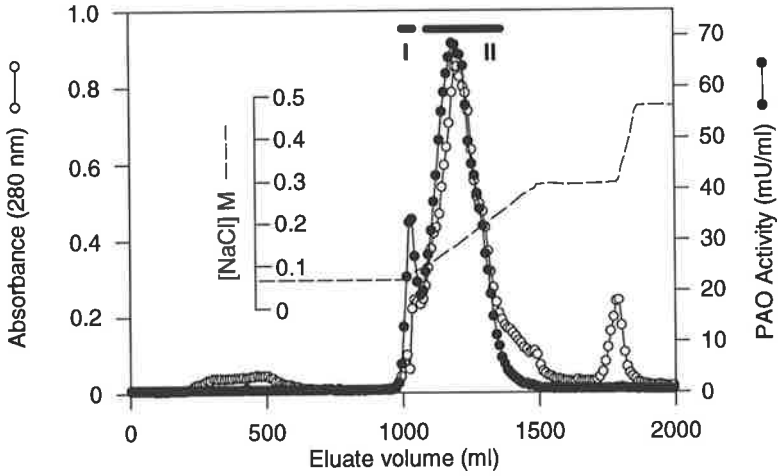
DEAE Trisacryl peak fraction. *Lane 9,* inactive immunoaffinity column eluate from PAO I purification (Figure 2, panel A) fraction 4 (30 –40 ml). *Lane 10,* monoclonal antibody pao-2.21.

Figure 5. Enzymatic deglycosylation of polyamine oxidase. Immunoaffinity purified polyamine oxidase was deglycosylated as described in the text. *Lane a,* immunoaffinity purified PAO (4.2 µg) before PNGase digestion. *Lane b,c,d,* immunoaffinity purified PAO after carbohydrate removal (CHO) removal, 1.86 µg, 4.65 µg and 9.3 µg respectively. The proteins were stained with Coomassie Blue R.

Figure 6. Polyamine oxidase under reducing (–SH) and non-reducing (–S-S–) conditions. *Lanes a and c,* immunoaffinity purified enzyme; *lanes b and d,* pooled activity from the size-exclusion chromatography of the immunoaffinity purified enzyme. M, monomer subunit; D, homodimer; T, tetramer. The proteins were stained with Coomassie Blue R.

Figure 7. Polyamine oxidase reaction progress. Reaction progress with [^{14}C]spermine as the substrate for an affinity purified retroplacental serum enzyme preparation after the initiation of the reaction: sequentially at 30 s and 30, 60, 120, 240 min. Reversed phase, ion pairing HPLC and other experimental procedures are described in the text. The time dependent decrease of spermine, and increase in spermidine, putrescine and its metabolites are shown on the chromatograms. The uppermost chromatogram shows the retention times of radiolabelled polyamine standards. PUT, putrescine; SPD, spermidine; SPM, spermine.

Abbreviations used: TPQ, 2,4,5-trihydroxyphenylalanine quinone; PBS–T, PBS containing 0.05% Tween 20; ABTS, 2,2^{1}-azino-bis-(3-ethylbenzthiazoline-6-sulphonate); PAO, polyamine oxidase, ABP, amiloride binding protein; HRPO, horseradish peroxidase; PTH, phenylthiohydantoin.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0.2
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[NaCl] M
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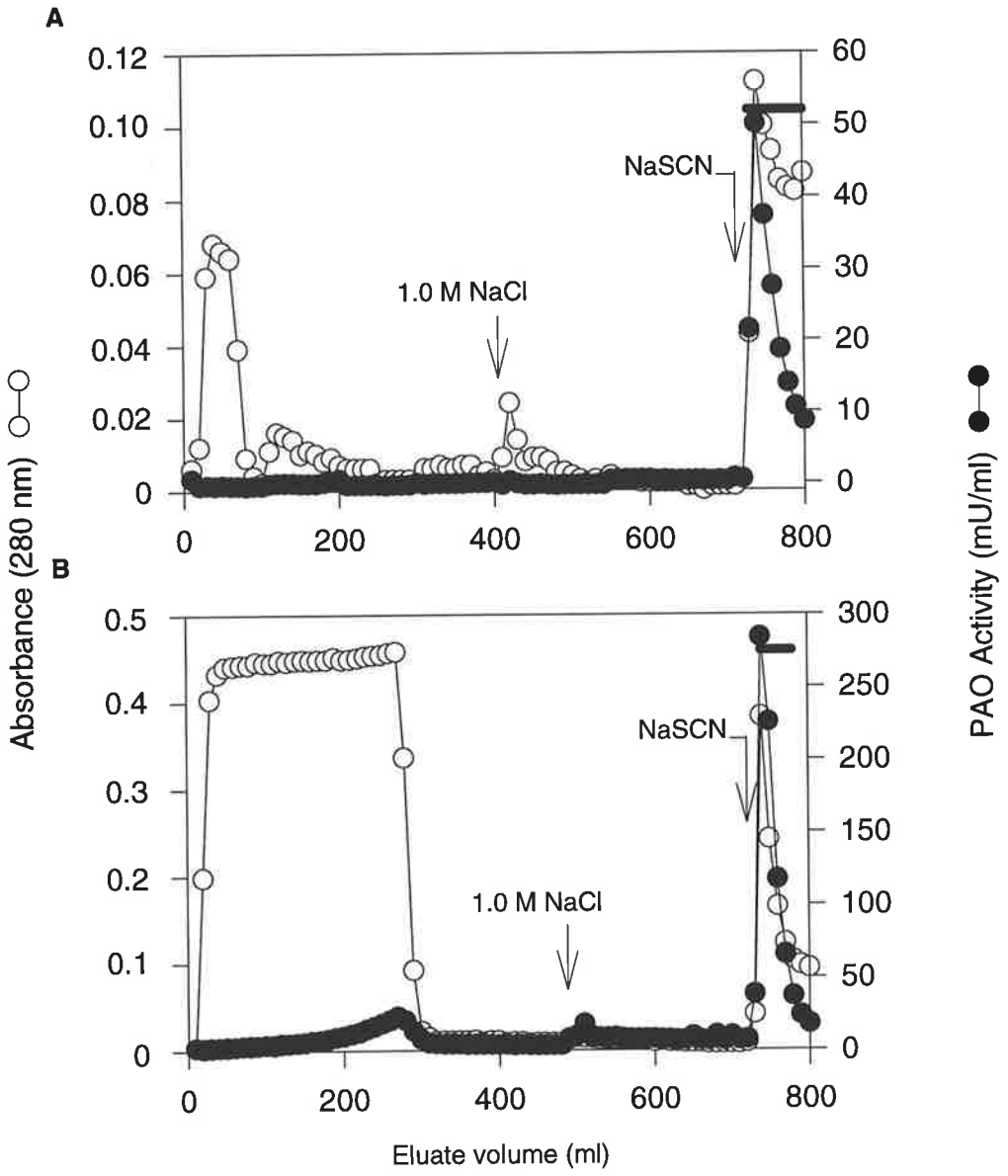
0.4
0.3
0.2
0.1
0
I
II
70
60
50
40
30
20
10
0
PAO Activity (mU/ml)
0
500
1000
1500
2000
Eluate volume (ml)

Figure. 1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0.10
0.08
0.06
0.04
0.02
0
60
50
40
30
20
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0
NaSCN
1.0 M NaCl
0
200
400
600
800
0.5
0.4
0.3
0.2
0.1
0
300
250
200
150
100
50
0
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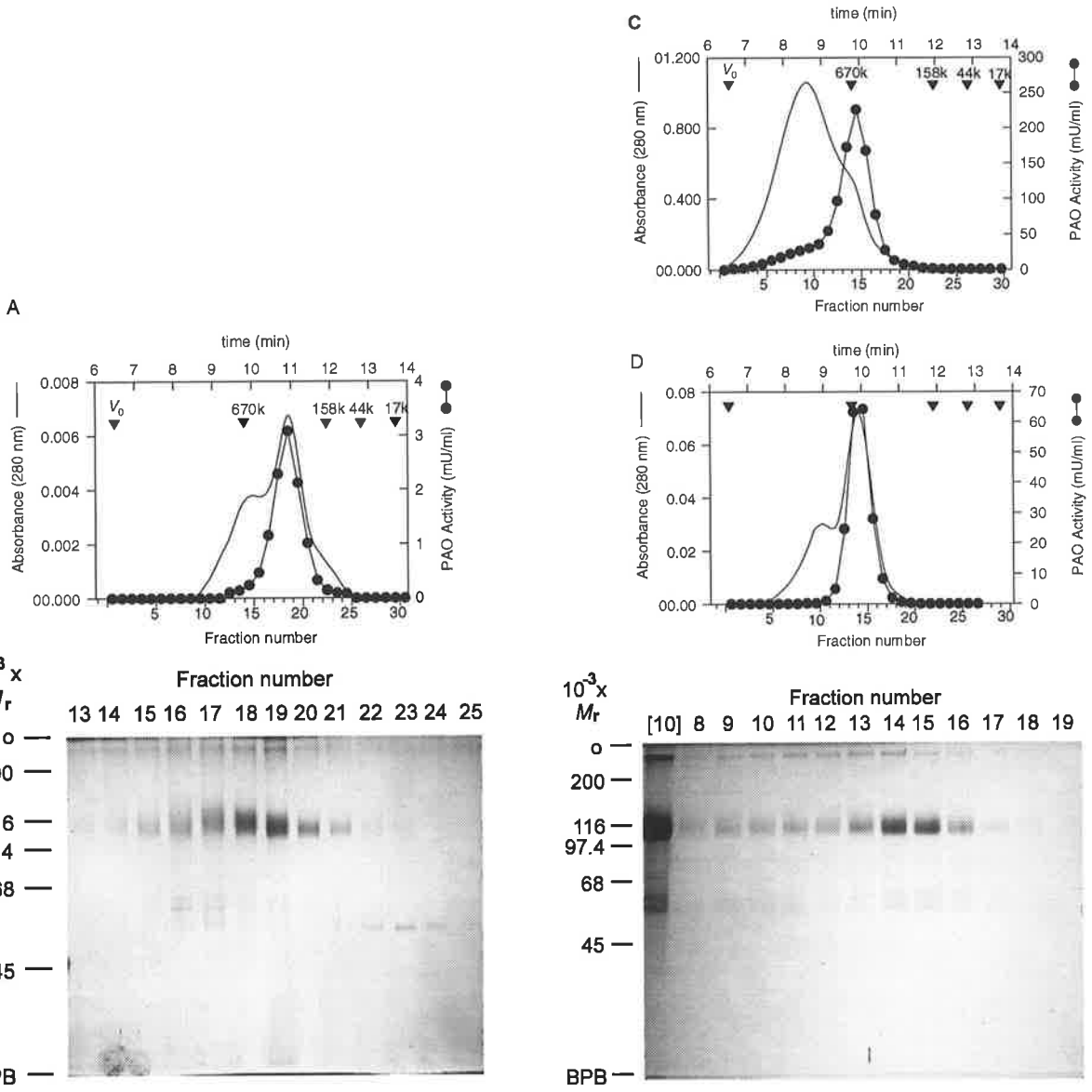
200
400
600
800
Absorbance (280 nm)
PAO Activity (mU/ml)
Eluate volume (ml)

Figure. 2


A
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PAO Activity (mU/ml)
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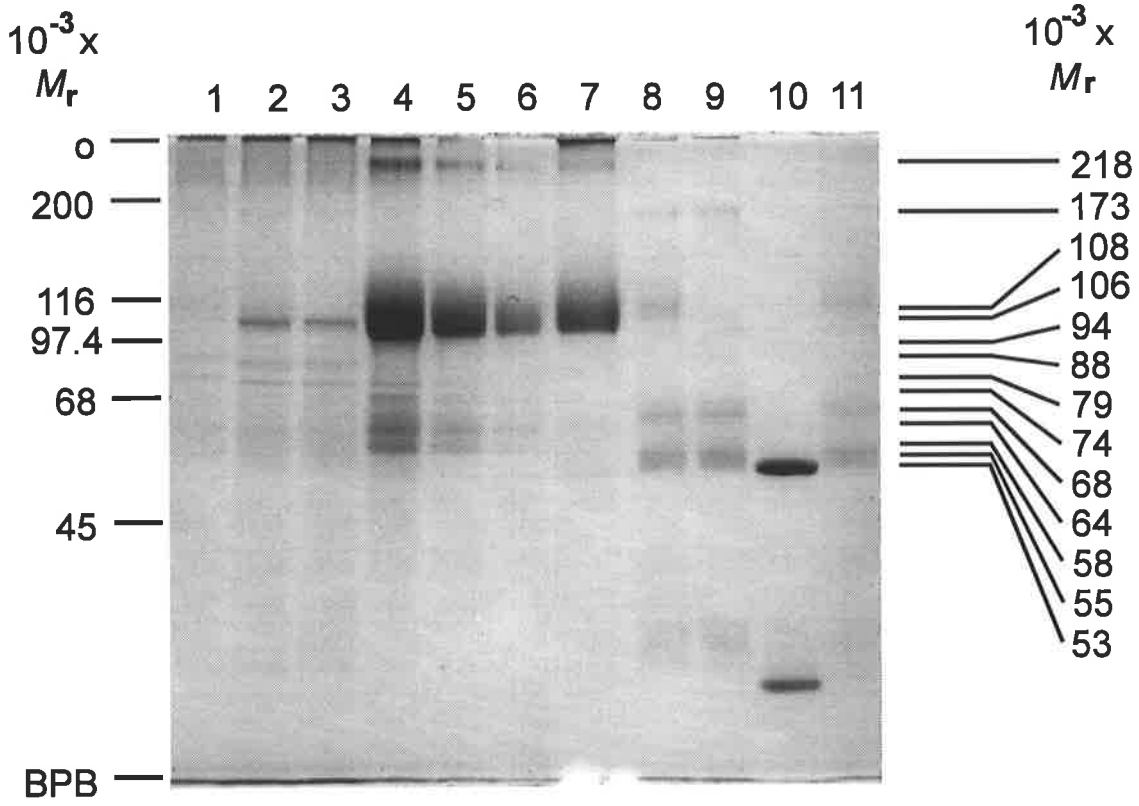
670k
158k
44k
17k
Fraction number
C
time (min)
Absorbance (280 nm)
PAO Activity (mU/ml)
V_0
670k
158k
44k
17k
Fraction number
D
time (min)
Absorbance (280 nm)
PAO Activity (mU/ml)
Fraction number
10^{-3} x M_r
Fraction number
13 14 15 16 17 18 19 20 21 22 23 24 25
200
116
97.4
68
45
BPB
10^{-3}x M_r
Fraction number
[10] 8 9 10 11 12 13 14 15 16 17 18 19
200
116
97.4
68
45
BPB

Figure. 3


10-3 x Mr
1 2 3 4 5 6 7 8 9 10 11
o
200
116
97.4
68
45
BPB
10-3 x Mr
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94
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79
74
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64
58
55
53

Figure. 4

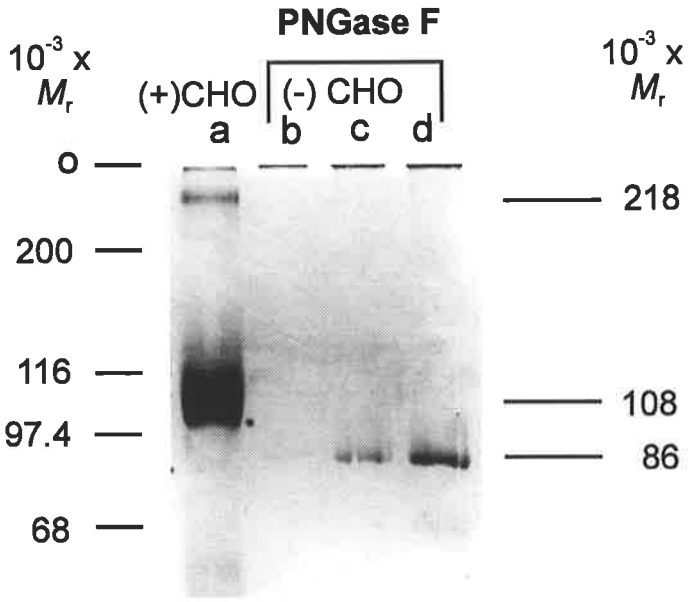


Figure. 5

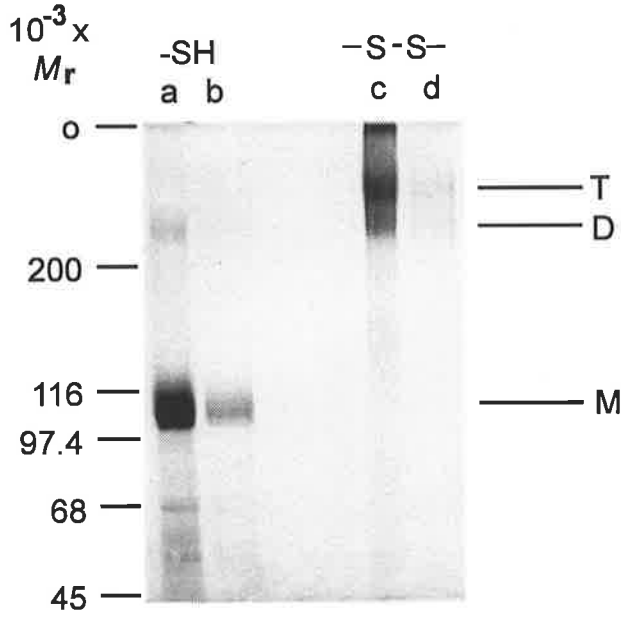
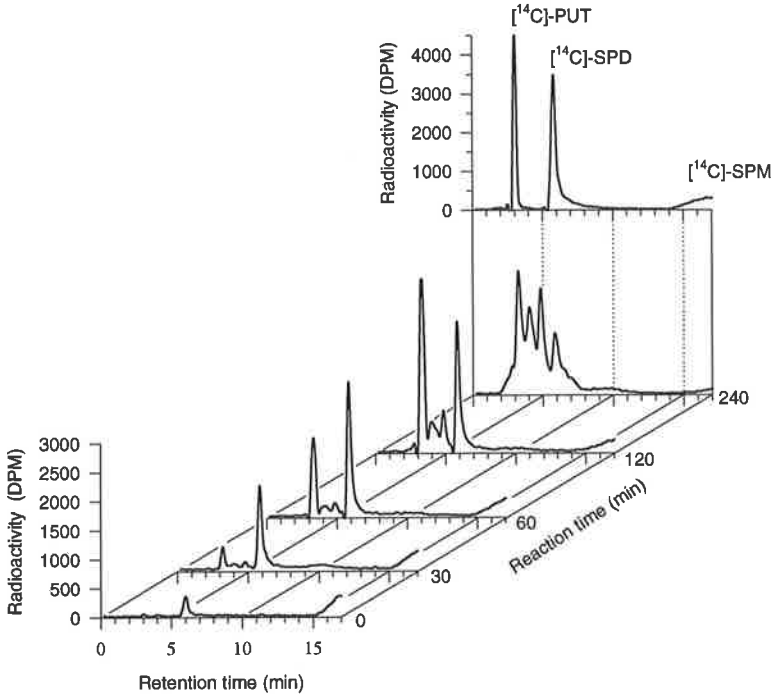


Figure. 6


[14C]-PUT
[14C]-SPD
[14C]-SPM
Radioactivity (DPM)
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3000
2000
1000
0
240
120
60
30
0
Reaction time (min)
3000
2500
2000
1500
1000
500
0
Radioactivity (DPM)
0
5
10
15
Retention time (min)

Figure. 7

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Polyamine oxidase activity in rheumatoid arthritis synovial fluid

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SUMMARY

Oxidation of polyamides by polyamine oxidases (PAO) leads to the generation of highly reactive aminoaldehydes which have been shown to have a variety of effects, including killing of pathogenic microorganisms and regulation of leucocyte functions. Data presented here show that PAO are present in synovial fluid from patients with rheumatoid arthritis. This finding may have important implications in the various properties attributed to synovial fluid which includes anti-inflammatory activity

Keywords polyamine oxidases rheumatoid arthritis

INTRODUCTION

Polyamine oxidases (PAO) have been tentatively defined as amine oxidases capable of catalysing the oxidation of the polyamines spermine and spermidine (Morgan, 1985a, 1989). In non-ruminants the enzymes are not usually detectable in the blood. However, significant levels may be found during infection, in 'pregnancy serum,' retroplacental serum and macrophages (Gaugas & Curzen, 1978; Morgan, Ferluga & Allison, 1980; Illei & Royston, 1983; Morgan, 1985b). These enzymes react with polyamines and generate highly reactive aminoaldehydes (Fig. 1), which have been shown to mediate intraerythrocyte death of malarial parasites (Morgan, Christensen & Allison 1981; Ferrante, Rzepczyk & Allison 1983; Rzepczyk, Saul & Ferrante 1984; Morgan *et al.*, 1986; Egan *et al.*, 1986), lysis of trypanosomes (Ferrante, Allison & Hirumi, 1982; Ferrante, Rzepczyk & Saul, 1984), killing of various helminth parasites (Ferrante *et al.*, 1986a), lysis of tumour cells (Storer *et al.*, 1988; Bachrach, Abzug & Bekierkurst, 1967), inhibition of lymphocyte proliferation (Byrd, Jabobs & Amos, 1977; Allen *et al.*, 1979; Gaugas, 1980; Hussain, Smith & Allen, 1983) and inhibition of neutrophil oxygen radical production and chemotaxis (Ferrante, 1985; Ferrante *et al.*, 1986b). Because of their effects on inflammatory cells such as neutrophils, it was postulated (Ferrante 1985; Ferrante *et al.*, 1986b) that the anti-inflammatory activity found in inflammatory exudates such as synovial fluid from rheumatoid arthritis patients (Capstick, Lewis & Cosh, 1975) may be explained in part by the presence of polyamine oxidizing enzymes. We now present evidence that PAO are present in synovial fluids from patients with rheumatoid arthritis.

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MATERIALS AND METHODS

Polyamines

^{14}C-spermine tetrahydrochloride (*N, N'*-bis-(3-aminopropyl)-^{14}C-tetramethylene-1,4-diamine) was obtained from Amersham Laboratories (Amersham, UK). ^{3}H-spermidine trihydrochloride (N-(3-aminopropyl) -1, 4-tetramethylene-1, 4-diamine), [terminal methylenes ^{3}H(N)] was from du Pont de Nemours & Co. (Boston, MA). Spermine tetrahydrochloride and spermidine trihydrochloride were from Sigma Chemical Co. (St Louis, MO).

^{14}C-spermine oxidation assay method

PAO activity was measured using the radiochemical method described by Morgan & Illei (1980b) with minor modifications. Briefly, 100 μl of sample were added to 200 μl 0·05 M Tris-HCl buffered spermine (46·6 kBq/μmol; 303 μM) plus 0·1% Triton X-100 (v/v), pH 7·4 at 37°C. The reaction mixture was incubated in a 37·0 $\pm$ 0·1°C water bath for 90 min after which the reaction was

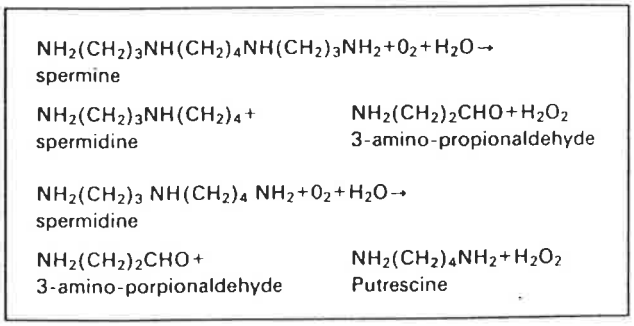

Fig. 1. The oxidation of polyamines by human polyamine oxidases.

Table 1. Polyamine oxidase activity in synovial fluids from rheumatoid arthritis patients

Fluid Serum type	Enzyme activity (mU/l) (m ± s.e.m.)
RA synovial fluid ($n = 12$)*	490 ± 44
RA serum ($n = 6$)*	89 ± 36
Retroplacental serum ($n = 58$)	1,722 ± 114
Pooled human serum	116
'Control' synovial fluid ($n = 3$)	144·9 ± 4·0

* Synovial fluid or serum from rheumatoid arthritis (RA) patients.

stopped by the addition of 50 μl trichloroacetic acid (50% w/v), precipitated protein was sedimented by centrifugation. Reaction products and substrate were separated by step-wise elution with hydrochloric acid 1·9 M (10 ml) and 4·0 M (10 ml) on Dowex 50W-X2, 200–400 mesh (BioRad laboratories, Richmond, CA) cation exchange resin (0·8 × 4 cm). Aliquots (200 μl) of the reaction mixture supernatant were applied to the columns followed by 200 μl deionized water. The eluates were collected into polypropylene tubes, aliquots of each fraction were transferred to scintillation vials amd mixed with scintillation cocktail (Beckman Ready-solv EP). For each sample d/min was determined by counting in a Mark III liquid scintillation counter (Searle Analytic, Des Plains, IO) and percent substrate conversion calculated. Controls and blanks were included in each assay. The separation of substrate and reaction products by this method was confirmed using ^{3}H-spermidine and ^{14}C-spermine with dual-channel scintillation counting. Enzyme activity is expressed as international units (i.e. μmol product formed/min).

Retroplacental fluid

Retroplacental fluid was collected from placentae after elective caesarean delivery at 38–40 weeks gestation as described by Klopper & Hughes (1978). Serous blood stained fluid was obtained from the maternal surface of the placenta. This fluid, which is mainly intervillous blood plus decidual and placental interstitial fluid, was allowed to clot for 30 min at 37°C. The blood mixture was then centrifuged and the serum (termed retroplacental serum) was collected.

Synovial fluid

Samples were obtained under local anaesthesia (xylocaine i.o.) from patients with definite or classical rheumatoid arthritis by ARA criteria classification (Mitchell & Fries, 1982) attending the Rheumatology Unit at the Royal Adelaide Hospital. Control synovial fluids were obtained at autopsy.

RESULTS

The results showed that all 12 rheumatoid arthritis synovial fluid samples examined were positive for PAO activity, ranging from 200 to 800 mU/l (Table 1). Only low activity was detected in 'control' synovial fluids, similar to levels found in normal serum and the serum from rheumatoid arthritis patients. However, the levels of activity in synovial fluids of rheumatoid arthritis patients were low compared to those of retroplacental serum (Table 1).

DISCUSSION

These findings represent the first evidence that PAO are present in inflammatory exudates and it is possible that this enzyme(s) is part of the inflammation molecular complex which may be involved in tissue degradation and tissue regeneration in diseases such as rheumatoid arthritis. Polyamines are present in all tissues and their concentrations in tissue fluids significantly rise as a consequence of tissue damage and regeneration (Gaugas, 1980). The oxidation of these polyamines by PAO could contribute to the events occurring in various inflammatory sites. Their effects could range from stimulation and regulation of tissue cell growth to suppression of the leucocytic inflammation. PAO may be released from inflammatory macrophages and/or pannus tissue associated with affected joints of rheumatoid arthritis patients. The regulation of inflammation by PAO in disease such as rheumatoid arthritis is supported by the findings of Flescher *et al.* (1989), who obtained evidence suggesting that the polyamine–polyamine oxidase system is somehow involved in the down-regulation of immune reactivity associated with rheumatoid arthritis, in particular to a down-regulation of interleukin-2 production. The levels of PAO levels of 500 mU/l found in rheumatoid arthritis synovial fluid would appear to be sufficient to inhibit lymphocyte proliferation, since Morgan & Illei (1980a) showed that inhibition of lymphocyte proliferation occurred as PAO activity exceeded 200 mU/l. The capacity of oxidized polyamines to inhibit the migration and oxygen radical production of neutrophils (Ferrante, 1985; Ferrante *et al.*, 1986b) as well as cytokine production (Flescher *et al.*, 1989) could explain the anti-inflammatory activity found in arthritic rat plasma (Persellin, 1972; Lewis, Capstick & Bers, 1976) and human rheumatoid synovial fluid (Capstick *et al.*, 1975).

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