

**A study on the interactions of
synthetic IGF-II analogues with
the type 1 IGF and insulin
receptors**

A thesis

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Abstract

Insulin-like growth factor II (IGF-II) is a unique regulatory peptide containing 67 residues and three disulfide bonds. It binds with high affinity to three receptors, the insulin receptor (IR), the type 1 insulin-like growth factor receptor (IGF-1R) and the type 2 insulin-like growth factor receptor (IGF-2R). Binding of IGF-II to these receptors signals mitogenic responses, such as cell proliferation, differentiation and migration. The interactions of IGF-II with the IR and IGF-1R have recently been identified as potential therapeutic targets for the treatment of cancer. Thus, an increased understanding of the interactions of IGF-II with the IGF-1R and the IR-A is required for the improved design and development of potential anticancer therapeutics.

A crystal structure of IGF-II bound to either the IGF-1R or the IR-A has not been reported. Thus, the precise location of IGF-II within the receptor binding pocket remains undefined. A fluorescence resonance energy transfer (FRET) approach was proposed to investigate the binding location and orientation of IGF-II within the IGF-1R. Two fluorescent IGF-II analogues, the F19Cou IGF-II and F28Cou IGF-II proteins, were synthesised for use in the desired FRET studies.

These FRET experiments first required the synthesis of an appropriate coumarin-based probe for incorporation into IGF-II. The synthesis of a range of fluorescent coumaryl amino acids is described in Chapter 2, and an analysis of the spectroscopic properties of these coumaryl amino acids is also detailed.

Site-specific incorporation of the coumarin-based probe into IGF-II was then undertaken. Three complementary methods were used for the preparation of the desired fluorescent IGF-II analogues. Chapter 3 describes the use of the nonsense suppression methodology for the expression of the novel F19Cou IGF-II protein. This was followed by an improved chemical synthesis of the F19Cou IGF-II protein using a linear solid phase peptide synthesis (SPPS) approach and is detailed in Chapter 4. A robust native chemical ligation approach was developed in Chapter 5, which allowed for the facile incorporation of the coumarin-based

probe at various locations within the IGF-II protein. Chapter 5 also details the synthesis of the native IGF-II, F19Cou IGF-II and F28Cou IGF-II proteins. The biological activity of the resultant IGF-II analogues was evaluated by competition binding assays. The fluorescent IGF-II analogues bind with low nanomolar affinity to the IR and IGF-1R, and as such were deemed suitable for use in the desired FRET-based experiments.

The FRET-based investigation into the binding interactions of the native IGF-II, F19Cou IGF-II and F28Cou IGF-II proteins to the IGF-1R is described in Chapter 6. FRET interactions were observed for both the F19Cou IGF-II and F28Cou IGF-II proteins. The results show the fluorophore binds in close proximity to Trp residues within the IGF-1R receptor and suggest the location of IGF-II binding within the IGF-1R is consistent with what is proposed in the literature. These experiments provide a basis for further investigations for determining the precise binding location and orientation of IGF-II within the IGF-1R.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Date

Publications

Work in this thesis has appeared in the following publication:

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Abbreviations

[α] ²³ _D	specific rotation at the sodium D line (589 nm) at 23 °C	Cou	coumarin fluorophore 2.2
2-Br-Z	2-bromobenzyloxycarbonyl	CR	cysteine-rich domain
2-Cl-Z	2-chlorobenzyloxycarbonyl	CT	C-terminal domain
2-HED	2-hydroxyethyl disulfide	Cys (C)	cysteine
2xYT	2x yeast extract and tryptone	DCM	dichloromethane
4-MeBzl	4-methyl benzyl	DIC	<i>N,N'</i> -diisopropylcarbodiimide
aaRS	aminoacyl-tRNA synthetase	DIPEA	<i>N,N</i> -diisopropylethylamine
Acm	acetamidomethyl	Dmb	<i>N</i> - α -(2,4-dimethoxybenzyl)
AIM	auto-inducing medium	DMF	<i>N,N</i> -dimethylformamide
Ala (A)	alanine	DNA	deoxyribonucleic acid
Amp	ampicillin	Dnp	2,4-dinitrophenyl
approx.	approximately	DODT	3,6-dioxa-1,8-octanedithiol
Ar	aromatic	DTT	dithiothreitol
Arg (R)	arginine	<i>E</i>	efficiency of the energy transfer (in FRET)
Asn (N)	asparagine	EDTA	ethylenediaminetetraacetic acid
Asp (D)	aspartic acid	equiv.	equivalent
Bn	benzyl	ESI-MS	electrospray ionisation mass spectrometry
Boc	<i>tert</i> -butoxycarbonyl	Et ₂ O	diethyl ether
Br	broad (spectroscopy)	EtOAc	ethyl acetate
Bz	benzyl	EuIGF-II	europium labelled IGF-II
calcd.	calculated	Ex11	exon 11
Cbz	benzyloxycarbonyl	Fmoc	9-fluorenylmethyloxycarbonyl
CDI	1,1-carbonyldiimidazole	FnIII	fibronectin type III domain
cDNA	coding DNA	FRET	fluorescence resonance energy transfer
CH ₂ N ₂	diazomethane		
conc.	concentrated		

Gln (Q)	glutamine	IGF	insulin-like growth factor
Glu (E)	glutamic acid	IGF-1R	type 1 insulin-like growth factor receptor
Gly (G)	glycine	IGF-2R	type 2 insulin-like growth factor receptor
GnHCl	guanidine hydrochloride	IGFBP	insulin-like growth factor binding Protein
h	hour(s)	IGF-I	insulin-like growth factor I
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate	IGF-II	insulin-like growth factor II
HBTU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate	Ile (I)	isoleucine
HCTU	2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate	IPTG	isopropyl- β -D-thiogalactoside
HEPES	<i>N</i> -2-hydroxyethylpiperazine- <i>N</i> -2-ethanesulfonic acid	IR	insulin receptor
HF	hydrogen fluoride	IR-A	insulin receptor isoform A
His (H)	histidine	IR-B	insulin receptor isoform B
HOBt	<i>N</i> -hydroxybenzotriazole	JM	juxtamembrane
HPLC	high performance liquid chromatography	kan	kanamycin
HRMS	high resolution mass spectrometry	L1	large domain 1
Hz	hertz (in NMR)	L2	large domain 2
IB	inclusion bodies	LB	Luria Bertani
ID	insert domain	LCMS	liquid chromatography mass spectrometry
		Leu (L)	leucine
		lit.	literature value
		Lys (K)	lysine
		<i>m/z</i>	mass to charge ratio
		Me	methyl
		MeONH ₂	methoxyamine hydrochloride
		•HCl	
		Met (M)	methionine

MHz	megahertz (in NMR)	pet. spirit	petroleum spirit
MIN	minimal medium	PG	unspecified protecting group
min	minute(s)	Phe (F)	phenylalanine
mp	melting point	ppm	parts per million
MPAA	mercaptophenylacetic acid	Pro (P)	proline
mRNA	messenger RNA	r	distance between the donor and acceptor (in FRET)
NCL	native chemical ligation	RF1	release factor 1
NIM	non-inducing medium	RNA	ribonucleic acid
NIR	near infrared	R_0	Förster distance
NMM	<i>N</i> -methylmorpholine	RP-HPLC	reverse phase high performance liquid chromatography
NMP	<i>N</i> -methylpyrrolidine	rt	room temperature
NMR	nuclear magnetic resonance	SDS	sodium dodecyl sulfate
O-2-Ada	2-adamantyl	SDS-	sodium dodecyl sulfate
<i>o</i> -aaRS	orthogonal aminoacyl-tRNA synthetase	PAGE	polyacrylamide gel electrophoresis
OcHx	cyclohexyl ester	semi-prep	semi-preparative
OD _{600nm}	optical density at 600 nm	Ser (S)	serine
<i>o</i> -tRNA	orthogonal tRNA	SPE	solid phase extraction
PAL	5-[3,5-dimethoxy-4-(fmoc-aminomethyl)phenoxy]pentanoic acid	SPPS	solid phase peptide synthesis
PAM	4-hydroxymethylphenylacetamidomethyl	<i>t</i> Bu	<i>tert</i> -butyl
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl	TCEP	tris(2-carboxyethyl)phosphine hydrochloride
PDB	protein data bank	TEMED	<i>N,N,N,N'</i> -tetramethylethylenediamine
PEG	polyethylene glycol	tet	tetracycline
		TFA	trifluoroacetic acid

THF	tetrahydrofuran	Tyr (Y)	tyrosine
Thr (T)	threonine	Uaa	unnatural amino acid
TIPS	triisopropylsilane	UV	ultraviolet
TK	tyrosine-kinase domain	v/v	volume per unit volume
TLC	thin-layer chromatography	Val (V)	valine
TM	transmembrane domain	w/v	mass per unit volume
TNBSA	2,4,6-trinitrobenzene sulfonic acid	Xaa	amino acid
Tos	tosyl	Xan	xanthyl
Tris	tris(hydroxymethyl)aminomet hane	α CT	C-terminal region of the α - subunit
tRNA	transfer RNA	λ_{em}	emission maximum
Trp (W)	tryptophan	λ_{ex}	excitation maximum
Trt	trityl		
