Characterisation of Protein Structure and Interactions: Novel Applications to the Study of Bioactive Peptides

by

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Contents

Abstract

The studies of protein/peptide folding, misfolding, structure, and interactions are vital to understanding complex biological problems. The work presented in this thesis describes the development and application of a variety of biophysical techniques to investigate protein structure and interactions, with applications to the structure and function of several bioactive peptides.

Firstly, the development of a novel negative ion amenable chemical crosslinking-mass spectrometry (CX-MS) approach is described. CX-MS is a low-resolution technique to study protein structure and interactions. It involves covalent modification and tethering of a protein complex by a reactive reagent, followed by proteolytic digestion. The sites of the intra- and inter-molecular crosslinks provide distance restraints for modelling and enables conclusions to be drawn about the three-dimensional structure and binding interfaces within a protein complex. However, easy identification of crosslinks amongst the large quantity of proteolytic fragments remains challenging. In this study, the application of novel disulfide-based MS cleavable crosslinking reagents was investigated as a tool to easily identify crosslinked peptides by their highly reproducible and characteristic fragmentation patterns in the negative ion mode. MS3 analysis of the product anions allows easy sequencing and identification of crosslinking sites. Preliminary investigations validate these reagent as a tools to readily identify chemical crosslinks within proteins and their complexes, demonstrating that this approach is an effective and efficient means to determine aspects of the topologies of protein complexes of biological importance.

Secondly, the use of several biophysical methods is described to probe the structures of a variety of complexes involving the regulatory protein calmodulin (CaM) with bioactive amphibian peptides. CaM is ubiquitous in nature and plays a regulatory role in numerous biological processes, including some in amphibians and their predators; for example, it is involved in the upregulation of nitric oxide synthesis *in vivo*. Isothermal titration calorimetry was used to investigate the specific heats of the interactions, ion mobility-mass spectrometry was used to investigate the changes in collision cross section that occur as a result of complexation and nuclear magnetic resonance spectroscopy was used to track chemical shift changes upon binding. The results obtained confirm that these complexes adopt canonical collapsed structures and demonstrate the strength of the interaction between the peptides and CaM.

Next, work is presented which investigated the abilities of several bioactive amphibian peptides to inhibit fibril formation by disease related proteins. The peptide caerin 1.8 and several synthetic modifications were tested for their ability to inhibit fibril formation by the Alzheimer's related amyloid-β (1-42) peptide. The results obtained show that caerin 1.8 redirects the aggregation process of amyloid-β (1-42) toward the amorphous aggregation pathway. In addition, the self-assembly properties of the antimicrobial peptide uperin 3.5 were investigated using a variety of biophysical techniques, including transmission electron microscopy, ion mobility-mass spectrometry, circular dichroism, thioflavin T binding and cell viability assays. Similarities were observed between the fibrils formed by this peptide and those of disease related proteins, supporting the notion that information can be obtained about disease related amyloid fibril formation by studying amyloidogenic host-defence peptides.

Lastly, work detailing the effect of aspartic acid (Asp) isomerisation to isoAsp on the structure, activity and proteolytic cleavage susceptibility of three amphibian peptides, *Crinia* angiotensin II, uperin 1.1 and citropin 1.1 is presented. isoAsp formation has been shown to occur naturally as a result of age-related protein degradation, and is a consideration when preparing formulations of peptide therapeutics. isoAsp formation causes a 'kink' in the normally helical structure of citropin 1.1, as determined by nuclear magnetic resonance spectroscopy, which results in a reduction of its antimicrobial activity. The effect of this isomerisation process on the smooth muscle activities of *Crinia* angiotensin II and uperin 1.1 was different, with Asp isomerisation in *Crinia* angiotensin II causing a decrease in activity, and Asp isomerisation in uperin 1.1 causing greater contraction at lower concentrations. Proteolytic cleavage with trypsin was identical for each pair of Asp/isoAsp isomers, whilst cleavage with α-chymotrypsin was different for the two Asp/isoAsp citropin 1.1 isomers due to the presence of isoAsp adjacent to the cleavage site.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma 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 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.

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Abbreviations

1D	one dimensional
2D	two dimensional
3D	three dimensional
$\Delta\delta$	secondary shift
ΔC_p	specific heat capacity
ΔH	enthalpy change
ΔS	entropy change
Å	Angstrom
Aβ	amyloid-β
AFM	atomic force microscopy
AMP	antimicrobial peptide
apoCaM	calcium-free calmodulin
ARIA	Ambiguous Restraints for Iterative Assignment
ATD	arrival time distribution
Ca^{2+}CaM	calcium-bound calmodulin
Ca_4^{2+}CaM	calcium-saturated calmodulin
CaM	calmodulin
CD	circular dichroism
CCS	collision cross-section
Chol	cholesterol
CID	collision induced dissociation
CMC	critical micelle concentration
CX	chemical cross-linking
CX-MS	chemical cross-linking mass spectrometry
COSY	correlation spectroscopy
CSI	chemical shift index
CSP	chemical shift perturbation
Da	Dalton
DC	direct current
DMPC	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DMPG	1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (sodium salt)
DNA	deoxyribonucleic acid
DPC	dodecylphosphocholine
DQF	double-quantum filtered
DSA	dithiobis(succinimidyl acetate)

DSB	dithiobis(succinimidyl butanoate)
DSP	dithiobis(succinimidyl propionate)
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetraacetic acid
EGTA	ethylene glycol tetraacetic acid
EGCG	(-)-epigallocatechin-3-gallate
EHSS	exact hard sphere scattering
eNOS	endothelial nitric oxide synthase
ESI	electrospray ionisation
ESI-MS	electrospray ionisation mass spectrometry
FPLC	fast protein liquid chromatography
FID	free induction decay
HIC	hydrophobic interaction chromatography
HPLC	high performance liquid chromatography
HSQC	heteronuclear single-quantum coherence
Hz	Hertz
I	nuclear spin quantum number
IM	ion mobility
IM-MS	ion mobility mass spectrometry
iNOS	inducible nitric oxide synthase
isoAsp	isoaspartic acid
LC-MS	liquid chromatography mass spectrometry
MALDI	matrix assisted laser desorption/ionisation
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MS^n	multi-stage mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m/z	mass to charge ratio
nanoESI	nanoelectrospray ionisation
NHS	N-hydroxysuccinimide
NMR	nuclear magnetic resonance
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
PA	projection approximation
PAGE	polyacrylamide gel electrophoresis
PC12	pheochromocytoma-12
PDB	Protein Data Bank

PIR	protein interaction reporter
RF	radiofrequency
RMD	restrained molecular dynamics
RMSD	root-mean-square deviation
RNA	ribonucleic acid
SA	simulated annealing
SDS	sodium dodecylsulfate
SUV	small unilamellar vesicle
TEM	transmission electron microscopy
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
ThT	thioflavin T
TOCSY	total correlation spectroscopy
ToF	time of flight
TWIG	travelling wave ion guide
UV	ultraviolet