The isolation, structure, and membrane interactions of biologically active peptides

A thesis submitted for the degree of doctor of philosophy

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Statement of originality

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university or 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.

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Patrick James Sherman Date

Abstract

The host-defence secretions of amphibians and the venoms of arachnids are an abundant source of biologically active peptides with a great potential for use in therapeutic pharmacology. Over millions of years of evolution, the chemical arsenals of a multitude of species have produced a vast collection of peptides that have potent and selective activities. The research presented in this thesis details the isolation, structure determination and mechanistic pathways of a selection of biologically active peptides.

The southern brown tree frog *Litoria ewingi* occupies areas of the southeastern coast of Australia and Tasmania. Over a twelve month period, the peptide skin profile of a population of *L. ewingii* from Penola (South Australia) was determined using a combination of chromatography, tandem mass spectrometry and Edman degradation techniques. The peptide profiles of a *L. ewingi* from Penola show surprising differences relative to a population previously studied from the Adelaide hills, despite appearing to be morphologically identical. A total of six skin peptides were identified, four of which were unique; showing peptide sequence homology with peptides from Adelaide hills population. The evidence showed how a species can evolve separately after long periods of geographical isolation, how peptide profiling can be used to trace the migration of a species, and how new peptides can be discovered from different populations of a species.
The antimicrobial meucin peptides were first identified using cDNA cloning of DNA from the venom gland of the 'Lesser Asian scorpion' *Mesobuthus eupus mongolicus*. These peptides exhibit cytolytic effects against a number of eukaryotic and prokaryotic cells at micromolar concentrations, and their peptide sequences share similarities with other antimicrobial peptides from scorpions, arthropods and amphibian species. The secondary structures of the meucin peptides were determined using 2-D NMR and molecular dynamics calculations. Both meucin peptides exhibit α-helical structure, and are amphipathic in nature. The study further shows how the length of the α-helical structure can as an antibiotic affect the cytolytic activity of the peptide, since meucin-18 is more potent than meucin-13.

The C-terminal amide analogue of the peptide fallaxidin 4.l (fallaxidin 4.1a) isolated from the dermal secretions of *Litoria fallax*, is partially α-helical in nature, and shows potent activity against a wide range of yeast and bacteria (both Gram-positive and Gram-negative). This thesis uses solid-state NMR to detail the dynamic interactions of fallaxidin 4.1a with artificial lipid bilayers, and to explore the surface interactions of the peptides with eukaryotic (neutral) and prokaryotic (anionic) membranes. The solid state NMR and analysis using a quartz crystal microbalance indicated that the peptide acts via a surface interaction with neutral membranes and forms pores within anionic membranes at micromolar concentrations, indicating the specific pore forming mechanism by which the peptide interacts with anionic (prokaryotic) membranes.

Rothein 1, an 11 residue neuropeptide from the dermal secretions of *Litoria rothii*, and two alanine substituted analogues, rothein 1.4 and 1.5; show differing activities via binding to CCK2 receptors. The structures of rothein 1.4 and 1.5 were determined using 2-D NMR and molecular dynamics calculations. Each peptide has a largely extended structure, with similarities to the structure of rothein 1. Two 10 residue, disulfide-containing neuropeptides signiferin 1 and riparin 1 from dermal secretions of frogs of the *Crinia* genus, show potent smooth muscle and splenocyte activities. The dynamics of the interaction of signiferin 1, riparin1 and rothein 1 with artificial eukaryotic (neutral) lipid bilayer suspensions were probed using solid-state NMR, to emulate how a neuropeptide interacts with a cellular membrane surface prior to receptor binding. Solid-state NMR showed that rothein 1 had little effect on the mobility and orientation of the lipids, signiferin 1 interacted largely at the surface of the bilayers, and riparin 1 was partially inserted into the membrane. Rothein 1 is significantly less active than the disulphide peptides and more hydrophilic in nature; this is reflected in the interactions with bilayers. The disulphide peptides are more hydrophobic in character and the solid-state NMR indicated that they adhere to membranes.

Abbreviations

1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
ARIA	Ambiguous Restraints for Iterative Assignment
CCK	Cholecystokinin
CCK1R	Type I cholecystokinin receptor
CCK2R	Type II cholecystokinin receptor
CID	Collision induced dissociation
C_L	Lethal concentration
CNS	Crystallography and NMR system
COSY	Correlation spectroscopy
CSA	Chemical shift anisotropy
DC	Direct current
DMPC	Dimyristoyl phosphatidylcholine
DMPG	Dimyristoyl phosphatidylglycerole
DNA	Deoxyribonucleic acid
DPC	Dodecylphosphotydylcholine
DPI	Dual polarisation interferometry
DQF	Double quantum filtered
ESI	Electrospray ionisation
ESMS	Electrospray mass spectrometry
ETD	Electron transfer dissociation
GUV	Giant unilamellar vesicle
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum coherence
HV	High volume
IC_{50}	Half maximal (50%) inhibitory concentration
IRMPD	Infrared multiphoton dissociation
L_α	Lamellar phase
L-NNA	L-N-nitroarginine
MCP	Microchannel Plate
MIC	Minimum inhibitory concentration
MLV	Multi-lamellar vesicle
MOPS	3-(*N*-morpholino)propanesulfonic acid
MPA	3-mercaptopropionic acid

mRNA	Mature ribonucleic acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NMR	Nuclear magnetic resonance
nNOS	Neuronal nitric oxide synthase
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy
PC	Phosphatidylcholine
PG	Phosphatidylglycerole
QCM	Quartz crystal microbalance
QCM-D	Quartz crystal microbalance with dissipation monitoring
RF	Radiofrequency
RMD	Restrained molecular dynamics
RMSD	Route mean standard deviation
RNA	Ribonucleic acid
SA	Simulated annealing
S_{CD}	Carbon-deuterium order parameter
SES	Surface electrical stimulation
SLB	Supported lipid bilayer
TFE	Trifluoroethanol
TMS	tetramethylsilane
TOCSY	Total correlation spectroscopy
TOF	Time of flight
TQF	Triple quantum filtered
VMD	Visual molecular dynamics
YM022	(R)-1-[2,3-dihydro-1-(2'-methyl-phenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea