

# Structural and Mechanistic Studies of Bioactive Peptides

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A thesis submitted for the Degree of Doctor of Philosophy

by

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August 2006

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## ~ ACKNOWLEDGMENTS ~

First and foremost I would like to offer a sincere thank you to my supervisor, Prof. John Bowie, for allowing me to undertake this research. This project has presented me with many interesting challenges and opportunities for which I am extremely appreciative, and I owe a great deal to his knowledgeable guidance and considerate advice.

I also gratefully acknowledge the Ferry Trust and the University of Adelaide for the Ferry and George Fraser Scholarships respectively, which provided financial support during my Ph.D studies.

In addition, I would like to recognise the help of a number of external collaborators. Many thanks to Assoc. Prof. Frances Separovic from the University of Melbourne for introducing me to the world of solid state NMR, and to past and present members of her group for all of their assistance during my visits. Thanks also to Dr. Jennifer Wilson of Griffith University for providing the resources and skills to undertake cancer cell work, and also for welcoming me into her home during my stay. Much appreciation also goes to Dr. Jenny Beck from the University of Wollongong for assistance with the calmodulin work and time on the mass spectrometer, and to Dr. Lucia Kuhn-Nentwig from the University of Bern for kindly providing the opportunity to leap from frogs to spiders.

A big thank you must also go to the academic, research and technical staff at the University of Adelaide for all of their advice and assistance, in particular Phil Clements for his help with NMR spectroscopy and mass spectrometry, Prof. John Carver for many valuable NMR discussions, Jeff Borkent for help with numerous computer problems, and Dr. Chris Cursaro for operating the Edman sequencer. Thanks also to Dr. Stephen Donnellan and Dr. Terry Bertozzi for providing the resources and guidance necessary for the hybrid DNA studies, as well as Dr. Grant Booker, in addition to past and present members of the Booker research group, for helping with everything biochemistry.



Much appreciation also goes to Prof. Michael Tyler for assistance in collecting the frog secretions and samples, as well as sharing an incredible wealth of amphibian knowledge. I would also like to say thank you to Harvey and Margaret Vaux for breeding and caring for the hybrids, as well as allowing me to visit for regular ‘milkings’.

Many thanks to Dr. Craig Brinkworth, Dr. Mark Fitzgerald, Dr. Margit Apponyi and Hayley Andrezza for making lab 2 an enjoyable place to work over the years. Thanks also to remaining members of the Bowie group, in particular Daniel Bilusich and Rebecca Jackway for help with proofreading.

To Brett Miller, I thank you with all my heart for the patience, love and happiness that you have given me over the past few years. I look forward to discovering life after Ph.D with you.

Finally, I would like to thank my family for the amazing support they have provided throughout my life. Sincere love and gratitude to Mum, Dad and Joshua for the encouragement and assistance which has allowed me to reach my goals and dreams.

## ~ 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 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.

I give consent for a copy of this thesis, when deposited in the University Library, to be available for loan and photocopying.

Tara Louise Pukala

August 2<sup>nd</sup> 2006

## ~ ABSTRACT ~

Venoms, toxins and host-defence systems constitute rich sources of biologically active molecules, many of which have enormous therapeutic and biotechnological potential. In particular, peptides are often a significant component of these chemical arsenals, and are fundamentally important as biological effector molecules. The research presented in this thesis is centred on the isolation and investigation of peptides from both frogs and spiders, and endeavours to probe the important structural and mechanistic features of these bioactive compounds.

The skin peptide profiles of interspecific hybrids between the green tree frog *Litoria caerulea* and the magnificent tree frog *Litoria splendida* have been investigated in a nine-month survey. Fourteen peptides were characterised primarily using mass spectrometry, of which three had not been identified previously in the skin secretions of either parent. A number of these peptides are antibacterial agents, while others effectively inhibit the formation of nitric oxide by neuronal nitric oxide synthase. Implications for the genetics and expression of amphibian dermal peptides are also discussed.

The majority of frogs of the genus *Litoria* contain at least one peptide in their glandular secretion capable of inhibiting the formation of nitric oxide by the enzyme neuronal nitric oxide synthase. This was proposed to occur by preventing the association of the regulatory cofactor, Ca<sup>2+</sup>-calmodulin, with its binding site on the enzyme. Non-covalent binding of the amphibian peptides to calmodulin in the presence of Ca<sup>2+</sup> has been confirmed using electrospray ionisation mass spectrometry, by the observation of complexes in the gas phase with a 1:1:4 calmodulin/peptide/Ca<sup>2+</sup> stoichiometry. In addition, the structure and binding interactions of caerin 1.8, a potent nitric oxide synthase inhibitor, have been further probed using mass spectrometry and nuclear magnetic resonance spectroscopy techniques.

Recently a number of small, disulfide-containing neuropeptides of the signiferin and riparin families have been characterised from the skin secretion of frogs of the *Crinia* genus. Of these, signiferin 1 and riparin 1.1 are both ten residue peptides with similar primary sequences, however appear to have a significantly different spectrum of bioactivity. Although both act at cholecystokinin-2 receptors, signiferin 1 is smooth muscle active while riparin 1.1 is not, and instead causes proliferation of lymphocytes. The three-dimensional structures of these peptides were determined using nuclear magnetic resonance spectroscopy and restrained molecular dynamics calculations. Both signiferin 1 and riparin 1.1 adopt  $\beta$ -turn type conformations, however differences in these structures may be responsible for the variation in biological activity noted for these peptides.

The dermal secretions of most Australian frogs contain at least one broad-spectrum peptide antibiotic, and often a series of peptides with differing activity to afford greater protection against microbial pathogens. Solid state nuclear magnetic resonance spectroscopy studies were carried out to investigate the interaction of a number of these antibacterial peptides with anionic model membranes, and the results are compared with work previously reported using neutral lipids. It appears the peptides may have a different mode of interaction with the membranes depending upon the charge of the lipid head group.

The cupiennin 1 peptides have been identified in the venom of the neotropical wandering spider, *Cupiennius salei*, and demonstrate potent wide-spectrum antibacterial activity. Primary sequence analysis of these peptides suggests a unique amphipathic structure distinctly different from that of other potentially helical cationic antimicrobial peptides isolated thus far. Using nuclear magnetic resonance spectroscopy and restrained molecular dynamics calculations, cupiennin 1a was found to adopt an  $\alpha$ -helical structure with a flexible central hinge region in membrane mimicking solvents. Following this, nuclear magnetic resonance spectroscopy methods were used to further probe the antibacterial and the newly identified neuronal nitric oxide synthase inhibitory activity of this peptide.