Amphibian skin peptides which inhibit nNOS: Structure and binding studies using heteronuclear NMR

A thesis submitted for the degree of Doctor of Philosophy

by

Margit Anneliese Apponyi B.Sc. (Hons)

from
The Department of Chemistry
The University of Adelaide

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Table of contents

Abstract

Using 2-D NMR spectroscopy, the structure of the sex pheromone from *Litoria splendida* has been determined, in order to elucidate its mode of transport through the aquatic environment. The peptide was found form an α-helical structure, with a central flexible hinge region. The mode of transport through the aquatic environment has been discussed in relation to the structure.

Previous work indicated that the Australian amphibian host defence skin peptides that inhibit neuronal nitric oxide synthase (nNOS) were likely to act indirectly on the enzyme, by binding to the co-enzyme of nNOS, calmodulin. ^{15}N labelled calmodulin was expressed and purified *via* a bacterial protein expression system and a series of 2-D NMR ^{15}N-HSQC titrations was performed with Australian amphibian host defence skin peptides. in order to determine whether these peptides bind to calmodulin. The three peptides tested were found to bind, and with differing strengths of interaction. One of these was selected for further study.

^{15}N and ^{13}C doubly labelled calmodulin was then prepared in order to study the complex between this protein and the selected peptide, caerin 1.8, an Australian amphibian skin peptide isolated from *Litoria chloris*. A series of 3-D NMR spectra has been recorded on this complex. The backbone atom resonances have been assigned for free calmodulin and for the calmodulin-peptide complex, using a combination of main chain directed and sequential assignment strategies. By analysing the changes in chemical shift that occur upon binding the peptide, it was determined that the mode of binding involves a stronger interaction with the C-terminal domain than the N-terminal domain..

Statement of originality

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

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

Margit Anneliese Apponyi

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List of figures

List of tables