Amphibian Peptides: Their Structures and Bioactivity

A thesis submitted for the Degree of Doctor of Philosophy

by

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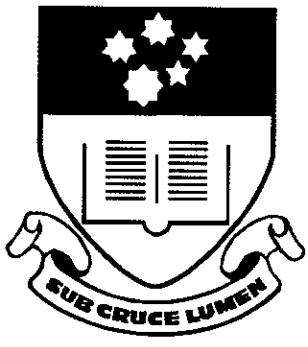

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Abstract

Amphibians secrete a cocktail of chemicals onto their skin in response to stressful stimuli, such as attack by predators. The compounds secreted include bioactive peptides, which can protect against infection, relieve pain and regulate aspects of the animal's physiology. Such peptides are potential therapeutic agents and can also be used to investigate the relationships between different amphibian species.

The skin secretion of the northern Australian frog *Litoria dahlii* has been investigated, with eleven novel peptides identified. These peptides have moderate biological activity, including antibacterial and anticancer actions, as well as the capacity to inhibit the enzyme neuronal nitric oxide synthase. The similarities between the isolated peptides and those from *L. aurea* and *L. raniformis* from southeast Australia, suggest these species are related, despite their geographical separation.

Many potent broad-spectrum antibiotics have previously been isolated from Australian amphibians. These peptides are believed to act by disrupting the bacterial cell membrane by forming transmembrane 'barrel-stave' type ion channels, lipid-incorporating toroidal pores or by assembling as a 'carpet' over the membrane surface.

The structures of several antibacterial peptides were determined using nuclear magnetic resonance spectroscopy and restrained molecular dynamics calculations. The short peptides citropin 1.1 (16 residues) and aurein 1.2 (13 residues) both form linear amphipathic α-helices, while caerin 1.1 and caerin 1.4 (both 25 residues) form amphipathic α-helical structures with flexible hinge regions around proline 15.

Oriented circular dichroism and solid-state nuclear magnetic resonance spectroscopy studies were used to investigate the orientation of citropin 1.1 and aurein 1.2 in neutral lipid bilayers. The experimental results suggest these peptides operate by the 'carpet' mechanism.