The Structure of Caerin 1.1, a Novel Antibiotic Peptide from Australian Tree Frogs

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The structure of caerin 1.1, a novel peptide with biological activity from Australian tree frogs, is determined on the basis of mass spectrometric data; the peptide's primary sequence prevents it from achieving a complete α-helix.

Amphibians live in environments where there are numerous microorganisms and animal predators, because of this they have evolved dermal secretions that contain a variety of host defence chemicals, including toxins and antibiotics, many of which are peptides.[1] Previous studies have often reported the killing of large numbers of animals in order to isolate peptides from their skins.[1,2] Recently, however, a benign method utilising injection of norepinephrine has been used to stimulate the release of glandular secretions from *Xenopus laevis*[3] and *Bombina orientalis*.[4]

In this study, we have used mild electrical stimulation[5] of the surface of the parotoid glands of the closely related Australian tree frogs *Litoria caerulea*, *L. splendida* and *L. gilleni*[6] to effect release of their glandular secretions, a method that allows regular 'milking' with no apparent adverse effects on the animal. We have characterised thirty five peptides following HPLC separation of the secretions. One of these, the hypotensive peptide caerulin **1** has been reported[7] and has found a number of clinical applications.[8]

Pyr Gln Asp Tyr(SO_3H) Thr Gly Trp Met Asp Phe(NH_2)

1

Gly Leu Leu Ser Val Leu Gly Ser Val Ala Lys His Val Leu Pro His Val Val Pro Val Ile Ala Glu His Leu(NH_2)

2

The major component of the glandular secretion of each of the three *Litoria* species is a novel peptide of molecular weight 2582, which we call caerin 1.1.[9] Caerin 1.1 **2** has twenty-five amino acid residues and bears no more than 38% identity with any peptide reported previously.[10] Although structurally quite different, it may nevertheless be placed in the same general category of amphibian peptides as the magainins, isolated from the African clawed frog *Xenopus laevis*,[3,11] and the bombinins from *Bombina* species.[4,12]

The structure of caerin 1.1 has been determined primarily by fast atom bombardment mass spectrometry (FABMS). Manual Edman degradation/FABMS identified the first ten amino acid residues, with the proviso that the method does not differentiate between isomeric Leu and Ile. Tryptic digest of caerin 1.1 cleaves the peptide between Lys(11) and His(12), yielding two peptides of molecular weights 1043 and 1558, whose structures were investigated by manual Edman/FABMS and collision-induced tandem (MS–MS) mass spectrometry. For example, sequential Edman degradation/FABMS of the latter peptide gives the sequence His Val Leu(or Ile) Pro His Val Val. The peptide remaining after the removal of these amino acids, has an $(M + H)^+$ ion at m/z 777. The amino acid sequence of this species is suggested to be Pro Val Leu(or Ile) Ala Glu His Leu(or Ile)(NH_2) by MS–MS (fragmentation) data. The final structural assignment for caerin 1.1, particularly the differentiation between Leu and Ile, has been confirmed using an automated sequencer,[13] and the molecule has been synthesised using the *tert*-BOC method.[14] The amphiphilic nature of caerin 1.1 may permit the molecule to adopt a helical conformation in solution, except that Pro(15) and Pro(19) will cause some distortion of the helix in their vicinity. A model of the peptide indicates that it may be a bent rod, 4.0 nm in length.

Caerin 1.1 shows promising antibiotic and antiviral activity: for example against *Staphylococcus aureus* (both the natural and synthetic peptides show activity within the same range) and also *Herpes simplex*.

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