Amphibian Antimicrobial Peptides: Their Structures and Mechanisms of Action.


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Corrections made in pencil (by JD B) following examiner comments for B C
Abstract

In the last decade or so, there has been an alarming increase worldwide in the number of antibiotic-resistant strains of pathogens. Efforts to find new drugs to combat this scourge have so far been of limited success. Amphibian skin is a rich source of potent antimicrobial peptides that has the potential to be developed into a new class of antibiotics. Most are specific only to pathogens and are found to exert their effects by disrupting their cytoplasmic membranes. The mechanisms by which they exert their biological effects are still a subject of controversy. Currently, two mechanisms have been proposed: (i) the channel mechanism where the peptides aggregate on the membrane surface to form a transmembrane pore. This disrupts the cell's osmoregulatory capability and results in osmolyis, and (ii) the carpet mechanism where the peptides orient themselves parallel to the membrane surface, forming a 'carpet' layer(s) before immersing themselves into the bacterial membrane, causing cell lysis.

Recently, three antimicrobial peptides, maculatin 1.1, uperin 3.6, caerin 4.1 were isolated from the respective skin glands of the Australian amphibians Litoria genimaculata, Uperoleia mjobergii and Litoria caerulea. To gain a deeper insight in their mechanism of action, three-dimensional structural studies have been conducted using circular dichroism, two-dimensional nuclear magnetic resonance and computer modelling techniques.

Experimental results reveal that all three peptides adopt an overall amphipathic α-helical conformation in membrane-mimetic environments. One of the peptides (maculatin 1.1) forms a helical structure with a 'kink' in the central region due to Pro15. Orientation studies using solid-state NMR studies suggest that 15N-labelled Ala7 maculatin 1.1 interacts with bacterial membranes via the carpet mechanism. Further investigations involving structure-activity relationship studies and the solution structures of synthetic variants were also conducted and compared. Results suggest that: (i) the bactericidal mechanism did not involve stereospecific binding sites or enzymes and (ii) the synthetic Ala15 analogue of maculatin 1.1 has markedly reduced activity, suggesting that the kink is important for biological activity. A
comparison was also made between using trifluoroethanol (TFE), a membrane-mimicking solvent and dodecylphosphocholine (DPC) micelles in water to investigate the reliability of TFE as a membrane-mimicking solvent. Results indicate that the three-dimensional structures produced in both systems are very similar, suggesting that membrane-interacting peptides in trifluoroethanol/water mixtures are representative of those adopted in a membrane environment. The role of central flexibility within antibiotic peptides in their interaction with bacterial membranes is also discussed.

Like maculatin 1.1, uperin 3.6 and caerin 4.1 also adopt overall α-helical conformations in TFE/water mixtures. Substituting the cationic residues in uperin 3.6 for neutral amino acid residues result in the loss of bioactivity, suggesting that electrostatic interactions are involved in the mechanism of bactericidal action. Caerin 4.1 is shown to possess narrow-spectrum antibiotic activities. Although it does not contain a central proline kink along the length of the helix like maculatin 1.1, it was found to have a central flexible region between Gly11 to Gly16. It is believed that its relatively rigid helical backbone conformation can only undergo limited conformational changes and thereby loses its wide-spectrum antibiotic capability.