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Biochimica et Biophysica Acta 1758 (2006) 1178–1183
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbamem

Review

Membrane interactions of antimicrobial peptides from Australian tree frogs

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Received 2 December 2005; received in revised form 4 February 2006; accepted 11 February 2006
Available online 9 March 2006

Abstract

The skin secretions of amphibians are rich in host defence peptides. The membrane interactions of the antimicrobial peptides, aurein 1.2, citropin 1.1 and maculatin 1.1, isolated from Australian tree frogs, are reviewed. Although all three peptides are amphipathic α-helices, the mode of action of these membrane-active peptides is not defined. The peptides have a net positive charge and range in length from 13 to 21 residues, with the longest, maculatin 1.1, having a proline at position 15. Interestingly, alanine substitution at Pro-15 leads to loss of activity. The effects of these peptides on phospholipid bilayers indicate different mechanisms for pore formation and lysis of model membranes, with the shorter peptides exhibiting a carpet-like mechanism and the longest peptide forming pores in phospholipid bilayer membranes.

Keywords: Peptide–lipid interactions; Model membranes; Antibacterial peptides; Solid-state NMR; Pore formation

Contents

1. Introduction

Host defence peptides are known to protect amphibians against a variety of pathogens [1]. The skin secretions of Australian tree frogs are rich in anti-bacterial peptides [2]. In order to exert their bioactive effects, the peptides must penetrate the cell membrane and the means by which they destroy bacteria is possibly by membrane lysis. The membrane interactions of peptides from Australian tree frogs have been studied, in particular, maculatin 1.1, citropin 1.1 and aurein 1.2; and also the peptides caerin 4.1 and caerin 1.1 but to a lesser extent. The amino acid sequences of these peptides [3–6] are given in Table 1.

The focus of this review is the antibacterial effect of these peptides. However, the peptides are known to demonstrate other bioactivity, including e.g. anti-cancer (aurein 1.2, caerin 1.1, citropin 1.1, maculatin 1.1) and both fungicidal and specific neuronal nitric oxide synthase inhibition (caerin 1.1, citropin 1.1, maculatin 1.1) [2]. Four of the peptides demonstrate anti-bacterial activity against both Gram-positive and Gram-negative species. The remaining peptide, caerin 4.1, shows a more specific range of antibacterial effect, preferentially lysing Gram-negative bacteria, including *Pasteurella haemolytica*, which causes swine fever. The antibacterial effects of the peptides are listed in Table 2.

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doi:10.1016/j.bbamem.2006.02.010

Table 1
Amino acid sequence of selected antibacterial peptides from Australian tree frogs

Peptide	Amino acid sequence	MW	AA	Net charge
Aurein 1.2 [3]	GLFDIIKKIAESF-NH_2	1478	13	+1
Caerin 1.1 [4]	GLLSVLGSVAKHVLPHVVPVIAEHL-NH_2	2582	25	+3
Caerin 4.1	GLWQKIKSAAGDLASGIVEGIKS-NH_2	2326	23	+4
Citropin 1.1 [5]	GLFDVIKKVASVIGGL-NH_2	1613	16	+2
Maculatin 1.1 [6]	GLFGVLAKVAAHVVPAIAEHF-NH_2	2145	21	+3

Host defence peptides are produced by Australian tree frogs as inactive three part peptides: a signal peptide, a spacer peptide and the anti-bacterial peptide. After synthesis the peptides are transported to storage glands on the dorsal surface of the animal, where the signal peptide is cleaved by an endopeptidase. The resulting spacer-active peptide combination does not exhibit antibacterial effect. Upon appropriate stimulation, the spacer peptide is cleaved by a second endopeptidase and the active peptide is secreted onto the dorsal surface of the amphibian [7]. The peptides are deactivated by a third endopeptidase, deactivation occurring between 5–30 min after secretion depending upon the species of frog [8]. The enzymes appear to be membrane proteins [8] and deactivate these membrane-active peptides by removal of residues from the N-terminus [5]. The peptides, many of which have major wide-spectrum antibacterial properties, are expressed in the skin secretions when the frog is stressed.

2. Common structural motifs

The peptides discussed in this study consist of between 13 (aurein 1.2) and 25 (caerin 1.1) amino acid residues. Each of the peptides are cationic around neutral pH, with a net positive charge between +1 (aurein 1.2) and +4 (caerin 1.1). Features common to these peptides include a tendency towards random coil arrangement in aqueous solution and an α-helical structure in membrane mimetic environments [3–6]. The helixes are amphipathic with polar side chains aligning along one face of the α-helix.

The primary structures of the peptides have several notable features. The N-terminus of each peptide consists of two common residues, glycine and leucine. Aurein 1.2, citropin 1.1 and maculatin 1.1 share a third common N-terminal amino acid, phenylalanine. Three of the peptides share the motif of adjacent basic amino acids that are essential for anti-bacterial activity [2]. Aurein 1.2 and citropin 1.1 contain lysine residues at positions 7 and 8, while caerin 1.1 contains a lysine–histidine arrangement at positions 11 and 12. Maculatin 1.1 does not contain adjacent basic amino acids, but does have a lysine residue at position 8. Each of the peptides is also C-terminal aminated and, again, this functional group is essential for anti-bacterial action [9].

These peptides may be divided into two groups based upon peptide length and conformation when in membrane mimetic environments. The shorter peptides aurein and citropin both adopt a single continuous α-helix upon membrane binding. The longer peptides comprise a flexible hinge region separating two α-helices. Maculatin 1.1 contains one proline residue while caerin 1.1 has two proline residues, which act to form a hinge region [6,10]. The presence of the proline residue is known to modulate the efficacy of maculatin 1.1 [11] and caerin 1.1. [12]. The region of conformational flexibility of caerin 4.1, on the other hand, contains two glycine residues [13].

3. Models of peptide interaction with lipid membranes

Two principal modes of action for membrane-perturbing peptides have been proposed: pore formation across the lipid bilayer or a ‘carpet’ mechanism, lysing the membrane in a detergent-like manner [14]. The transmembrane models involve the peptides forming pores through the bacterial outer membrane: the ‘barrel-stave’ [15] and toroidal pore [16,17] mechanisms. In these models, the peptides oligomerize to form pores through the membrane. The pores act as non-selective channels for ions, toxins and metabolites, thus preventing the bacterium from maintaining homeostasis. Peptides with 20 or more amino acids lend themselves to these mechanisms, as they are able to span the lipid bilayer when in an α-helical conformation.

A key difference between these two mechanisms is the positioning of the head group region of the lipid molecules with respect to the peptide. In the barrel-stave mechanism, the headgroups remain located along the membrane surface, while the pore is formed by the interaction of the peptide within the hydrophobic core of the membrane. The transmembrane pore is lined by the hydrophilic surface of the peptide. By contrast, toroidal pores are formed when the peptides insert in such a way as to cause the inner and outer membrane leaflets to curve and

Table 2
Antibacterial activity of selected peptides from Australian tree frogs [2]

	Aurein 1.2	Caerin 1.1	Caerin 4.1	Citropin 1.1	Maculatin 1.1
Bacillus cereus	50	50	–	50	25
Leuconostoc lactis	6	1.5	–	6	3
Listeria innocua	6	25	–	25	100
Micrococcus luteus	100	12	12	12	12
Staphylococcus aureus	–	3	–	25	6
Staphylococcus epidermidis	50	12	–	12	12
Streptococcus uberis	100	12	–	25	3
Escherichia coli [a]	–	–	25	–	–
Pasteurella multocida [a]	–	25	–	–	50

Minimum inhibitory concentration (μg/mL).
[a] Gram negative bacteria.