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Review

Membrane interactions of antimicrobial peptides from Australian frogs

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ABSTRACT

The membrane interactions of four antimicrobial peptides, aurein 1.2, citropin 1.1, maculatin 1.1 and caerin 1.1, isolated from Australian tree frogs, are reviewed. All four peptides are amphipathic α-helices with a net positive charge and range in length from 13 to 25 residues. Despite several similar sequence characteristics, these peptides compromise the integrity of model membrane bilayers via different mechanisms; the shorter peptides exhibit a surface interaction mechanism while the longer peptides may form pores in membranes.

Contents

1. Introduction

Antibiotics are typically directed at structural or enzymatic targets that are unique to bacteria. The traditional target for a majority of current antibiotics (e.g. beta-lactam antibiotics such as the penicillin and cephalosporin series [1]), is the cell wall structure and machinery. Alternate classes of antibiotics exploit differences in the prokaryotic and eukaryotic ribosomes (e.g. the aminoglycosides [2]), and other critical biosynthetic machinery, such as the enzyme inhibiting quinolones [3,4]. As multiple drug resistant bacterial strains proliferate against this current range of antibiotics, research is underway to identify and capitalize on other differences between infectious and eukaryotic cells. Differences between prokaryotic and eukaryotic membrane compositions offer one such possibility, whereby molecular agents would preferentially interact with and disrupt microbial membrane integrity.

Many amphibians have evolved to secrete a wealth of novel compounds from their skin [5], which possess potent activity toward a range of microbial targets [6]. These sources may provide the necessary leads for the rational development of this new class of specific and broad-banded antibacterial agents. We focus on four natural peptides, aurein 1.2, citropin 1.1, maculatin 1.1 and caerin 1.1, which have been isolated from the skin secretions of the frog species: *Litoria aurea*, *L. raniformis*, *L. citropa*, *L. genimaculata*, *L. splendida*, *L. caerulea* and *L. gilleni*. While these species secrete a number of related peptides, we concentrate on the membrane interactions of these four, which represent much of the sequence homology as well as the contrast in length among these peptides. Our studies aim to characterise the sequence and structure determinants of corresponding mechanisms and reported activity in a number of applications, including broad spectrum antibacterial [6–11], anti-fungal [6], anti-cancer [6,12], and neuronal nitric oxide synthase inhibitory activity [6].

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

Aurein 1.2	GLFD I I	KK	I A					ESF
Citropin 1.1	GLFD VI	KK	VA	SV	I			GGL
Maculatin 1.1	GLFG VL	AK	VA	AH			VVPAIA	EHF
Caerin 1.1	GLLS VL	GS	VA	KH	VL	PH	VVPVIA	EHL

The amino acid sequences of selected antimicrobial peptides from Australian tree frogs are shown [19,23,63–65]. Hydrophilic residues are depicted in bold and underlined. The sequences are separated into sections based on similarities in polarity and residue type.

With respect to antimicrobial activity, effectiveness against a wide range of bacteria makes it highly unlikely that these peptides act via specific receptor mediated processes, but rather by membrane disruption [7,13,14]. Furthermore, maculatin 1.1 and several similar cationic antimicrobial peptides synthesised from all D-amino acids have essentially equivalent activity to the natural peptides [8,11,15,16], thus indicating that chirality has no influence on activity and lending support for the membrane disruption hypothesis.

Each of these four peptides is cationic with a calculated pI between 9.9 and 10.6. Each peptide behaves similarly in aqueous solution, existing in an unstructured random coil conformation, and re-arranges into an amphipathic α-helix on partitioning into membrane or membrane mimetic environments [8,17–19]. All four peptides (Table 1) have significant sequence homology in the N- and C-termini, as well as in the intervening sequences even as this central segment increases from aurein (13 residues), to citropin (16 residues), maculatin (21 residues), and caerin (25 residues). Each of the peptides maintains an amphipathic residue pattern, with a polar (or proline) residue every three to four residues in the sequence. Within the context of an α-helix, this places all polar residues, including the interruption in hydrogen bonding pattern introduced by the prolines, along one surface of the helix. If the helical turns are indexed according to the polar residues, the hydrophobic residues contained within the second and subsequent turns are rich in the β-branched amino acids valine and isoleucine. The C-terminus of each peptide is also amidated, which is essential for activity [8,10].

2. Biological activity

The peptides studied are all active against a range of organisms. The activities of these peptides towards several different bacterial species are given in Table 2. In some cases, the activities of the peptides have been recorded using different techniques and with different isolates of bacteria. For example, two values for activity of maculatin 1.1 are quoted in Table 2. One set of values was calculated using the method of zone inhibition [20] on agarose plates containing the bacteria, while the second set of values was determined using the serial dilution technique [21], in a cell suspension containing a known quantity of bacteria. Dennison et al. [22] found no association between the minimum inhibitory concentration (MIC) and the sequence length or molecular weight of α-helical antimicrobial peptides; nevertheless in these four specific peptides, while individual activities vary significantly among the organisms surveyed, there remains a trend for activity to increase with increasing peptide length [6].

The increase in antimicrobial activity is, however, also accompanied by a four-fold increase in haemolytic activity on going from aurein 1.2 [23] to caerin 1.1 [6]. Haemolytic activities of these peptides have been reported in several publications that often do not fully specify the method or blood source [9]. Therefore, there is a need to standardise measurement of peptide activity. Despite the trend for haemolytic activity to follow antimicrobial potency, the largest of the four peptides still displays a very useful therapeutic index (calculated by the ratio of haemolytic to anti-microbial activity). The peptides also display some anti-cancer activity [6], possibly owing to an increase in anionic surface charge relative to healthy cells [24–26], similar to bacterial membranes,which suggests that membrane interaction rather than receptor binding is the mode of peptide action. Aurein 1.2, in particular, has been found to be active against at least 55 human cancers [12]. In lipid monolayers containing anionic phosphatidylserine (PS) and phosphatidylglycerol (PG), used to mimic cancer cells, similar interactions are observed with aurein 1.2 as for mimics of cancer cell membranes [12]. Furthermore, removal of PG and PS from the monolayers reduces the interaction of aurein by 67% [12]. Interactions with anionic lipids appear to be the major determinant in the peptide affinity towards cancer cells, reinforcing the view that negatively charged membrane components form the basis of peptide selectivity towards certain cell types. The presence of negatively charged sialic acids in cancer cells would further enhance the peptide interaction relative to model bilayer systems.

While each displays a broad range of activity, the peptides are far more specific towards Gram-positive bacteria [6]. The lack of activity towards Gram-negative organisms can perhaps be attributed to the more complex protective structures, including the outer membrane and periplasm, which is lacking in Gram-positive species and would allow the cationic peptides freer access to the net negatively charged bacterial membrane [27].

3. Mechanisms of membrane disruption

Two general mechanisms for membrane disrupting peptides have been proposed [28,29]. Peptides of greater than 20 amino acid residues can form α-helices of sufficient length to individually span a lipid

Table 2
Antibacterial activity of selected peptides from Australian tree frogs.

Organism	MIC (μg/mL)[a]						
	Aurein 1.1 [6,7]	Citropin 1.1 [6,7,66]	Maculatin 1.1 [6–8,10]	MaculatinP15A [8,9]	Caerin 1.1 [6–8,10,11,67]	Caerin P→G [6,11]	Caerin P→A [6]
Bacillus cereus	100	50	25	>100	50	50	>100
Leuconostoc lactis	12	6	3	12	1.5	12	25
Listeria innocua	100	25	100	100	25	50	>100
Micrococcus luteus	100	12	12	50	12	12	>100
Staphylococcus aureus	50	25	6, 17*	50–100, 8*	3	25–50	>100
Staphylococcus epidermis	50	12	12	100	12	100	>100
Streptococcus faecalis			25	>100	25		
Streptococcus uberis	50	25	3	50	12	12	>100
Escherichia coli	>100	>100	>100, 68*	>100, 135*	>100	50	>100
Pasteurella multocida	100	>100	50	>100	25	100	>100

[a] Activities stated above are based on the measurement of inhibition zones caused by the applied peptide to thin agarose plates containing the organism, following the method of Jorgensen et al. [20].

* Activities measured using the technique of Yoshida et al. [21] and are based on a cell suspension containing 10^4 cells/mL, with the peptide solution added at different dilutions. Following a 24 hour incubation at 37 °C, the absorbance was measured at 620 nm.