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Peptides 24 (2003) 1681–1691
PEPTIDES
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The relationship between peptide structure and antibacterial activity

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Received 30 April 2003; accepted 8 August 2003

Abstract

Cationic antimicrobial peptides are a class of small, positively charged peptides known for their broad-spectrum antimicrobial activity. These peptides have also been shown to possess anti-viral and anti-cancer activity and, most recently, the ability to modulate the innate immune response. To date, a large number of antimicrobial peptides have been chemically characterized, however, few high-resolution structures are available. Structure–activity studies of these peptides reveal two main requirements for antimicrobial activity, (1) a cationic charge and (2) an induced amphipathic conformation. In addition to peptide conformation, the role of membrane lipid composition, specifically non-bilayer lipids, on peptide activity will also be discussed.

Keywords: Antimicrobial cationic peptide; Polyphemusin; Structure

1. Introduction

Cationic antimicrobial peptides are generally defined as peptides of less than 50 amino acid residues with an overall positive charge, imparted by the presence of multiple lysine and arginine residues, and a substantial portion (50% or more) of hydrophobic residues. These peptides can possess antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi [69] and protozoa [1] and have demonstrated minimal inhibitory concentrations (MIC) as low as 0.25–4 μg/ml [32]. Certain cationic peptides have been shown to inhibit the replication of enveloped viruses such as influenza A virus [71], vesicular stomatitis virus (VSV) and human immunodeficiency virus (HIV-1) [59,70]. Cationic peptides may also possess anticancer activity [3,44] or promote wound healing [24]. Recent studies have also indicated a role for cationic peptides as effectors of innate immune responses. It is these properties that make cationic peptides exciting candidates as new therapeutic agents.

2. Structure

Currently, more than 500 cationic antimicrobial peptides have been isolated from a wide range of organisms and can be found in the Antimicrobial Sequences Database (http://www.bbcm.univ.trieste.it/~tossi/antimic.html). Peptides are classified based on their structures of which there are four major classes: β-sheet, α-helical, loop, and extended peptides [32], with the first two classes being the most common in nature. For clarity, representative structures from each of these classes are indicated in Fig. 1. In addition to the natural peptides, thousands of synthetic variant peptides have been produced which also fall into these structural classes. A common trait shared amongst the cationic antimicrobial peptides is the ability to fold into amphipathic or amphiphilic conformations, often induced by interaction with membranes or membrane mimics.

Of the known cationic antimicrobial peptides, the majority have been characterized by circular dichroism spectroscopy. While this imparts relevant information as to the structural class a peptide may belong to, it is not equivalent to the determination of a detailed three-dimensional conformation. It is therefore interesting to determine a high-resolution structure for each compound, so that structure activity relationships can be explained. To this end, the structures of 50 cationic antimicrobial peptides have been elucidated, primarily by two-dimensional proton NMR. A list of the peptides for which structures are available is recorded as Table 1. The peptides in this list have been grouped according to structural class and the Protein Data Bank (PDB) ID codes are indicated. In compiling this list it was necessary to eliminate the many partial structural analyses that have been published. In addition, only published structures with coordinates that have been deposited in the PDB are reported.

Of the 50 peptide structures determined to date, 36 are naturally occurring and the remaining 14 are synthetic

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doi:10.1016/j.peptides.2003.08.023

Table 1
Cationic antimicrobial peptides with available high-resolution three-dimensional structures

Peptide	Class	Host/synthetic	PDB ID	Reference
CA-MA	α-Helix	Synthetic	1D9J	[73]
CA-MA analogue (P1)	α-Helix	Synthetic	1D9L	[74]
CA-MA analogue (P2)	α-Helix	Synthetic	1D9M	[74]
CA-MA analogue (P3)	α-Helix	Synthetic	1D9O	[74]
CA-MA analogue (P4)	α-Helix	Synthetic	1D9P	[74]
Carnobacteriocin B2	α-Helix	*Carnobacterium piscicola*	1CW5	[94]
G-10 Novispirin	α-Helix	Synthetic	1HU6	[83]
Magainin 2	α-Helix	*Xenopus laevis*	2MAG	[26]
Magainin 2 analogue	α-Helix	Synthetic	1DUM	[35]
Moricin	α-Helix	*Bombyx mori*	1KV4	[36]
Ovispirin-1	α-Helix	Synthetic	1HU5	[83]
Sheep myeloid antimicrobial peptide (Smap-29)	α-Helix	*Ovis aries*	1FRY	[89]
T-7 Novispirin	α-Helix	Synthetic	1HU7	[83]
γ-1-P thionin	β-Sheet	*Triticum turgidum*	1GPS	[8]
θ-Defensin 1	β-Sheet	*Macaca mulatta*	1HVZ	[92]
A. hippocastanum antimicrobial protein 1 (Ah-Amp1)	β-Sheet	*Aesculus hippocastanum*	1BK8	[16]
Androctonin	β-Sheet	*Androctonus australis*	1CZ6	[57]
Bovine neutrophil β-defensin 12 (BNBD-12)	β-Sheet	*Bos taurus*	1BNB	[102]
Circulin A	β-Sheet	*Chassalia parviflora*	1BH4	[10]
Drosomycin	β-Sheet	*Drosophila melanogaster*	1MYN	[53]
Gomesin	β-Sheet	*Acanthoscurria gomesiana*	1KFP	[55]
Heliomicin	β-Sheet	*Heliothis virescens*	1I2U	[52]
Heliomicin analogue	β-Sheet	Synthetic	1I2V	[52]
Hepcidin-20	β-Sheet	*Homo sapiens*	1M4E	[41]
Hepcidin-25	β-Sheet	*Homo sapiens*	1M4F	[41]
Human β-defensin 1 (Hbd-1)	β-Sheet	*Homo sapiens*	1KJ5	[84]
			1E4S	[4]
			1IJV	[40]
Human β-defensin 2 (Hbd-2)	β-Sheet	*Homo sapiens*	1E4Q	[4]
Human β-defensin 3 (Hbd-3)	β-Sheet	*Homo sapiens*	1KJ6	[84]
Human defensin (HNP-3)	β-Sheet	*Homo sapiens*	1DFN	[37]
Insect defensin A	β-Sheet	*Protophormia terraenovae*	1ICA	[9]
Lactoferricin B	β-Sheet	*Bos taurus*	1LFC	[43]
Leucocin A	β-Sheet	*Leuconostoc gelidum*	2LEU	[18]
			3LEU	
Mediterranean mussel defensin (MGD-1)	β-Sheet	*Mytilus galloprovincialis*	1FJN	[96]
Mouse β-defensin 7 (Mbd-7)	β-Sheet	*Mus musculus*	1E4T	[4]
Mouse β-defensin 8 (Mbd-8)	β-Sheet	*Mus musculus*	1E4R	[4]
P. sativum defensin 1 (Psd1)	β-Sheet	*Pisum sativum*	1JKZ	[2]
Pafp-S	β-Sheet	*Phytolacca americana*	1DKC	[25]
Protegrin-1 (Pg1)	β-Sheet	*Sus scrofa*	1PG1	[14]
Rabbit kidney defensin (RK-1)	β-Sheet	*Oryctolagus cuniculus*	1EWS	[68]
Ramoplanin	β-Sheet	*Actinoplanes* sp.	1DSR	[50]
Sapecin	β-Sheet	*Sarcophaga peregrina*	1LV4	[34]
Tachyplesin I	β-Sheet	*Tachypleus tridentatus*	1MA2	[51]
			1MA5	
Tachyplesin I analogue (Tpy4)	β-Sheet	Synthetic	1MA4	[51]
			1MA6	
Tachystatin A	β-Sheet	*Tachypleus tridentatus*	1CIX	[22]
Ac-AMP2	Extended	*Amaranthus caudatus*	1MMC	[58]
Indolicidin	Extended	*Bos taurus*	1G89	[82]
			1G8C	
Indolicidin analogue (CP10A)	Extended	Synthetic	1HR1	[21]
Pw2	Extended	Synthetic	1M02	[91]
Tritrpticin	Extended	Synthetic (potential porcine cathelicidin)	1D6X	[85]
Thanatin	Loop	*Podisus maculiventris*	8TFV	[56]

For the purposes of compiling this list, cationic antimicrobial peptide has been defined as less than 50 amino acid residues with an overall positive charge. Only published structures with PDB deposited coordinates are indicated.

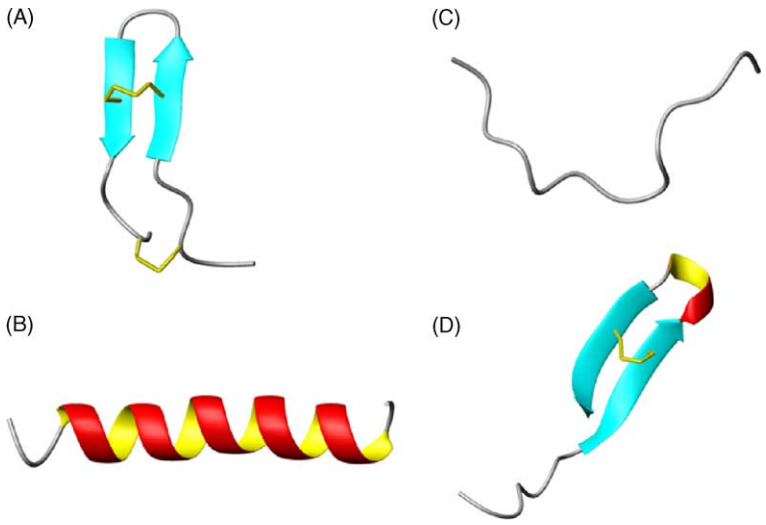

Fig. 1. Structural classes of antimicrobial peptides: (A) β-sheet, tachyplesin I [51]; (B) α-helical, magainin 2 [26]; (C) extended, indolicidin [82]; (D) loop, thanatin [56]. Disulfide bonds are indicated in yellow. Figure prepared with MOLMOL [48].

analogues. The naturally occurring peptides have been isolated from a wide variety of organisms: eight vertebrate, six arthropod, six plant, three bacteria, three insect and one mollusc species are represented. Of these organisms, humans are the most represented species with the structures of six peptides determined.

3. Mechanism of action

The mechanism of action of cationic antimicrobial peptides is being actively studied and the available information continues to grow. The majority of experiments to date have focused primarily on the interaction of cationic peptides with model membrane systems. Additional studies have also been conducted on whole microbial cells predominantly utilizing membrane potential sensitive dyes and fluorescently labeled peptides. These studies have indicated that all antimicrobial peptides interact with membranes and tend to divide peptides into two mechanistic classes: membrane disruptive and non-membrane disruptive. An alternative perspective is that as a group, cationic antimicrobial peptides have multiple actions on cells ranging from membrane permeabilization to cell wall and division effects to macromolecular synthesis inhibition and that the action responsible for killing bacteria at the minimal effective concentration varies from peptide to peptide and from bacterium to bacterium for a given peptide [20]. While this review will briefly discuss mechanisms, more detailed reviews can be consulted [13,31,33,42,75,87].

The mechanism of action on Gram-negative bacteria will be discussed since this has been best studied. An overview of the interaction of peptide with a Gram-negative bacterial envelope is shown as Fig. 2. The initial association of peptides with the bacterial membrane occurs through electrostatic interactions between the cationic peptide and anionic LPS in the outer membrane leading to membrane perturbation. It has been shown that cationic peptides have a higher affinity for LPS in the outer leaflet of the outer membrane of Gram-negative bacteria than do native divalent cations such as Mg^{2+} and Ca^{2+} [31]. The cationic peptides displace these cations from the negatively charged LPS leading to a local disturbance in the outer membrane. This facilitates the formation of destabilized areas through which the peptide translocates the outer membrane in a process termed self-promoted uptake [30]. Access to the cytoplasmic membrane is now possible. The peptides then associate with the outer monolayer of the cytoplasmic membrane. It is at this point that membrane disruptive and non-membrane disruptive mechanisms diverge, depending on whether this reorientation leads to perturbation of the integrity of the cytoplasmic membrane or peptide translocation into the cytoplasm.

4. Membrane disruptive peptides

Membrane disruptive peptides are generally reported to be of the α-helical structural class although, it should be strongly cautioned, that not all α-helical peptides are membrane disruptive. For example, buforin [76], CP10A [21] and a pleurocidin analogue [78] clearly do not have their primary action on membranes. Three mechanistic models, the “barrel stave”, “micellar aggregate” and “carpet” models, have been developed to explain membrane disruption. In the barrel-stave model [12], the amphipathic peptides reorient perpendicular to the membrane and align (like the staves in a barrel) in a manner in which the hydrophobic sidechains face outwards into the lipid environment while the polar sidechains align inward to form transmembrane pores. These pores are proposed to allow leakage of cytoplasmic components and also disrupt the membrane

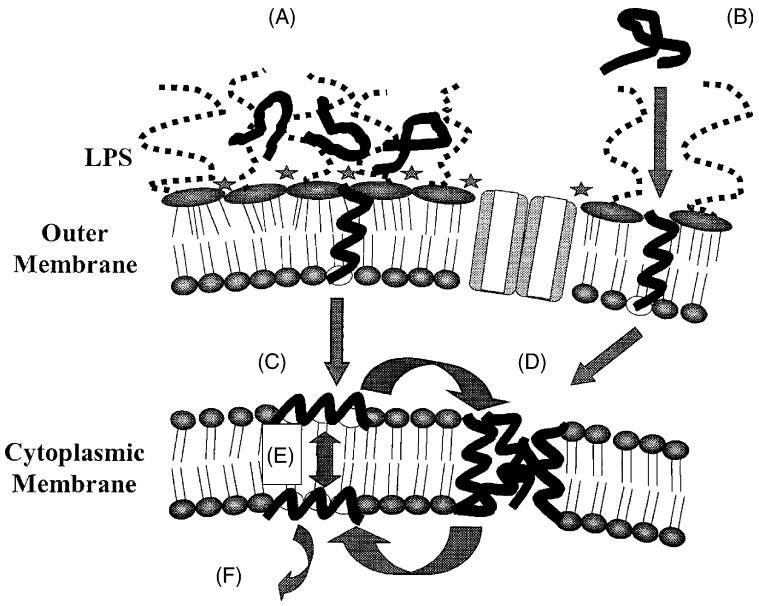

Fig. 2. Proposed mechanism of interaction of cationic antimicrobial peptides with the cell envelope of Gram-negative bacteria. Passage across the outer membrane is proposed to occur by self-promoted uptake. According to this hypothesis, unfolded cationic peptides are proposed to associate with the negatively charged surface of the outer membrane and either neutralize the charge over a patch of the outer membrane, creating cracks through which the peptide can cross the outer membrane (A), or actually bind to the divalent cation binding sites on LPS and disrupt the membrane (B). Once the peptide has transited the outer membrane, it will bind to the negatively charged surface of the cytoplasmic membrane, created by the headgroups of phosphatidylglycerol and cardiolipin, and the amphipathic peptide will insert into the membrane interface (the region where the phospholipid headgroups meet the fatty acyl chains of the phospholipid membrane) (C). It is not known at which point in this process the peptide actually folds into its amphipathic structure (i.e. during transit across the outer membrane or during insertion into the cytoplasmic membrane). Many peptide molecules will insert into the membrane interface and are proposed to then either aggregate into a micelle-like complex which spans the membrane (D) or flip-flop across the membrane under the influence of the large transmembrane electrical potential gradient (approximately 140 mV) (E). The micelle-like aggregates (D) are proposed to have water associated with them, and this provides channels for the movement of ions across the membrane and possibly leakage of larger water-soluble molecules. These aggregates would be variable in size and lifetime and will dissociate into monomers that may be disposed at either side of the membrane. The net effect of (D) and (E) is that some monomers will be translocated into the cytoplasm and can dissociate from the membrane and bind to cellular polyanions such as DNA and RNA (F). This figure is reproduced with permission from [31].

potential. The major argument against this model is the lack of preferred stoichiometries for the “pores” as demonstrated by the wide variability in conductance increases induced by peptides in model membranes [95].

The alternative micellar aggregate model [31,66] suggests that the peptides reorient and associate in an informal membrane-spanning micellar or aggregate-like arrangement and further indicates that collapse of these micellar aggregates can explain translocation into the cytoplasm.

In the alternative carpet model [79], the peptides do not insert into the membrane but align parallel to the bilayer, remaining in contact with the lipid head groups and effectively coating the surrounding area. This orientation leads to a local disturbance in membrane stability, causing the formation of large cracks, leakage of cytoplasmic components, disruption of the membrane potential and, ultimately, disintegration of the membrane.

Regardless of which model is correct, the net result of membrane disruption would be the rapid depolarization of the bacterial cell leading to rapid cell death, with total killing occurring within 5 minutes for the most active peptides [19]. It should be noted that membrane depolarization is not a lethal event per se, and in the absence of evidence of a catastrophic collapse of cytoplasmic membrane integrity, the specific way in which membrane disruption results in cell death is yet to be determined. It should also be noted that each of the above models might be correct depending on the peptide examined, such that certain peptides may function through a barrel-stave mechanism, while others may function through a micellar aggregate or carpet mechanism. It has been recently shown that sub-inhibitory concentrations of cecropin A, classified as a lytic peptide, induce transcriptional changes within bacteria [39]. Other studies have indicated that magainin 2 can translocate into the bacterial cytoplasm [63]. While the significance of these changes is yet to be determined, they may suggest a role for these peptides in a non-membrane disruptive fashion.

5. Other peptide mechanisms

Peptides that do not appear to act on membranes are thought to act on cytoplasmic targets. Translocation across membranes is proposed to occur by a process related to the micellar aggregate mechanism and has been demonstrated

for the frog-derived antimicrobial peptide buforin II since, rather than causing large membrane perturbations, the disruption is transient and permeabilization does not occur [77]. Other peptides demonstrate similar results [101]. Analogous translocation studies using eukaryotic cells have found that some arginine rich peptides are capable of translocating across both the cellular and nuclear membranes and can serve as delivery agents for conjugated compounds [23]. Once present in the bacterial cytoplasm, cationic peptides are thought to interact with DNA, RNA and/or cellular proteins and to inhibit synthesis of these compounds. Indeed, DNA and RNA binding has been demonstrated in vitro [76,97] and other studies have demonstrated the inhibition of macromolecular synthesis after treatment with sub-lethal peptide concentrations [54,78]. In addition, specific enzymatic targets have been identified for certain peptides. The proline-rich insect peptide, pyrrhocoricin, has been shown to bind the heat shock protein DnaK inhibiting chaperone-assisted protein folding [49] while the *Bacillis* lantibiotic, mersacidin has been demonstrated to bind lipid II leading to the inhibition of peptidoglycan biosynthesis [7]. For these peptides, loss of viability is much slower than with membrane-acting peptides, which exert their antimicrobial effects within minutes [27,28]. For pyrrhocoricin, the ability of the peptide to interfere with protein folding in live cells is not observed until 1 h after exposure [49] and observable cell lysis as a result of mersacidin treatment is not seen until 3 h after treatment [7].

6. Structure–activity relationships

Rather than attempt to sum up the great number of structure–activity relationship (SAR) studies that have been conducted to date, a representative peptide from each structural class is chosen for discussion below. For a more detailed review of specific peptides and structural classes there are numerous reviews that may be consulted.

6.1. β-Sheet peptides

This class of peptides is characterized by the presence of an antiparallel β-sheet, generally stabilized by disulfide bonds. Larger peptides within this family may also contain minor helical segments. Perhaps the best characterized β-sheet peptides are the small 17–18 residue tachyplesins (Fig. 1A). Isolated from the haemocytes of the Japanese horseshoe crab, *Tachypleus tridentatus* [72], the tachyplesins represent a convenient scaffold for structure–activity studies due to their small size and availability of a high-resolution ^{1}H NMR structure. The conformation of tachyplesin I is that of an antiparallel β-sheet (residues 3–8 and 11–16) connected by a type I β-turn (residues 8–11) stabilized by two disulfide bonds (residues 3 and 16 and residues 7 and 12) with an amidated C-terminus [47]. Tachyplesin I possesses moderate antimicrobial activity (<12.5 μg/ml MIC against *Escherichia coli* K12) [72] as well as a high affinity for lipopolysaccharides [38].

Although the structure and in vitro activity of the tachyplesins are well characterized, the exact mechanism of antimicrobial activity remains poorly understood. While it is known that the tachyplesins have a high affinity for LPS, it is thought that intracellular targets also exist. Indeed, it has been shown that tachyplesin I binds the minor groove of DNA [97]. Additional studies involving the related β-sheet peptide, polyphemusin I, demonstrate that these peptides are effective at inducing lipid flip-flop and undergoing membrane translocation but do not cause substantial calcein release from model membrane systems [101]. This suggests these peptides disrupt lipid organization leading to the translocation of peptide molecules across the bilayer but do not form long-lived pores or channels. Thus, these peptides may function through a micellar-aggregate or related model of translocation.

Several SAR studies have focused on the requirement of the disulfide bonds for the antimicrobial activity of these compounds. Linearization has been accomplished through adding chemical protecting groups [65,67,90] as well as amino acid substitution [80,90]. Studies involving linear tachyplesin chemically protected with acetomidomethyl groups (T-Acm) demonstrate reduced antimicrobial and antiviral activity of the linear compound [90] as well as a reduction in calcein release from model membranes [65]. Interestingly, although T-Acm was less effective at permeabilization of model membranes, it possessed greater membrane disrupting ability as assayed by measuring lipid chain orientation [65]. Additional studies, using liposomes and planar lipid bilayers, demonstrated that the linear analogue completely lacks the ability of the parent peptide to translocate across membranes [67]. Structural characterization of T-Acm by CD spectroscopy indicated a random coil conformation in H_2O [90] while polarized attenuated total reflection spectroscopy suggested an antiparallel β-sheet conformation in lipid environments [65].

Tachyplesin analogues linearized through amino acid substitution possessed similar properties to T-Acm. Cysteine residues were simultaneous substituted with aliphatic (A, L, I, V, M), aromatic (F, Y) or acidic (D) residues [80]. Structural analysis by CD spectroscopy indicated that the analogues primarily adopt unordered and α-helical patterns in aqueous and hydrophobic environments, respectively. In acidic liposomes, an isoleucine analogue was the only peptide to display a spectrum characteristic of β-sheet content, but this peptide was found to have reduced antimicrobial activity against *E. coli*.

From these studies it is apparent that, although the stabilizing disulfide bonds of tachyplesin are not absolutely required for antimicrobial activity, they are necessary to permit membrane translocation in model systems. Due to the observed differences in membrane disruption and permeabilization, it may be concluded that the mechanism of antimicrobial activity is different for the parent and linear peptides.

Recently, the solution and micelle-bound structures of tachyplesin and a linear analogue were determined by ^{1}H NMR and revealed major differences between the two forms [51]. Specifically, the association of tachyplesin with micelles (a membrane-like environment) triggers a conformational change leading to the bending of the molecule about the central arginine residues along with an associated exposure of specific hydrophobic side chains. A linear tachyplesin analogue in which the cysteine residues are substituted with tyrosine was randomly arranged in free solution but, when bound to micelles, adopted a conformation that differs from the hinged structure formed by the native tachyplesin. This indicates that the disulfide bonds impart a stabilizing force to the overall molecule and allow the (hinge-like) bending to occur and that this structural flexibility in what has been traditionally thought of as a rather rigid β-hairpin conformation permits or drives translocation across membranes. These studies thus highlight the need for high-resolution peptide structures, rather than simple conformational analyses by circular dichroism, to provide detailed structure–activity information.

6.2. α-Helical peptides

Peptides of the α-helical class are characterized by their α-helical conformation, and often contain a slight bend in the center of the molecule. In one study, this bending was critical for selectivity by suppressing haemolytic activity [100]. The α-helical magainins are representative of this structural class (Fig. 1B). Isolated from the skin of the African clawed frog, *Xenopus laevis*, magainin 1 and 2 are 23 residues in length and possess modest antimicrobial activities (e.g. MIC of 50 μg/ml versus *E. coli*) [98]. The structure of magainin 2 has been determined by ^{1}H NMR in the presence of DPC and SDS micelles. The peptide adopts an amphipathic α-helical conformation with a slight bend centered at residues 12 and 13 [26].

The antimicrobial mechanism of magainin has been proposed to involve selective permeabilization of bacterial membranes leading to disruption of the membrane potential [60]. This mechanism is further supported by the observation that there are no differences in activity between D- and L-enantiomeric peptides, ruling out the involvement of a chiral receptor or an enzyme as the target [5,93]. A model has been proposed to explain the mechanism of action of magainin 2 [66] and follows the micellar-aggregate model of antimicrobial activity. In this model, magainins interacting with negatively charged phospholipids spontaneously form transient, membrane spanning pores, which, upon collapse, permit peptide translocation to the inner leaflet [63,66]. Indeed, membrane disruption has been demonstrated in model systems [61,62,64] and magainin induced depolarization has been shown in *E. coli* and model systems [45,46].

Various structure–activity studies have been conducted on the α-helical magainins. N-Terminal truncation of magainin 2 indicates that the first 3 residues do not play a major role in antimicrobial activity but the deletion of residue 4 (K) greatly reduces activity and further truncation of residues 5 and 6 (F and L) eliminates activity altogether [99]. It is thought that truncation of the peptide to fewer than 20 residues (i.e. deletion of residue 4 and above) results in a compound that is unable to span the lipid bilayer and thus, from a mechanistic perspective, explains the corresponding loss of antimicrobial activity [99]. However, α-helical peptides with as few as 13 residues can possess antimicrobial activity so an ability to span a lipid bilayer is not an obligate requirement for activity of α-helical peptides [101].

In both the membrane-disruptive and non-membrane-disruptive mechanisms of peptide antimicrobial activity, the initial step is the interaction of the cationic peptide with the negatively charged cell surface. It thus remains of key interest to determine the forces leading to favorable association, as well as to ascertain if this step is simply driven by electrostatic attraction. To this end, the contribution of charge toward the activity of magainin 2 has been investigated using analogues with varying cationic charges [11]. It was determined that charge increase to +5 is accompanied by a corresponding increase in antimicrobial activity. Further increase of charge to +7 did not alter the maximal activity observed at +5, however, haemolytic activity was found to increase. Interestingly, experiments using model membranes composed of the anionic lipid phosphatidylglycerol found that an increase in charge actually led to a decrease in membrane permeabilizing ability. This is likely a result of the corresponding decrease in hydrophobicity that accompanies an increase in charge.

6.3. Extended peptides

The extended class of peptides lack classical secondary structures, generally due to their high proline and/or glycine contents. Indeed, these peptides form their final structures not through interresidue hydrogen bonds but by hydrogen bond and Van der Waals interactions with membrane lipids. Perhaps the best characterized representative of the extended family of cationic peptides is the tryptophan and proline-rich indolicidin (Fig. 1C). Indolicidin is a 13-residue, C-terminal amidated peptide isolated from the cytoplasmic granules of bovine neutrophils [86]. Of these 13 residues, 5 are tryptophan thus making indolicidin the peptide with the highest known proportion of tryptophans [86]. The conformation of indolicidin is dependent on its environment. The structure of indolicidin has been determined by ^{1}H NMR in both anionic SDS and zwitterionic dodecylphosphocholine (DPC) micelles [82]. In both lipid environments, the molecule exists in an extended conformation, however, in neutral DPC micelles, the molecule takes on a more bent conformation due to two half-turns about residues 5 and 8. Indolicidin possesses reasonable antimicrobial activity (MIC of 10 μg/ml against *E. coli*) but does not have a high affinity for LPS [15] when compared to other peptides such as the β-hairpin tachyplesins [38].

The antimicrobial mechanism of indolicidin has yet to be unambiguously identified. It was first hypothesized that indolicidin acts by disrupting the cytoplasmic membrane by voltage-induced channel formation driven by membrane potential [15]. This hypothesis is certainly plausible given the size of indolicidin (25×32 Å) making it possible to span biological membranes [82]. However, intact cell experiments demonstrated that, under conditions where greater than 99% of cells were killed, indolicidin was unable to completely depolarize the cytoplasmic membrane of *E. coli* [95] and *S. aureus* [20] arguing against membrane disruption as a mechanism. In addition to its channel forming ability, indolicidin has also been shown to induce filamentation of *E. coli*, which is thought to be a result of DNA synthesis inhibition [88]. In order for this mechanism to be effective, membrane translocation must obviously occur. It is interesting to note that, in accordance with the micellar-aggregate model of antimicrobial activity, both hypotheses combine to explain the actions of indolicidin; the formation of informal aggregate channels that, upon collapse, lead to translocation of the peptide into the cytoplasm.

In model membrane studies, indolicidin is not effective at translocating across membranes and we assume that in bacteria the trans-cytoplasmic membrane electrical potential gradient of -140 mV is required to drive translocation. To improve upon and understand the structural requirements required for the antimicrobial activity of indolicidin, various improved analogues have been synthesized. Two particular analogues, CP-11, which possesses an increased cationic charge, and CP10A, in which all proline residues are replaced with alanine, with improved activity versus Gram-negative and Gram-positive bacteria, respectively, are of particular interest. With CP-11, the increase in charge results in a decrease in monolayer insertion, lipid flip-flop and calcein release, and membrane translocation (in the absence of a membrane potential) remained poor [101]. In the case of CP10A, monolayer insertion, lipid flip-flop and membrane translocation were increased while calcein release was reduced [101]. Structural analysis by ^{1}H NMR revealed that the substitution of proline with alanine enables CP10A to adopt a helical conformation [21] rather than the extended structure of the parent indolicidin [82]. Thus, in the case of the indolicidin family of peptides, it appears to be conformational changes rather than changes in charge or hydrophobicity that account for differences in activity. The change in conformation from extended to helical, led to increased membrane insertion and improved membrane translocation, allowing CP10A better access to the cytoplasm and cytoplasmic targets.

6.4. Loop peptides

This class of peptides is characterized by their loop structure imparted by the presence of a single bond (either disulfide, amide or isopeptide). The only member of the loop family of peptides with an available high-resolution structure is thanatin (Fig. 1D). Thanatin is a 21-residue, loop peptide isolated from the spined soldier bug, *Podisus maculiventris* [17]. The solution structure of thanatin has been determined by ^{1}H NMR and is that of an anti-parallel β-sheet, formed by residues 8–21, stabilized by the single disulfide bond between residues 11 and 18 [56]. Thanatin possesses reasonable antimicrobial activity against Gram-negative and -positive bacteria as well as fungi [17] and is comparable in activity to members of the β-sheet family of peptides.

While the exact antimicrobial mechanism of thanatin remains unknown, it is thought to involve targets other than membranes, as treatment with peptide does not induce changes in permeability [17]. The mechanism of killing is believed to be dependent on the organism and, while both D- and L-enantiomers are equally active against Gram-positive and fungal species, only L-thanatin is active against Gram-negative bacteria [17]. This suggests that a stereospecific target such as a receptor may be involved in Gram-negative bacteria while non-specific interactions dominate in both fungi and Gram-positive bacteria [17]. Structure–activity studies have revealed that truncation of the C-terminus or beyond the third N-terminal residue greatly reduces activity and the loop region alone is completely inactive [17].

7. Lipids and peptide activity

While great interest in the influence on peptide activity of structure has been evident, very little focus has been directed at other factors directly influencing peptide–membrane interactions. Recently, there has been increased attention on membrane lipids and their potential role in peptide activity. While the number of studies investigating the relationship between antimicrobial peptides and membrane lipids remain few and limited in their scope, related compounds, namely cationic lipids, have been investigated. These compounds are similar to antimicrobial peptides in charge, amphipathicity and ability to deliver compounds intracellularly.

Recently, Hafez et al. have proposed a mechanistic model explaining the intracellular delivery of polynucleic acids by cationic lipids [29]. Briefly, plasmid-cationic lipid complexes taken up by endocytosis act to destabilize the endosomal membrane. This is driven by the association of cationic lipid headgroups with the anionic phospholipid headgroups of the inner endosomal membrane resulting in the formation of an ion pair with a supramolecular structure effectively resembling that of a type II lipid. The overall effect is the disruption of the endosomal membrane through hexagonal (H_{II}) phase formation. Based upon this mechanism, it is conceivable that cationic peptides act in a similar manner by binding anionic phospholipids that are abundant in bacterial membranes.

7.1. Type II lipids and translocation

Studies focusing on membrane lipid composition have indicated the importance of specific lipids for normal membrane function. Rietveld et al. demonstrated that *E. coli* mutants deficient in phosphatidylethanolamine synthesis are greatly diminished in their ability to transport proteins across the plasma membrane but this could be increased by the addition of divalent cations or the type II lipid DOPE, both of which induce non-bilayer phase formation [81]. In a similar study, Bogdanov et al. showed this mutant does not produce a properly folded lactose permease but renaturation of the protein in the presence PE induces proper folding [6].

These studies indicate the importance of non-bilayer-forming lipids to membrane translocation and protein folding. It is therefore conceivable that specific phospholipids or membrane phases are required for peptide translocation and may play as significant a role as peptide structure in determining translocation efficiency.

8. Conclusions

While the number of antimicrobial peptides that have been chemically characterized continues to grow, the number of those with available high-resolution structures remains relatively small. To date, structure–activity analyses of a broad range of peptides reveal two main requirements for antimicrobial activity, (1) a cationic charge and (2) an induced amphipathic conformation. Indeed, conformational change leading to an active structure seems to be needed as, even the β-hairpin peptide tachyplesin, a peptide once thought to be rigid in conformation, undergoes a major change in a lipid environment.

To date, studies focused on mechanism of action have concentrated primarily upon the chemical and structural properties of peptides and relatively little interest has been placed upon other factors. Specifically, membrane components may play a significant role in the activity of peptides. Indeed, studies focused upon the translocation of other cationic compounds have revealed major contributions from non-bilayer forming lipids and thus, suggest the importance of these compounds in the mechanism of action of antimicrobial peptides. The diversity of lipids among microorganisms may very well explain the differences in activity of a single peptide between these species and thus, further study of the interactions between antimicrobial peptides and lipids are required to propose an accurate mechanism of activity for each peptide and organism.

Acknowledgments

We acknowledge funding from the Canadian Bacterial Diseases Network to R.E.W.H. R.E.W.H. is the recipient of a Canada Research Chair and J.P.S.P. is the recipient of an NSERC Industrial Postgraduate Scholarship supported by Helix BioMedix Inc. The authors would like to thank Joseph McPhee and David Jung for critical help in preparing this review.

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