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Review

Antimicrobial Peptides (AMPs): Peptide Structure and Mode of Action

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Antimicrobial peptides (AMPs) have been isolated and characterized from tissues and organisms representing virtually every kingdom and phylum. Their amino acid composition, amphipathicity, cationic charge, and size allow them to attach to and insert into membrane bilayers to form pores by 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms. Although these models are helpful for defining mechanisms of AMP activity, their relevance to resolving how peptides damage and kill microorganisms still needs to be clarified. Moreover, many AMPs employ sophisticated and dynamic mechanisms of action to carry out their likely roles in antimicrobial host defense. Recently, it has been speculated that transmembrane pore formation is not the only mechanism of microbial killing by AMPs. In fact, several observations suggest that translocated AMPs can alter cytoplasmic membrane septum formation, reduce cell-wall, nucleic acid, and protein synthesis, and inhibit enzymatic activity. In this review, we present the structures of several AMPs as well as models of how AMPs induce pore formation. AMPs have received special attention as a possible alternative way to combat antibiotic-resistant bacterial strains. It may be possible to design synthetic AMPs with enhanced activity for microbial cells, especially those with antibiotic resistance, as well as synergistic effects with conventional antibiotic agents that lack cytotoxic or hemolytic activity.

Keywords: Antibiotic agents, Antimicrobial peptides (AMPs), Barrel-stave, Carpet, Toroidal-pore mechanisms

Introduction

Antimicrobial peptides (AMPs) are small gene-encoded peptides that show a broad range of activity against Gram-negative and -positive bacteria, fungi, and mycobacteria (Zasloff, 2002). Certain cationic peptides have been shown to inhibit the replication of enveloped viruses, such as influenza A virus (Murakami *et al.*, 1991), vesicular stomatitis virus and human immunodeficiency virus (Lee *et al.*, 2004). AMPs may also possess anticancer activity (Baker *et al.*, 1993) or promote wound healing (Gallo *et al.*, 1994). Originally identified in insects, they have subsequently been extracted from plants, crustaceans, ascidians, and vertebrates (Boman, 1995; Ganz and Lehrer, 1998; Otvos, 2000; Jakob *et al.*, 2005), and it is now clear that these molecules constitute a key component of the innate immune system in all multicellular organisms.

Amphibian skin has proved to be an especially rich source of such peptides. It contains a remarkably wide variety of AMPs that collectively have a broad spectrum of antimicrobial activity and little sequence similarity (Simmaco *et al.*, 1998). The peptides are normally stored in the dermal glands of Anurans (frogs and toads) and are released into skin secretions in a holocrine fashion upon stress or injury, thus acting as the first line of defense against invading pathogens. Bombinins and magainins, isolated from skin secretions of *Bombina* species (Gibson *et al.*, 1991) and *Xenopus laevis* (Zasloff, 1987), respectively, are well-known examples of amphibian AMPs. In most cases, several peptides of the same family with overlapping sequence and structural features but distinct spectra of antimicrobial activity can be found in a single specimen, which would protect the animal from a wide range of pathogens.

AMPs are typically less than 50 amino acids in length and have an overall positive charge imparted by multiple lysine and arginine residues as well as a substantial portion (≥50%) of hydrophobic residues. These peptides have minimal inhibitory concentrations (MIC) as low as 0.25-4 μg/ml (Hancock and Lehrer, 1998). Recent studies have also indicated a role for AMPs as effectors of innate immune responses. It is these properties that make AMPs exciting candidates as new therapeutic agents.

AMP Diversity

AMPs are a unique and diverse group of molecules (Tables 1

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and 2) that are divided into subgroups on the basis of their amino acid composition and structure (Vizioli and Salzet, 2002). The NMR solution structures of selected peptides of these subgroups are shown in Fig. 1.

One subgroup contains anionic AMPs. Among these are small (721.6-823.8 Da) peptides present in surfactant extracts, bronchoalveolar lavage fluid, and airway epithelial cells (Brogden *et al.*, 1998). They are produced in millimolar concentrations, require zinc as a cofactor for antimicrobial activity, and are active against both Gram-positive and -negative bacteria. They are similar to the charge-neutralizing propeptides of larger zymogens, which also have antimicrobial activity when synthesized alone (Brogden *et al.*, 1997).

A second subgroup contains approximately 290 short (<40 amino acids) cationic peptides that lack cysteine residues and sometimes have a hinge or 'kink' in the middle (Gennaro *et al.*, 2000). In aqueous solutions many of these peptides are disordered, but in the presence of trifluoroethanol, sodium dodecyl sulfate (SDS) micelles, phospholipid vesicles, liposomes, or Lipid A, all or part of the molecule is converted to an α-helix (Gennaro *et al.*, 2000). A good example is LL-37. In water, it exhibits a circular dichroism (CD) spectrum consistent with a disordered structure (Johansson *et al.*, 1998). However, in 15 mM HCO_3^-, SO_4^{2-}, or $CF_3CO_2^-$, the peptide adopts a helical structure. For buforin II (Kobayashi *et al.*, 2004), its congeners, and LL-37, the extent of α-helicity correlates with the antibacterial activity against both Gram-positive and -negative bacteria (Park *et al.*, 2000).

A third subgroup contains approximately 44 cationic peptides that are rich in certain amino acids (Otvos *et al.*, 2002) (see Antiinfective peptides in the Online links box). This group includes the bactenecins and PR-39, which are rich in proline (33-49%) and arginine (13-33%) residues; prophenin, which is rich in proline (57%) and phenylalanine (19%) residues; and indolicidin, which is rich in tryptophan residues (Otvos *et al.*, 2002). These peptides lack cysteine residues and are linear, although some can form extended coils.

Approximately 380 members form a fourth subgroup of anionic and cationic peptides. These contain cysteine residues and form disulfide bonds and stable β-sheets (see Antiinfective peptides in the Online links box). This subgroup includes protegrin from porcine leukocytes (which comprises 16 amino

Table 1. Classes of AMPs

Anionic peptides • Maximin H5 from amphibians. • Small anionic peptides rich in glutamic and aspartic acids from sheep, cattle and humans. • Dermcidin from humans.
Linear cationic α-helical peptides • Cecropins (A), andropin,moricin, ceratotoxin and melittin from insects. • Cecropin P1 from *Ascaris* nematodes. • Magainin (2), dermaseptin, bombinin, brevinin-1, esculentins and buforin II from amphibians. • Pleurocidin from skin mucous secretions of the winter flounder. • Seminalplasmin, BMAP, SMAP (SMAP29,ovispirin), PMAP from cattle, sheep and pigs. • CAP18 from rabbits. • LL37 from humans.
Cationic peptides enriched for specific amino acids • Proline-containing peptides including abaecin from honeybees. • Proline- and arginine-containing peptides including apidaecins from honeybees; drosocin from *Drosophila*28; pyrrhocoricin from the European sap-sucking bug; bactenecins from cattle (Bac7), sheep, and goats; and PR-39 from pigs. • Proline- and phenylalanine-containing peptides including prophenin from pigs. • Glycine-containing peptides including hymenoptaecin from honeybees. • Glycine- and proline-containing peptides including coleoptericin and holotricin from beetles. • Tryptophan-containing peptides including indolicidin from cattle. • Small histidine-rich salivary polypeptides including the histatins from man and some higher primates.
Anionic and cationic peptides that contain cysteine and form disulphide bonds • Peptides with 1 disulphide bond including brevinins. • Peptides with 2 disulphide bonds including protegrin from pigs and tachyplesins from horseshoe crabs. • Peptides with 3 disulphide bonds including α-defensins from humans (HNP-1,HNP-2, cryptidins), rabbits (NP-1) and rats154; β-defensins from humans (HBD1, DEFB118), cattle,mice, rats, pigs, goats and poultry12; and rhesus θ-defensin (RTD-1) from the rhesus monkey. • Insect defensins (defensin A). • SPAG11/isoform HE2C, an atypical anionic β-defensin. • Peptides with >3 disulphide bonds including drosomycin in fruit flies and plant antifungal defensins.
Anionic and cationic peptide fragments of larger proteins • Lactoferricin from lactoferrin. • Casocidin I from human casein. • Antimicrobial domains from bovine α-lactalbumin, human haemoglobin, lysozyme and ovalbumin.

Table 2. Characteristics that affect antimicrobial activity and specificity

Size The size of AMPs varies from 6 amino acid residues for anionic peptides to greater than 59 amino acid residues for Bac7. Even di- and tripeptides with antimicrobial activity have been reported.
Sequence Peptides often contain the basic amino acid residues such as lysine or arginine, the hydrophobic residues including alanine, leucine, phenylalanine or tryptophan, and other residues such as isoleucine, tyrosine and valine. Some peptides contain amino acid repeats. Ratios of hydrophobic to charged residues can vary from 1 : 1 to 2 : 1.
Charge Anionic peptides are rich in aspartic and glutamic acids and cationic peptides are rich in arginine and lysine. Anionic peptides that are complexed with zinc, or highly cationic peptides, are often more active than neutral peptides or those with a lower charge.
Conformation and structure AMPs can assume a variety of secondary structures including α-helices, relaxed coils and antiparallel β-sheet structures. Amphipathic α-helical peptides are often more active than peptides with less-defined secondary structures. Peptides with a γ-core motif (two antiparallel β-sheets with an interposed short turn in defensin-like molecules) are often very active.
Hydrophobicity This characteristic enables water-soluble AMPs to partition into the membrane lipid bilayer.
Amphipathicity A trait by which peptides contain hydrophilic amino acid residues aligned along one side and hydrophobic amino acid residues aligned along the opposite side of a helical molecule. For α-helical peptides, amphipathicity is often expressed as a hydrophobic moment,which is the vector sum of hydrophobicity indices, treated as vectors normal to the helical axis. Other peptides often show spatial separation of polar and hydrophobic residues that is less easy to quantify.

acid residues, including four cysteines that are linked by two intramolecular disulfide bonds), and a diverse family of defensins. There are approximately 55 α-defensins, including human neutrophil peptides and cryptdins. These peptides are 29-35 amino acid residues in length and include six cysteines that are linked by three intramolecular disulfide bonds (Lehrer *et al.*, 1993). There are also approximately 90 β-defensins from both humans and animals that are 36-42 amino acids long and include six cysteines that are linked by three intramolecular disulfide bonds (Schutte and McCray, 2002). Furthermore, there are approximately 54 arthropod (insect) defensins, approximately 58 plant defensins, and one rhesus θ-defensin (RTD-1), which is an 18-residue peptide that forms a circular molecule crosslinked by three disulfide bonds (Tang *et al.*, 1999). SPAG11/isoform HE2C is an atypical anionic β-defensin-like peptide (von Horsten *et al.*, 2004).

Finally, there are anionic and cationic peptides that are fragments of larger proteins. These fragments have antimicrobial activity and are similar in composition and structure to the AMPs described above. However, their role in innate immunity is not yet clear.

Structure

Currently, more than 500 AMPs 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). The peptides can be separated into four major classes based on their structures: β-sheet, α-helical, loop, and extended peptides (Hancock and Lehrer, 1998). The first two classes are the most common in nature. Representative structures from each of these classes are illustrated in Fig. 1.

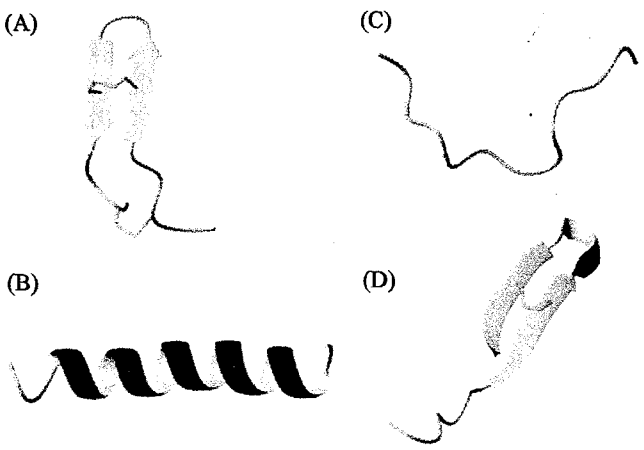

Fig. 1. Structural classes of AMPs: (A) β-sheet, tachyplesin I; (B) α-helix, magainin 2; (C) extended, indolicidin; (D) loop, thanatin.

Structure-activity Relationships (SARs)

Rather than attempt to summarize the large number of structure-activity relationship (SAR) studies that have been conducted to date, we discuss a representative peptide from each structural class. For a more detailed review of specific peptides and structural classes there are numerous reviews that may be consulted.

β-Sheet peptides This class of peptides is characterized by the presence of an antiparallel β-sheet, which is 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- to 18-residue tachyplesins (Fig. 1A). Isolated from the hemocytes of the Japanese horseshoe crab, *Tachypleus tridentatus* (Nakamura *et al.*, 1988), 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 (Kawano *et al.*, 1990). Tachyplesin I possesses moderate antimicrobial activity (<12.5 μg/ml MIC against *Escherichia coli* K12) [72] as well as a high affinity for lipopolysaccharides (Hirakura *et al.*, 2002).

Although the structure and *in vitro* activity of the tachyplesins are well characterized, the exact mechanism of antimicrobial activity remains poorly understood. The tachyplesins have a high affinity for lipopolysaccharide, but it is thought that intracellular targets also exist. Indeed, it has been shown that tachyplesin I binds to the minor groove of DNA (Yonezawa *et al.*, 1992). 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 they do not cause substantial calcein release from model membrane systems (Zhang *et al.*, 2001). This suggests that 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 by adding chemical protecting groups (Matsuzaki *et al.*, 1993) as well as by amino acid substitution (Rao, 1999). Studies involving linear tachyplesin chemically protected with acetomidomethyl groups (T-Acm) demonstrate that the linear compound has reduced antimicrobial and antiviral activity (Tamamura *et al.*, 1993) and results in less calcein release from model membranes (Matsuzaki *et al.*, 1993). Interestingly, although T-Acm was less effective than tachyplesin at permeabilizing model membranes, it possessed greater membrane-disrupting ability as determined by measuring lipid chain orientation (Matsuzaki *et al.*, 1993). Additional studies using liposomes and planar lipid bilayers have demonstrated that the linear analogue lacks the ability of the parent peptide to translocate across membranes (Matsuzaki *et al.*, 1997). Structural characterization of T-Acm by CD spectroscopy indicated that it has a random coil conformation in H_2O (Tamamura *et al.*, 1993), whereas polarized attenuated total reflection spectroscopy suggested an antiparallel β-sheet conformation in lipid environments (Matsuzaki *et al.*, 1993).

Tachyplesin analogues linearized through amino acid substitution possessed properties similar to those of T-Acm. Cysteine residues were simultaneous substituted with aliphatic (A, L, I, V, or M), aromatic (F or Y), or acidic (D) residues (Rao, 1999). Structural analysis by CD spectroscopy indicated that the analogues primarily adopt unordered and β-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 for 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. These revealed major differences between the two forms (Laederach *et al.*, 2002). 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. This also demonstrates that this structural flexibility, in what has been traditionally thought to be 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 CD for determination of detailed structure-activity information.

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 hemolytic activity (Zhang *et al.*, 1999). 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 *vs. E. coli*) (Zasloff, 1987). The structure of magainin 2 has been determined by ^{1}H NMR in the presence of DPC and SDS micelles. This peptide adopts an amphipathic α-helical conformation with a slight bend centered at residues 12 and 13 (Gesell *et al.*, 1997).

The antimicrobial mechanism of magainin has been proposed to involve selective permeabilization of bacterial membranes leading to disruption of the membrane potential (Matsuzaki, 1998). This mechanism is 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 (Bessalle *et al.*, 1990). A model based on the micellar-aggregate model of antimicrobial activity has been proposed to explain the mechanism of action of magainin 2 (Matsuzaki *et al.*, 1997). In this model, magainins interact with negatively charged phospholipids to spontaneously form transient membrane-spanning pores, which, upon collapse, permit peptide translocation to the inner leaflet (Matsuzaki *et al.*, 1997). Indeed, membrane disruption has been demonstrated in model systems (Matsuzaki, 1998), and magainin induced depolarization has been shown in *E. coli* and model systems (Juretic *et al.*, 1989).

Various structure-activity studies have been conducted on the α-helical magainins. N-terminal truncation of magainin 2 indicates that the first three residues do not play a major role in antimicrobial activity, but that the deletion of residue 4 (K) greatly reduces activity, and further truncation of residues 5 and 6 (F and L) eliminates activity altogether (Zasloff *et al.*, 1988). It is thought that truncation of the peptide to fewer than 20 residues (*i.e.*, N-terminal truncation at residue 4) 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 (Zasloff *et al.*, 1988). However, α-helical peptides with as few as 13 residues can possess antimicrobial activity, so an ability to span a lipid bilayer is not essential for the activity of α-helical peptides (Zhang *et al*, 2001).

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. Thus, determining the forces that lead to a favorable association and ascertaining whether this step is simply driven by electrostatic attraction remains a key question. To this end, the contribution of charge toward the activity of magainin 2 has been investigated using analogues with varying cationic charges (Dathe *et al.*, 2001). A charge increase to +5 was found to be accompanied by a corresponding increase in antimicrobial activity. A further increase of charge to +7 did not alter the maximal antimicrobial activity beyond that observed at a charge of +5, but the hemolytic activity was found to increase. Interestingly, experiments using model membranes composed of the anionic lipid phosphatidylglycerol revealed that an increase in charge actually leads to a decrease in the ability to permeabilize membranes. This is likely a result of the corresponding decrease in hydrophobicity that accompanies an increase in charge.

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 rather 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 (Selsted *et al.*, 1992). Of its 13 residues, 5 are tryptophan, making indolicidin the peptide with the highest known proportion of tryptophans (Selsted *et al.*, 1992). The conformation of indolicidin is dependent on its environment, as shown by ^{1}H NMR studies carried out in both anionic SDS and zwitterionic DPC micelles (Rozek *et al.*, 2000). 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 around residues 5 and 8. Indolicidin possesses reasonable antimicrobial activity (MIC of 10 μg/ml against *E. coli*). This peptide also does not have a high affinity for lipopolysaccharide (Falla *et al.*, 1996) when compared to other peptides such as the β-hairpin tachyplesins (Hirakura *et al.*, 2002).

The antimicrobial mechanism of indolicidin has yet to be unambiguously identified. It was first hypothesized that indolicidin acts by disrupting the cytoplasmic membrane by forming a voltage-induced channel (Falla *et al.*, 1996). This hypothesis is certainly plausible given the size of indolicidin (25 × 32Å), making it possible to span biological membranes (Rozek *et al.*, 2000). However, intact cell experiments have demonstrated that, under conditions where greater than 99% of cells were killed, indolicidin was unable to completely depolarize the cytoplasmic membrane of *E. coli* (Wu *et al.*, 1999) or *S. aureus* (Friedrich *et al.*, 2000), 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 due to the inhibition of DNA synthesis (Subbalakshmi and Sitaram, 1998). 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; both are consistent with the formation of informal aggregate channels which, upon collapse, lead to translocation of the peptide into the cytoplasm.

In model membrane studies, indolicidin is unable to translocate across membranes, and we assume that the transcytoplasmic membrane electrical potential gradient of 140 mV in bacteria is required to drive translocation. To improve upon and understand the structural requirements for the antimicrobial activity of indolicidin, various improved analogues have been synthesized. Two analogues are of particular interest: CP-11, which possesses an increased cationic charge; and CP10A, in which all proline residues were replaced with alanine. These peptides have improved activity against Gram-negative and -positive bacteria, respectively. The increase in charge in CP-11 results in a decrease in

Table 3. Membrane and intracellular models of AMP killing and lysis

Mode of Action	Examples of peptides
Barrel-stave model	Alamethicin
Carpet model	Dermaseptin S, cecropin, melittin, caerin and ovispirin
Toroidal-pore model	Magainin, protegrin, melittin, LL-37 and MSI-7890
Membrane translocation model	Buforin II

monolayer insertion and lipid flip-flop, whereas calcein release and membrane translocation in the absence of a membrane potential remained poor (Zhang *et al.*, 2001). In contrast, for CP10A, monolayer insertion, lipid flip-flop, and membrane translocation were increased and calcein release was reduced (Zhang *et al.*, 2001). Structural analysis by ^{1}H NMR revealed that the substitution of proline with alanine enables CP10A to adopt a helical conformation (Friedrich *et al.*, 2001) rather than the extended structure of the parent indolicidin (Rozek *et al.*, 2000). Thus, in the case of the indolicidin family of peptides, it appears that conformational changes rather than changes in charge or hydrophobicity account for the 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.

Loop peptides This class of peptides is characterized by their loop structure, which is imparted by the presence of a single bond (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* (Fehlbaum *et al.*, 1996). The solution structure of thanatin, determined by ^{1}H NMR, is an antiparallel β-sheet formed by residues 8-21 and stabilized by a single disulfide bond between residues 11 and 18 (Mandard *et al.*, 1998). Thanatin possesses reasonable antimicrobial activity against Gram-negative and -positive bacteria and fungi (Fehlbaum *et al.*, 1996), and has similar activity to other members of the β-sheet family of peptides.

The exact antimicrobial mechanism of thanatin remains unknown, but it is thought to involve targets other than membranes because the peptide does not induce changes in permeability (Fehlbaum *et al.*, 1996). The mechanism of killing is believed to be dependent on the organism and, although both d- and l-enantiomers are equally active against Gram-positive and fungal species, only l-thanatin is active against Gram-negative bacteria (Fehlbaum *et al.*, 1996). This suggests that a stereospecific target such as a receptor may be involved in Gram-negative bacteria whereas nonspecific interactions dominate in both fungi and Gram-positive bacteria (Fehlbaum *et al.*, 1996). Structure-activity studies have revealed that truncation of the C-terminus or beyond the third N-terminal residue greatly reduces activity and that the loop region alone is completely inactive (Fehlbaum *et al.*, 1996).

Mode of Action

At low peptide/lipid ratios, peptides bind parallel to lipid bilayers (Yang *et al.*, 2001). As the peptide/lipid ratio increases, peptides begin to orient perpendicular to the membrane. At high peptide/lipid ratios, the peptides are perpendicularly oriented, and they insert into the bilayer, forming transmembrane pores (referred to as the I state). The I state peptide/lipid ratio varies with both the peptide and the target lipid composition (Lee *et al.*, 2004), and a number of models have been proposed to explain membrane permeabilization (Table 3).

Barrel-stave model In the 'barrel-stave model' (Fig. 2), peptide helices form a bundle in the membrane with a central lumen, much like a barrel composed of helical peptides as the staves (Yang *et al.*, 2001). This unique type of transmembrane pore is induced by alamethicin. Oriented CD (Yang *et al.*, 2001), neutron scattering (Yang *et al.*, 2001), and synchrotron-based X-ray scattering (Spaar *et al.*, 2004) have shown that alamethicin adopts an α-helical configuration and that it attaches to, aggregates, and inserts into oriented bilayers that have been hydrated with water vapor. The hydrophobic peptide regions align with the lipid core region of the bilayer, and the hydrophilic peptide regions form the interior region of the pore. The alamethicin-induced transmembrane pores can contain 3-11 parallel helical molecules, and the inner and outer diameters have been calculated to be approximately 1.8 and 4.0 nm, respectively (Spaar *et al.*, 2004). The walls of the channel are approximately 1.1 nm thick, which is approximately the diameter of the alamethicin helix and is consistent with eight alamethicin monomers arranged according to the barrel-stave model (Yang *et al.*, 2001). However, changes in bilayer lipid composition can modulate peptide aggregation equilibria and the number of peptides in the aggregate (Cantor, 2002).

Carpet model In the 'carpet model' (Fig. 3), peptides accumulate on the bilayer surface (Pouny *et al.*, 1992). This model explains the activity of AMPs such as ovispirin (Yamaguchi *et al.*, 2001) that orient parallel ('in-plane') to the membrane surface (Bechinger, 1999). Peptides are electrostatically attracted to the anionic phospholipid head groups at numerous

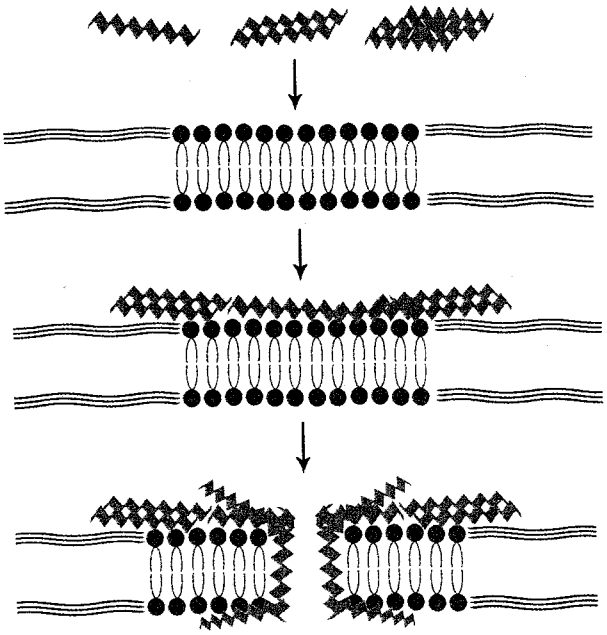

Fig. 2. The barrel-stave model of antimicrobial-peptide-induced killing. In this model, the attached peptides aggregate and insert into the membrane bilayer so that the hydrophobic peptide regions align with the lipid core region and the hydrophilic peptide regions form the interior region of the pore.

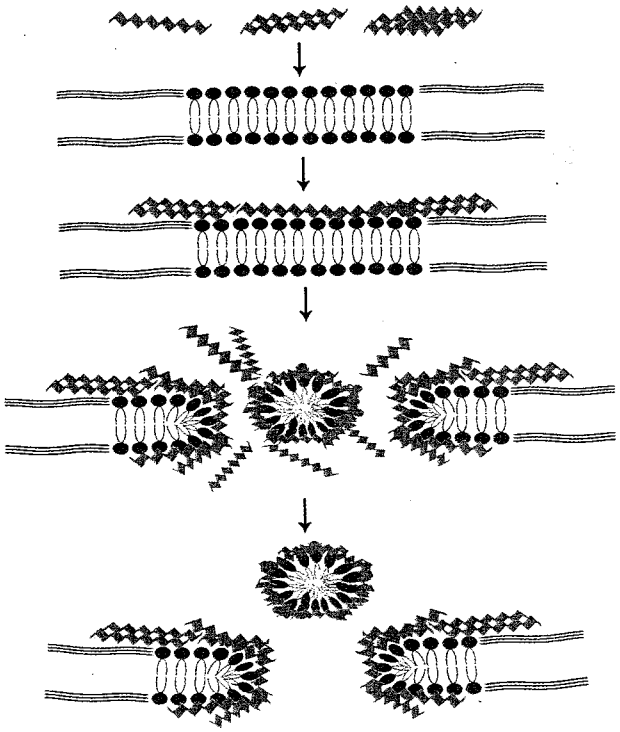

Fig. 3. The carpet model of antimicrobial-peptide-induced killing. In this model, the peptides disrupt the membrane by orienting parallel to the surface of the lipid bilayer and forming an extensive layer or carpet.

sites, covering the surface of the membrane in a carpet-like manner. At high peptide concentrations, surface-oriented peptides are thought to disrupt the bilayer in a detergent-like manner, eventually leading to the formation of micelles (Shai, 1999). At a critical threshold concentration, the peptides form toroidal transient holes in the membrane, allowing additional peptides to access the membrane. Finally, the membrane disintegrates and forms micelles after disruption of the bilayer curvature (Oren and Shai, 1998).

Toroidal-pore model In the 'toroidal-pore model' (Fig. 4), AMP helices insert into the membrane and induce the lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid head groups (Matsuzaki *et al.*, 1996). This type of transmembrane pore is induced by magainins, protegrins, and melittin (Matsuzaki *et al.*, 1996). In forming a toroidal pore, the polar faces of the peptides associate with the polar head groups of the lipids (Yamaguchi *et al.*, 2002). The lipids in these openings then tilt from the lamellar normal and connect the two leaflets of the membrane, forming a continuous bend from the top to the bottom in the fashion of a toroidal hole; the pore is lined by both the peptides and the lipid head groups, which are likely to screen and mask cationic peptide charges (Yang *et al.*, 2001). The toroidal model differs from the barrel-stave model in that the peptides are always associated with the lipid head groups even when they are perpendicularly inserted in the lipid bilayer (Yang *et al.*, 2001). Otherwise, the presence of several monomers in a toroidal pore would result in a COULOMB ENERGY that is too high for pore formation (Yang *et al.*, 2001). Magainin-induced toroidal pores are larger and have a more variable pore size than alamethicin-induced pores (Yang *et al.*, 2001): they have an inner diameter of 3.0-5.0 nm and an outer diameter of approximately 7.0-8.4 nm, and each pore is thought to contain only 4-7 magainin monomers and approximately 90 lipid molecules (Yang *et al.*, 2001).

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. This has been demonstrated for the frog-derived AMP buforin II, which does not cause large membrane perturbations and the disruption is transient and occurs in the absence of permeabilization (Fig. 5) (Park *et al.*, 2000). Other peptides have similar properties (Zhang *et al.*, 2001). 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 (Futaki *et al.*, 2001). 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

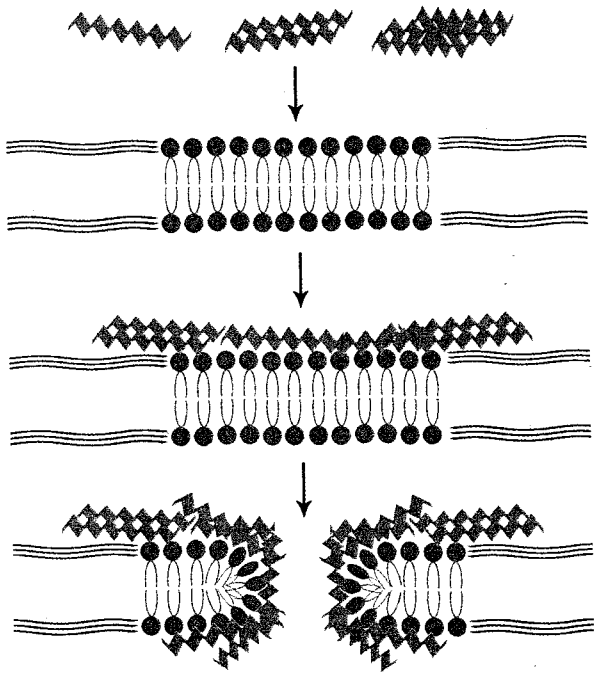

Fig. 4. The toroidal model of AMP induced killing. In this model the attached peptides aggregate and induce the lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid head groups.

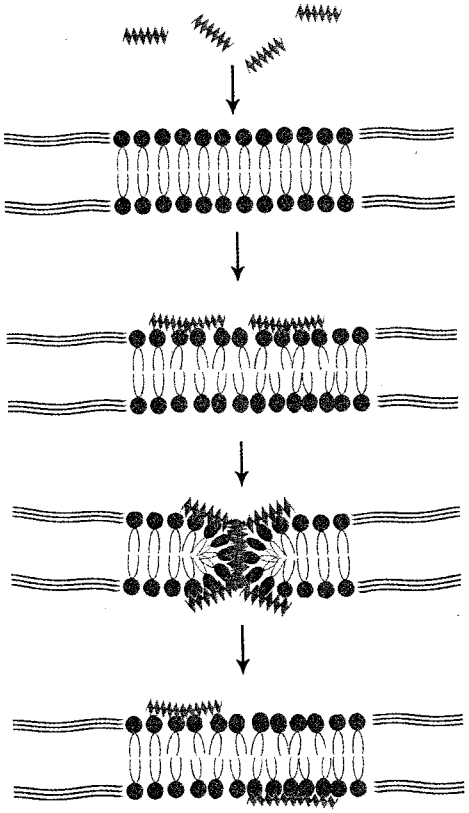

Fig. 5. Membrane translocation mechanism of the AMP buforin 2.

compounds. Indeed, DNA and RNA binding has been demonstrated *in vitro* (Yonezawa *et al.*, 1992), and other studies have demonstrated the inhibition of macromolecular synthesis after treatment with sublethal concentrations of peptides (Patrzykat *et al.*, 2002). In addition, specific enzymatic targets have been identified for certain peptides. The proline-rich insect peptide, pyrrhocoricin, binds the heat shock protein DnaK, inhibiting chaperone-assisted protein folding (Kragol *et al.*, 2001), whereas the *Bacillis* lantibiotic, mersacidin, binds lipid II leading to the inhibition of peptidoglycan biosynthesis (Brotz *et al.*, 1998). The loss of viability with these peptides is much slower than with membrane-acting peptides, which exert their antimicrobial effects within minutes (Giacometti *et al.*, 1999). For pyrrhocoricin, the ability of the peptide to interfere with protein folding in live cells is not observed until 1 h after exposure (Kragol *et al.*, 2001), and cell lysis by mersacidin is not observed until 3 h after treatment (Brotz *et al.*, 1998).

Conclusions

Research focusing on the structures and functions of AMPs from diverse sources has grown dramatically in recent years. The composition and conformation of AMPs yield signature three-dimensional distributions of charge and hydrophobicity. Investigations in this area have identified compelling themes among mechanisms of AMP action. Many AMPs have rapid and potent antimicrobial effects that are mediated by mechanisms that are distinct from their influences on the phospholipid bilayer.

Thus, it may be possible to develop AMPs as pharmacologic agents that target strategic microbial structures or functions, suppress pathogen resistance to host defenses, and restore or potentiate the activities of conventional antibiotics against drug-resistant pathogens. Understanding the mechanism of AMP action and resistance should yield unique and useful information for the development of novel antimicrobial agents. Thus, AMPs are excellent candidates as lead compounds for the development of novel antiinfective agents.

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