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MINI-REVIEW

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Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action

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Abstract Ribosomally synthesized peptides with antimicrobial activity are produced by prokaryotes, plants, and a wide variety of animals, both vertebrates and invertebrates. These peptides represent an important defense against micro-organisms. Although the peptides differ greatly in primary structures, they are nearly all cationic and very often amphiphilic, which reflects the fact that many of these peptides kill their target cells by permeabilizing the cell membrane. Moreover, many of these peptides may roughly be placed into one of three groups: (1) those that have a high content of one (or two) amino acid(s), often proline, (2) those that contain intramolecular disulfide bonds, often stabilizing a predominantly β-sheet structure, and (3) those with amphiphilic regions if they assume an α-helical structure. Most known ribosomally synthesized antimicrobial peptides have been identified and characterized during the past 15 years. As a result of these studies, insight has been gained into fundamental aspects of biology and biochemistry such as innate immunity, membrane-protein interactions, and protein modification and secretion. Moreover, it has become evident that these peptides may be developed into useful antimicrobial additives and drugs. This review presents a broad overview of the main types of ribosomally synthesized antimicrobial peptides produced by eukaryotes and prokaryotes.

Key words Antimicrobial peptides · Defensins · Cathelicidins · Magainins · Cecropins · Bacteriocins · Lantibiotics

Abbreviations *CD* Circular dichroism · *LAP* Lingual antimicrobial peptide(s) · *LPS* Lipopolysaccharide · *TAP* Tracheal antimicrobial peptide(s)

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Introduction

Gene-encoded, ribosomally synthesized antimicrobial peptides are widely distributed in nature, being produced by mammals, birds, amphibia, insects, plants, and microorganisms (Cammue et al. 1994; Sahl 1994; Boman 1995). Although they form a diverse group of peptides as judged by their primary structures, they are often cationic and amphiphilic, and most of them kill bacteria by permeabilizing the target-cell membrane. Their positive charge presumably facilitates interactions with the negatively charged bacterial phospholipid-containing membranes and or acidic bacterial cell walls, whereas their amphiphilic character enables membrane permeabilization.

In mammals, antimicrobial peptides are expressed in phagocytes and mucosal epithelial cells (Lehrer et al. 1993; Boman 1995; Martin et al. 1995). In insects, bacterial infection induces the release of antibacterial peptides into the haemolymph (Boman 1995). In frogs, the skin contains glands that produce antimicrobial peptides enabling frogs with wounds to thrive in water dense with bacteria (Zasloff 1987; Kreil 1994; Barra and Simmaco 1995). Thus, antimicrobial peptides in animals are thought to be key effector molecules in the innate immunity that are particularly important in early defense against invading micro-organisms. As pointed out by Boman (1995, 1996), antimicrobial peptides are an ideal first line of defense since peptides are made much more rapidly than proteins: antibacterial peptides may be made more than 100 times faster than IgM, assuming a constant rate of peptide bond formation. Moreover, small peptides diffuse more rapidly than large proteins and immune cells. The production of antimicrobial peptides by bacteria may also be thought of as a type of defense since the peptides kill invading bacteria that compete with the producer for nutrients.

Most ribosomally synthesized antimicrobial peptides presently known have been identified during the past 15 years, the insect cecropins and the defensins isolated in the early 1980s being the first animal antimicrobial pep-

Table 1 Antimicrobial peptides discussed in this review that are produced by eukaryotes (grouped according to characteristic structural features)

Predominantly β-sheet structure and disulfide bonds		Amphiphilic α-helical structure[a]				High content of an amino acid			Miscellaneous[b]	
		Peptide	Source	Peptide	Source	Peptide	Rich in	Source	Peptide	Source
Peptide	Source									
α-Defensins	Humans, rats, rabbits, guinea pigs, mice	C18[c]	Rabbits	Bombinin	Frogs	Bac-5, Bac-7[c]	Pro, Arg	Cattle, sheep, goats	Brevinins-1[e]	Frogs
		PMAP-36[c]	Pigs	Brevinins-2[e]	Frogs				Ranalexin[e]	Frogs
β-Defensins	Cattle	PMAP-37[c]	Pigs	Esculentins-1,	Frogs	PR-39[c]	Pro, Arg	Pigs	Bombinins H	Frogs
Protegrins[c]	Pigs	SC5[c]	Sheep	Esculentins-2[e]		Prophenin[c]	Pro, Phe	Pigs	Thanatin	Insects
Tachyplesins	Crabs	LL-37[c]	Humans	Cecropins	Insects[f]	Indolicidin[c]	Trp	Cattle		
Insect defensins	Insects	Magainins	Frogs	Andropin	Insects	Apidaecins	Pro, Arg	Insects		
Drosomycin[d]	Insects	Dermaseptins	Frogs	Moricin	Insects	Abaecin	Pro	Insects		
Plant defensins	Plants					Drosocin	Pro	Insects		
Cyclic dodeca-peptide[c, d]	Cattle									

[a] Contains a region that becomes amphiphilic if it assumes an α-helical structure
[b] Not easily placed in the 3 other groups: Brevinin-1, ranalexin, and bombinin H are all hydrophobic, and thanantin is similar in sequence to brevinin-1
[c] Belongs to the group of cathelin-associated antibacterial peptides
[d] It has not been shown that this peptide has a β-sheet structure, but it is placed in this group because it contains a disulfide bond
[e] Contains the „Rana box" motif with two cysteines (one of which is C-terminal) that are separated by five residues and joined by a disulfide bond
[f] A cecropin, cecropin P1, has also been found in the intestine of pigs

tides thoroughly characterized (Steiner et al. 1981; Selsted et al. 1983). Since then there has been an increasing interest in ribosomally synthesized antimicrobial peptides. This is, perhaps, in part due to the importance of developing new types of antimicrobial agents because of the increase in antibiotic-resistant bacterial strains resulting from extensive use of antibiotics. One ribosomally synthesized antimicrobial peptide, the lantibiotic bacteriocin nisin produced by some strains of lacticocci, is in fact presently being used as a food preservative (Delves-Broughton et al. 1996). Antimicrobial peptides from the skin of frogs have been tested in human phase-III clinical trials for the treatment of skin infections (Jacob and Zasloff 1994). Some of these frog peptides also have potential as anticancer agents since they kill a variety of tumor cells at concentrations that are tenfold lower than that required to kill normal cells. They also exhibit activity against malignant melanoma and ovarian cancer cells in mouse models (Jacob and Zasloff 1994). The interest in antimicrobial peptides is, of course, also due to their general biological and biochemical importance. By studying these peptides, insight has been gained into host defense systems, membrane-protein interactions, and protein modification and secretion. Some antimicrobial peptides contain D-amino acids (Mignogna et al. 1993; Skaugen et al. 1994), and they consequently represent an excellent model system for studying how D-amino acids may be formed in ribosomally synthesized polypeptides.

We present a general overview of some of the main types of ribosomally synthesized antimicrobial peptides produced by eukaryotes and prokaryotes. Readers interested in more details on these peptides should refer to a recent monograph on antimicrobial peptides (Boman et al. 1994) and to recent reviews on antimicrobial peptides produced by eukaryotes (Lehrer et al. 1993; Boman 1995; Martin et al. 1995) and prokaryotes (Klaenhammer 1993; Sahl 1994; Sahl et al. 1995; Nes et al. 1996). In Tables 1 and 2 the antimicrobial peptides discussed in this article are listed according to characteristic structural features.

Antimicrobial peptides in mammals

The defensins in phagocytic cells and mucosal tissues are effector molecules in innate immunity

At micromolar concentrations, the defensins show antimicrobial activity against a wide range of gram-positive and gram-negative bacteria, fungi, and some enveloped viruses (Lehrer et al. 1993; Martin et al. 1995; Selsted and Ouellette 1995). They are arginine-rich, amphiphilic β-sheet peptides containing 29–45 amino acid residues, 6 of which are cysteines that are linked by three intramolecular disulfide bonds. Their predominant β-sheet structure stabilized by the three disulfide bonds is a unifying feature that distinguishes the defensins from other antimicrobial peptides, many of which form amphiphilic α-helices. NMR and crystallographic studies revealed that defensins may exist as noncovalently linked dimers shaped like bas-

Table 2 A selection of antimicrobial peptides (peptide-bacteriocins) produced by gram-positive bacteria

Group I: modified bacteriocins, the lantibiotics[a]		Group II: unmodified bacteriocins	
Type A	Type B	One-peptide bacteriocins	Two-peptide bacteriocins
Nisin	Mersacidin	Pediocin-like bacteriocins:	Lactococcin G
Lactocin S	Actagardine	Pediocin PA1, Leucocin A, Sakacin P,	Lactacin F[b]
Subtilin	Cinnamycin	Mesentericin Y105, Carnobacteriocin-BM1,	Plantaricin E/F[c]
Epidermin	Duramycin	Carnobacteriocin-B2, Enterocin A,	Plantaricin J/K[c]
Pep 5		Piscicolin 126	
Gallidermin			
Lacticin 481		Nonpediocin-like bacteriocins:	
Epilancin K7		Lactococcin A	

[a] See Sahl et al. (1995) for a review on the listed lantibiotics and for a more complete list

[b] See Klaenhammer (1993)

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kets that have a hydrophobic bottom and a polar top (White et al. 1995). The initial step in the permeabilization of the target-cell membrane involves electrostatic interactions between the positively charged arginine residues and the negatively charged moieties on the target-cell surface. The hydrophobic part of the peptide is then probably pulled into the membrane by the trans-membrane potential, after which several dimers or monomers form a membrane-spanning pore estimated to have a diameter of 25 Å (Selsted and Ouellette 1995; White et al. 1995).

The defensins found in mammals have been divided into the α-defensins (or classical defensins) and the β-defensins (White et al. 1995). They differ in number of residues, the location and connectivities of their cysteine residues, and in their unique consensus sequences. The β-defensins are 38–42 residues long and somewhat larger than the α-defensins, which have 29–35 residues (White et al. 1995). About 30% of the residues are well-conserved among the α-defensins, and 60–70% are well-conserved among the β-defensins. Upon optimal alignment, the α- and β-defensins have identical residues at eight positions, including the six cysteines.

The α-defensins have been found in azurophil granules of neutrophils and of some macrophages in humans, rabbits, rats, and guinea pigs and may account for more than 5% of the total cellular protein (Lehrer et al. 1993; Selsted and Ouellette 1995). These granule-associated defensins presumably kill phagocytized micro-organisms when the defensin-containing granules fuse with phagocytic vacuoles containing the ingested micro-organisms. In fact, mutations that rendered *Salmonella typhimurium* bacteria 1,000-fold more sensitive to defensins simultaneously rendered the bacteria avirulent to mice, and the bacteria lost their ability to survive within murine macrophages (Fields et al. 1989).

In humans and in mice, α-defensins are also expressed and secreted to the intestine by Paneth cells, granule-rich secretory epithelial cells of the small intestinal crypts with a presumed host defense function (Bevins 1994; Selsted and Ouellette 1995; Ouellette and Selsted 1996). Two isoforms are expressed in the human small intestine, whereas 16 or more are expressed in mice. These enteric α-defensins are thought to constrain the intestinal bacterial flora.

The β-defensins have been found in neutrophiles and in the respiratory tract/tongue of cattle (Bevins 1994; Schonwetter et al. 1995; Diamond et al. 1996), and in the leukocytes of chickens (Harwig et al. 1994). Enhanced expression of β-defensins of the bovine respiratory tract and tongue [sometimes referred to as tracheal antimicrobial peptides (TAP) and lingual antimicrobial peptides (LAP)] is obtained upon wounding or exposure to lipopolysaccharides (LPS), suggesting that they play an important role in host defense of the respiratory tract.

The defensins are synthesized as preproproteins containing approximately 95 residues including a typical 19-residue signal sequence at the N-terminus followed by a 40 to 45-residue anionic prosequence (Lehrer et al. 1993; Ganz 1994; Martin et al. 1995; Selsted and Ouellette 1995). The signal sequence functions to target the preproprotein to the endoplasmic reticulum, whereas the biological role of the prosequence is not known. It may function to mask the membrane-permeabilizing activity during intracellular processing and transport (prodefensin lacks microbicidal activity), assist in proper folding, and/or help target the proprotein to the approp-riate organelle. The genes for the neutrophil defensins from humans, guinea pigs, and rabbits all span about 3 kb and contain three exons. The last exon encodes the mature defensin peptide, and the second-to-last encodes the preprodomain (Ganz 1994; Martin et al. 1995; Selsted and Ouellette 1995). The human and mouse enteric defensin genes contain only two exons that correspond to the last two exons of the neutrophil defensin genes (Ganz 1994).

Cathelin-associated antimicrobial peptides: a diverse group of antimicrobial peptides with a common proregion

The cathelin-associated antimicrobial peptides constitute a very diverse group of antimicrobial peptides differing

greatly in sequence, structure, and number of residues. Nevertheless, they all have a common N-terminal preprosequence (Zanetti et al. 1995). The conserved proregion consists of about 100 amino acid residues, and it is homologous to the cysteine protease inhibitor, cathelin. Proteins with this conserved proregion and a highly variable C-terminal cationic antimicrobial domain are consequently termed cathelicidins (Zanetti et al. 1995). The C-terminal antimicrobial domain is cleaved off upon formation of the mature antimicrobial peptide, which is referred to as a cathelin-associated antimicrobial peptide. Cathelin-associated antimicrobial peptides are bactericidal at micromoler concentrations and are, with a few exceptions, active against both gram-positive and gram-negative bacteria.

The cathelin-associated antimicrobial peptides that will be discussed below may roughly be placed into three groups: (1) those that have a high content of one (or two) amino acid(s), often proline, (2) those that contain intramolecular disulfide bonds, and (3) those with amphiphilic regions if they assume an α-helical structure.

The group of cathelin-associated antimicrobial peptides that are rich in one or two amino acids (group 1) includes Bac-5 (bactenecin, mol. mass 5 kDa, 42 residues), Bac-7 (bactenecin, mol. mass 7 kDa, 59 residues), PR-39 Pro-Arg-rich peptide with 39 residues), and prophenin Pro-Phe-rich peptide, 79 residues), which all have a high content of proline (> 45%) (Frank et al. 1990; Agerberth et al. 1991; Harwig et al. 1995). Moreover, Bac-5, Bac-7, and PR-39 are also rich in arginine (ca. 25%), whereas prophenin is rich in phenylalanine (ca. 20%). Bac-5 has the triplet Arg-Pro-Pro at 7 places interspaced by Arg, Pro, Phe, Ile, Leu, and/or Tyr residues, while Bac-7 has the triplet Pro-Arg-Pro at 12 places interspaced with Arg, Pro, Gly, Ile, Leu, and/or Phe residues. The predicted secondary structure and model building indicates that Bac-5 and Bac-7 contain a series of turn/coil-segments interspersed with extended structures that may allow for asymmetrical clustering of polar and nonpolar amino acids (Frank et al. 1990). Such an amphiphilic structure suggests that the peptides interact with membranes, which is consistent with the observation that the peptides permeabilize the *Escherichia coli* membrane. A polyproline helical type-II structure that may interact with cell membranes has been suggested for PR-39 (Cabiaux et al. 1994).

PR-39 and prophenin are found in the intestine and/or in leukocytes of pigs (Agerberth et al. 1991; Harwig et al. 1995), whereas Bac-5 and Bac-7 are found in the large cytoplasmic granules present in neutrophils of cattle, goat, and sheep, but not in neutrophils of most other animals (Zanetti et al. 1990). In contrast to defensins, Bac-5 and Bac-7 are stored in the granules as inactive precursors (proproteins) with molecular masses of 20 and 16 kDa, respectively, and they become activated by proteases in the azurophil granules when these granules fuse with the probactenecin-containing granules (Zanetti et al. 1990).

Indolicidin is a cathelin-associated antimicrobial peptide that is novel because of a high content of tryptophan, from which the name of the peptide is derived (Selsted et al. 1992). This cationic peptide is found in granules of bovine neutrophils, and it contains 13 residues, of which 5 are tryptophan, 3 are proline, and 3 are basic residues. Its carboxyl-terminal arginine is carboxamidated, and it apparently forms a polyproline type-II helix that disrupts the cytoplasmic membrane by channel formation (Falla et al. 1996).

The group of cathelin-associated antimicrobial peptides that contain two or more cysteines joined by intramolecular disulfide bonds (group 2 mentioned above) includes cyclic dodecapeptide – found in bovine neutrophil granules – and the protegrins – found in porcine leukocytes. Cyclic dodecapeptide contains only 12 residues, of which there are 4 arginines and 2 cysteines linked by an intramolecular disulfide bond (Romeo et al. 1988). The remaining residues are the hydrophobic amino acids Val, Ile, and Leu. Its mode of action is not known. The protegrins contain 16–18 residues, 4 of which are cysteines joined by two intramolecular disulfide bonds (Kokryakov et al. 1993). Although only half their size, the protegrins show sequence similarity to some defensins: a sequence of 8–10 residues that includes three cysteines is almost identical in the two peptides (Kokryakov et al. 1993). The protegrins also resemble the tachyplesins, a family of antimicrobial peptides recently identified in cytoplasmic granules of horseshoe crab haemocytes (Iwanaga et al. 1994). Both have a broad spectrum of antimicrobial activity. They contain a similar number of residues, including four cysteines that form intramolecular disulfide bonds, and both have an amidated C-terminus as is the case for indolicidin (Kokryakov et al. 1993; Iwanaga et al. 1994; Aumelas et al. 1996). Structural studies have shown that both contain two antiparallel β-strands that are linked by a β-turn and stabilized by two disulfide bonds (Iwanaga et al. 1994; Aumelas et al. 1996). Moreover, both have hydrophilic and hydrophobic clusters that presumably are of importance in their interaction with target-cell membranes.

The third group of cathelin-associated antimicrobial peptides mentioned above are those that will become amphiphilic if they assume an α-helical structure. As discussed below, the amphiphilic α-helix is a structure found in many antimicrobial peptides that kill cells by permeabilizing the target cell membrane. This third group of peptides includes: C18, a 21-residue sequence derived from the C-terminal region of CAP18 (a cathelicidin expressed in rabbit neutrophils; Tossi et al. 1994); PMAP-36 and PMAP-37 (porcine myeloid antibacterial peptide with 36 and 37 residues, respectively) expressed in pig myeloid cells (Storici et al. 1994; Tossi et al. 1995); SC5, expressed in sheep bone-marrow cells (Mahoney et al. 1995); and LL-37 (N-terminal residues are L-L, contains 37 residues), which is present in human granulocytes (Gudmundsson et al. 1996). Although they differ in primary structures, they all have a high content of the basic amino acids arginine and lysine (25–35%) and apparently kill cells by permeabilizing their membranes.

The genes that encode PR-39, prophenin, the protegrins, and LL-37 are organized similarly, and this may

possibly be the case for all genes coding for cathelicidins. All four genes contain four exons. The first three exons code for the conserved signal peptide and the cathelin proregion, whereas the fourth exon encodes for processing site(s) and the variable C-terminal antimicrobial domain that is cleaved off upon formation of the mature antimicrobial peptide (Zhao et al. 1995a, b; Gumundsson et al. 1995, 1996). The promoter regions of these genes contain recognition sites that are also found in the promoter regions of genes that participate in immune responses, and this suggests that the expression of cathelicidin-encoding genes responds to cytokines, such as IL-6, that are produced early during infection (Zhao et al. 1995a, b; Gudmundsson et al. 1995, 1996).

Frog skin glands produce cationic antimicrobial peptides

The magainins form an amphiphilic α-helix, a structural motif common to many antimicrobial peptides

The magainins are produced by the frog *Xenopus laevis*. Their discovery was triggered by the observation that frogs with wounds thrive in water dense with bacteria (Zasloff 1987). Initially the magainins were isolated from the frog skin, but have since also been found in the stomach (Moore et al. 1991). At micromolar concentrations, magainins kill gram-positive bacteria, gram-negative bacteria, and fungi and protozoa (Jacob and Zasloff 1994). They contain 21–27 residues, none of which are cysteines, and are synthesized as polyprotein precursors consisting of several magainin segments that are cleaved off to yield the mature peptides (Zasloff 1987).

Upon binding to membranes, the magainins form an amphiphilic α-helix of which one side going down the helix is hydrophobic and the other side is hydrophilic (Zasloff 1987). In this respect the magainins are similar to some of the cathelin-associated antimicrobial peptides (group 3 discussed above) and to other antimicrobial peptides found in the skin secretions of frogs, the cecropins produced by insects, and a number of bacterial antimicrobial peptides. The detailed mechanism by which amphiphilic α-helical peptides permeabilize membranes is not completely elucidated, and it is debated. However, there is general agreement that upon interacting with membranes, amphiphilic α-helical peptides may initially lie parallel to the plane of the membrane with the hydrophobic side of the helix facing towards and shallowly penetrating the membrane. Recent biophysical studies (Matsuzaki et al. 1995) on the interaction of magainins with phospholipid bilayers suggest that membrane pores may then transiently form through a "barrel-stave" mechanism in which several magainin peptides form a bundle (possibly a pentamer) of membrane-spanning helices. The α-helical peptides are now oriented perpendicularly to the plane of the membrane, with the hydrophilic side of the helices facing into the transmembrane channel and the hydrophobic side facing the hydrophobic lipids surrounding the channel. Consistent with the view that membrane lipids are the main target is the observation that chemically synthesized all-D magainin 2 has the same antimicrobial activity as natural all-L magainin 2 (Wade et al. 1990). Thus, the magainins do not need to interact with chiral centres such as enzymes, classical receptors, or other membrane proteins in order to exert their antimicrobial activity.

Great diversity in frog-skin antimicrobial peptides

The magainins may be unique to *Xenopus* species since very different antimicrobial peptides are found in other frogs. The dermaseptins (28–34 residues) from *Phyllomedusa* species (Mor and Nicolas 1994) and the bombinins (25–27 residues) from *Bombina* species (Gibson et al. 1991; Simmaco et al. 1991) have sequence similarities neither with each other nor with the magainins. However, like the magainins, they are linear cationic peptides that can assume an amphiphilic α-helical conformation upon association with membranes.

Brevinins-2, esculentins-1, and esculentins-2 (containing up to 46 residues), all isolated from various Rana species, also have a sequence (in their N-terminal region) that can form an amphiphilic α-helix presumably involved in membrane permeabilization (Kreil 1994). Their C-terminal region contains two cysteine residues (one of which is the C-terminal residue) that are separated by five residues and joined by a disulfide bridge. This motif (the Rana box) is also present in brevinins-1 (24 residues) and ranalexin (20 residues), both isolated from Rana species (Clark et al. 1994; Barra and Simmaco 1995). However, their N-terminal region is predominantly hydrophobic and cannot form an amphiphilic α-helix. Brevinins-1 and ranalexin, consequently, permeabilize membranes by a mechanism different from that used by the amphiphilic helical peptides. This must also be the case for the predominantly hydrophobic bombinins H isolated from *Bombina* species. Surprisingly, some of the bombinins H contain D-alloisoleucine as the second amino acid (Mignogna et al. 1993). The primary product of translation contains L-isoleucine, which is then converted to a D-isomer. As is the case for protegrins, tachyplesins, and indolicidin, the C-terminal residues of bombinins H, bombinins and dermaseptins are amidated (Kreil 1994), thereby increasing their positive charge. The great variety of antimicrobial peptides found in the limited number of frog species studied indicates that the skin of amphibians is a rich source for antimicrobials.

Antimicrobial peptides are effector molecules in insect immunity

Wounding or exposure to certain bacterial components such as lipopolysaccharide (LPS) induces insect blood cells and the insect fat body (functionally the insect counterpart to the mammalian liver) to transiently produce and release into the blood a number of different peptides that

kill bacteria at micromoler concentrations (Hultmark 1994; Boman 1995; Hoffman 1995). The synthesis of these antimicrobial peptides resembles the immune response and the acute-phase response in mammals: their gene expression is regulated by a κB-related *cis*-regulatory motif and by NF-κB-related transactivators similar to those that regulate expression of genes encoding immune and acute-phase response proteins in mammals (Hoffmann 1995). The insect antimicrobial peptides are cationic and can roughly be grouped into four major classes: (1) the cecropins, with 31–39 residues (none of which are cysteines) and an amphiphilic α-helical region enabling them to kill both gram-negative and gram-positive bacteria by membrane permeabilization; (2) the insect defensins, which resemble the defensins found in mammals; (3) the 2- to 4-kDa cationic proline-rich peptides such as the apidaecins, abaecin and drosocin; and (4) the somewhat larger 8- to 30-kDa glycine-rich peptides such as diptericins (ca. 9 kDa), attacin (ca. 20–22 kDa), sarcotoxin II (ca. 27 kDa), and coleoptericin (ca. 8 kDa). These larger polypetides, which are distantly related to each other (members of the attacin superfamily), will not be discussed further.

The cecropins (group 1) have been found in insects of the order Lepidoptera, which includes moths and butterflies, and the order Diptera, which includes flies (Hoffman 1995). They may be widespread in the animal kingdom since a cecropin-like peptide, cecropin P1, has also been isolated from the small intestine of pigs (Lee et al. 1989). The sequence of pig cecropin P1 is 33% identical and 65–75% similar to the sequence of cecropin A from *Drosophila*. All the cecropins have in common a basic N-terminal part, which may form an amphiphilic α-helix, and a relatively hydrophobic C-terminal helical part, which is joined by a hinge sequence containing proline and/or glycine (Boman 1995). The C-terminal carboxyl group is amidated in the insect cecropins, as is the case for several antimicrobial peptides in mammals, frogs, and crabs (see above). The cecropins are synthesized as preproproteins containing 62–64 residues with conserved signal- and pro-sequences. The encoding gene has one intron (Boman 1995). The bacterial membrane is the primary target of the cecropins. The cecropins bind electrostatically to the membrane surface, orienting their amphiphilic α-helix parallel to the plane of the membrane (Gazit et al. 1994) as discussed above for the magainins. However, it is not clear whether permeabilization then entails that the peptides become oriented perpendicularly to the plane of the membrane, transiently forming pores through a "barrel-stave" mechanism as is suggested for the magainins. Similarly to the magainins, the cecropins do not interact with chiral centers on membranes since chemically synthesized all-D cecropin A has the same antimicrobial activity as the natural all-L enantiomer counterpart (Wade et al. 1990).

Two other membrane-permeabilizing insect antimicrobial peptides with a cationic amphiphilic α-helical N-terminal region are andropin, expressed in the ejaculatory duct of the adult male *Drosophila* fly (Hultmark 1994), and moricin, recently isolated from the hemolymph of the silkworm *Bombyx mori* (Hara and Yamakawa 1995). They have no sequence similarity to other known peptides or to each other. Andropin, however, is predicted to form a similar secondary structure to cecropin. Its function is presumably to keep the seminal fluid free from bacterial growth. Moricin contains 42 residues and has a very cationic C-terminal sequence; 6 of the last 7 residues are positively charged.

The insect defensins (group 2 mentioned above) are widespread among different insects and so far have been found in Diptera, Coleoptera, Hymenoptera, Hemiptera, and Odonata. They permeabilize the target-cell membrane, primarily killing gram-positive bacteria (Hoffmann 1995). They owe their name to sequence and structural similarities to the mammalian defensins, although it is uncertain whether or not they are homologous polypeptides (White et al. 1995). The global three-dimensional structure of insect defensins also resembles that of some plant antimicrobial peptides, now termed plant defensins (Broekaert et al. 1995). The insect defensins are cationic and contain 38–43 residues, and similar to the mammalian defensins, they have a characteristic motif of six cysteines that form three disulfide bonds that stabilize a predominant β-sheet structure (White et al. 1995). However, unlike the mammalian defensins, the insect defensins also have an α-helical region (also present in the plant defensins), and the connectivities of their three intramolecular disulfide bonds differ from those found in the mammalian defensins (White et al. 1995). Also contrary to many of the mammalian defensins, the insect defensins do not act within phagocytes but are secreted into the blood of infected insects. The insect defensins are synthesized as preproproteins consisting of a 23-residue signal region, a 34-residue prosequence, and the defensin sequence (Dimarcq et al. 1990). Interestingly, the relatively newly isolated antimicrobial peptide drosomycin from *Drosophila* shows nearly 40% sequence similarity with plant defensin (Fehlbaum et al. 1994), suggesting an evolutionary relationship to the plant peptide and the possibility that drosomycin is an insect-defensin-like peptide.

Another group of insect antimicrobial peptides (group 3 mentioned above) is similar to some mammalian antimicrobial peptides such as PR-39, Bac-5, and Bac-7 in that its members are rich in proline and – in some cases – also in arginine. Two types of peptides found in honey bees belong to this group: the apidaecins, which contain 18 residues [33 and 17% of which are proline and arginine, respectively (Casteels et al. 1989)], and abaecin, which contains 34 residues [29% of which are proline (Casteels et al. 1990)]. They are primarily active against gram-negative bacteria, although some gram-positive bacteria are also sensitive. Their mode of action is unknown, although in the case of apidaecins it does not appear to involve the cell membrane. The D-enantiomer of apidaecin is not active, indicating that the peptide interacts with a chiral cellular component (Casteels and Tempst 1994). Another proline-rich insect antimicrobial peptide is drosocin, which has structural similarities to apidaecins. This 19-residue peptide found in *Drosophila* flies is unusual in that it is glycosylated (Bulet et al. 1993). The disaccharide

N-acetylgalactosamine-galactose is O-linked to a threonine residue and is necessary for full biological activity.

Interestingly, a newly identified, 21-residue-long insect antimicrobial peptide, thanatin, which does not belong to any of the groups defined above, is similar to antimicrobial peptides found in frogs (Fehlbaum et al. 1996). The sequence similarity between thanatin and brevinins-1 is nearly 50%, and thanatin has a C-terminally located disulfide bridge motif similar to that (the Rana box) found in brevinins-1 and brevinins-2, in esculentins-1 and esculentins-2, and in ranalexin.

From animals to bacteria

Antimicrobial peptides of eukaryotes and prokaryotes are produced and function in entirely different settings. However, apparently diverse biological systems often have many elements in common at a molecular level. The prokaryote has been an especially useful model system from which much of our general and fundamental knowledge in biochemistry and molecular biology has been obtained. Certainly, knowledge about prokaryotic antimicrobial peptides is relevant when studying the eukaryotic peptides, and vice versa.

Bacterial ribosomally synthesized antimicrobial polypeptides are generally referred to as bacteriocins. Antibiotics are not included in this group because they are not ribosomally synthesized and will, therefore, not be discussed. The bacteriocins produced by gram-negative bacteria – for instance, the colicins produced by *Escherichia coli* – are often proteins larger than 20 kDa with a narrow spectrum of action. Colicin V and the microcins, a heterogeneous group of peptides smaller than 10 kDa produced by Enterobacteriaceae, are exceptions. The microcins are often posttranslationally modified and have been divided into three classes based on their mode of action. Microcins of class A inhibit metabolic enzymes, those of class B inhibit DNA replication, and those of class D impair energy generation. Of these peptides, microcin B17 is perhaps the most thoroughly characterized. It is a glycine-rich, extensively modified (containing thiazole and oxazole rings) class-B microcin that inhibits DNA gyrase, causing double-stranded breaks and DNA degradation (Liu 1994). Because of its small size, colicin V (mol. wt. 8733) could be classified as a microcin rather than as a colicin. It has recently been suggested, on the basis of similarities in leader sequences, that colicin V belongs to a family of unmodified peptide bacteriocins originally found in gram-positive lactic acid bacteria (group-II bacteriocins discussed below; Nes et al. 1996).

The following discussion will be restricted to the bacteriocins produced by gram-positive bacteria. In contrast to the gram-negative bacteria, gram-positive bacteria most often produce peptide bacteriocins smaller than 6 kDa. They are often cationic, amphiphilic, membrane-permeabilizing peptides. In this manner they resemble many of the antimicrobial peptides produced by eukaryotes. However, they appear to have a much higher specific activity: antibacterial activity against sensitive strains can be detected at picomoler-to-nanomoler concentrations, although the action spectra is often narrow at these concentrations. A number of peptide bacteriocins produced by gram-positive bacteria, particularly from lactic acid bacteria, have been identified in the last few years. These peptide bacteriocins are classified into two main groups: group I consisting of modified bacteriocins (the lantibiotics), and group II consisting of the unmodified peptide bacteriocins (the nonantibiotics).

Unmodified bacteriocins: the pediocin-like bacteriocins and the two-peptide bacteriocins

The pediocin-like bacteriocins (36–48 residues) produced by a variety of lactic acid bacteria have similar amino acid sequences and constitute a subgroup of the unmodified group-II bacteriocins. The first characterized bacteriocins belonging to this subgroup were pediocin PA1 (Henderson et al. 1992; Nieto Lozano et al. 1992), sakacin P (Tichaczek et al. 1992), leucocin A (Hastings et al. 1991), curvacin A (Holck et al. 1992; Tichaczek et al. 1992), and mesentericin Y105 (Héchard et al. 1992). On the basis of their sequence, bacteriocins identified recently [e.g., carnobacteriocin BM1 and B2 (Quadri et al. 1994), enterocin A (Aymerich et al. 1996), and piscicolin 126 (Jack et al. 1996)], may also be placed in the group of pediocin-like bacteriocins. These bacteriocins have 40–60% sequence similarity. Especially the hydrophilic N-terminal region is well-conserved. The N-terminal region of all pediocin-like bacteriocins presently identified contains two cysteines (joined by a disulfide bridge) found in a „pediocin box“ motif: -Y-G-N-G-V-X_1-C-X_2-K/N-X_3-X_4-C-, where $X_{1–4}$ represent polar uncharged or charged residues. Despite similar primary structures, the pediocin-like bacteriocins differ in their target-cell specificity. For antimicrobial peptides in general, it is often observed that the specificity of similar peptides and the susceptibility of similar target-cells for a given peptide vary much more than one would expect simply on the basis of an interaction between a cationic amphiphilic peptide and the lipids of a cell membrane. Subtle structural differences in peptides may lead to marked differences in specificity, and subtle differences in target cells may lead to marked differences in their susceptibility to a peptide. Elucidation of what governs the specificity of peptides and the susceptibility of target cells is a major challenge and of great importance for future use of peptides as antimicrobial agents. The lipid composition of the target-cell membrane is presumably one important factor determining susceptibility. By constructing hybrid bacteriocins, it has been shown for the pediocin-like bacteriocin that the C-terminal region is an important determinant of target-cell specificity (Fimland et al. 1996). This region also appears to interact with the membrane, thereby causing membrane leakage (Chikindas et al. 1993) since the region (in contrast to the N-terminal region) is either hydrophobic or amphiphilic.

Group II also includes several unmodified nonpediocin-like bacteriocins such as Lactococcin A, a 55-residue membrane permeabilizing cationic peptide (Holo et al. 1991; Van Belkum et al. 1991), and the two-peptide bacteriocins. The two-peptide bacteriocins are novel in that they consist of two different cationic peptides of 25–40 residues each. Antibacterial activity requires the presence of both peptides, optimal activity being obtained when the two peptides are present in approximately equivalent amounts. Lactococcin G was the first of these two-peptide bacteriocins to be isolated and characterized (Nissen-Meyer et al. 1992). It forms potassium-selective channels in the target bacterial membrane (Moll et al. 1996b). The two peptides that constitute lactococcin G contain regions that become amphiphilic if they assume an α-helical structure, and circular dichroism (CD) studies have shown that these regions do in fact adapt an α-helical structure when the two peptides interact with each other in the presence of membrane-mimicking micelles (H. H. Hauge, V. Eijsink, I. F. Nes, J. Nissen-Meyer, unpublished results).

At least four genes (five for the two-peptide bacteriocins) found in close proximity are required for production of the unmodified bacteriocins: (1) the structural gene (two structural genes for the two-peptide bacteriocins) encoding the preform of the bacteriocins, (2) an immunity gene encoding a protein that protects the producer against its own bacteriocin, (3) a gene encoding a membrane-associated ABC transporter that transfers the bacteriocin across the membrane, and (4) a gene encoding an assessory protein also needed for secretion of the bacteriocin (Nes et al. 1996). The preform of these bacteriocins contains an N-terminal leader sequence – with 15–30 residues and a specific consensus sequence – that is cleaved off at a common Gly-Gly motif (Klaenhammer 1993). The immunity proteins for lactococcin A and carnobacteriocin B2 have been isolated, and a fraction of the cellular pool of the protein has been shown to be associated with the cell membrane (Nissen-Meyer et al. 1993; Venema et al. 1994; Quadri et al. 1995). However, it is not clear how immunity proteins endow resistance to bacteriocins. It has recently been shown that the production of some group-II bacteriocins (such as plantaricins of *Lactobacillus plantarum* C11, sakacin A, sakacin P, and possibly carnobacteriocin B2) are transcriptionally regulated through a signal transduction system that consists of three components: an induction factor, a histidine protein kinase, and a response regulator (Nes et al. 1996). The induction factor is a bacteriocin-like peptide with a double glycine leader processed and externalized presumably by the ABC transporter that transfers the bacteriocin across the membrane.

Modified bacteriocins: the lantibiotics and the formation of D-alanine in ribosomally synthesized polypeptides by conversion of L-serine

The modified bacteriocins (group I) are designated lantibiotics because they contain the thioether amino acid, lanthionine. The first step in the formation of lanthionine is a sequence-specific dehydration of serine residues resulting in the formation of the α,β-unsaturated amino acid, 2,3-didehydroalanine (Sahl et al. 1995). Lanthionine is then formed when the double bond in 2,3-didehydroalanine is attacked by the thiol group of a nearby cysteine residue. The resulting lanthionine may, therefore, be described as a D-alanine (derived from the serine residue) and an L-alanine (derived from the cysteine residue) linked by a sulfur, that forms a thioether bond. Lanthionine is consequently similar to cystine, which may be described as two L-alanine residues linked by two sulfurs, forming a disulfide bond. The thioether bond in lanthionine presumably functions to stabilize the three-dimensional structure of the lantibiotics and is, therefore, analogous to the function of the disulfide bonds in cystine often found in proteins. However, the thioether bond is more stable than the disulfide bond, lanthionine being more stable than cystine. Lantibiotics also contain methyl-lanthionine, the formation of which is similar to the formation of lanthionine except that it is derived from a threonine instead of from a serine residue. Other modified residues found are 2,3-didehydroalanine and 2,3-didehydrobutyrine (2,3-didehydro-threonine). Interestingly, one lantibiotic, lactocin S, has been shown to contain D-alanine in three defined positions where the gene encodes a serine (Skaugen at al. 1994). The conversion of serine – presumably by way of 2,3-didehydroalanine followed by a stereospecific hydrogenation to alanine – is, thus, a mechanism whereby a D-amino acid may be formed in a ribosomally synthesized protein.

The lantibiotics have been divided into two major subgroups based on their structure (Sahl et al. 1995). Type-A lantibiotics – of which nisin is perhaps the most thoroughly characterized – are elongated, screw-shaped, and amphiphilic; they have a molecular weight in the range 2,000–5,000. Type-B lantibiotics are more globular in shape and have a molecular weight of about 2,000. The type-A lantibiotics interact with the membrane of susceptible cells and form transient voltage-dependent pores (Moll et al. 1996a), whereas at least some type-B lantibiotics inhibit the functioning of enzymes. For instance, the type-B lantibiotics mersacidin and actagardine interfere with the cell-wall synthesis in gram-positive bacteria (Sahl et al. 1995).

As is the case with the unmodified (group-II) bacteriocins, the lantibiotics are synthesized from prepeptides with characteristic N-terminal leader peptides (Sahl et al. 1995). The leader peptides are anionic and are predicted to form an amphiphilic helix. It may possibly function to keep the bacteriocin inactive until export secretion, to facilitate interaction with the ABC transporter, and/or to promote interactions with the modification enzymes. Nearby the structural gene encoding lantibiotic prepeptides are genes encoding (1) one or more proteins involved in immunity, (2) an ABC transporter, (3) a serine protease that presumably functions to cleave off the leader sequence, (4) one or two proteins catalyzing dehydration and lanthionine ring formation, (5) two-component regu-

latory proteins (Sahl et al. 1995). The two-component regulatory proteins transmit an extracellular signal and thereby induce bacteriocin expression. Interestingly, it has been shown recently that the lantibiotic nisin acts as the extracellular signal that induces – via the two-component regulatory proteins – the expression of its own structural gene (Kuipers et al. 1995).

Conclusion

For the survival of an organism, an efficient defense system for protection against micro-organisms is of upmost importance. Thus, there has been strong selection pressure for the development and preservation of such systems. Insight into how these defense systems function will obviously have a positive impact on medicine and health. Just 15 years ago, the first ribosomally synthesized antimicrobial peptides from animals were characterized. Since then, a large number of antimicrobial peptides have been identified in many different organisms, and it is now evident that these peptides represent an important defense system against micro-organisms. New basic knowledge in biochemistry and biology has been gained from studying these peptides. Moreover, the peptides may be developed into useful antimicrobial additives and drugs. Bacteriocins produced by „food-grade“ lactic acid bacteria are now successfully used as food preservatives. It is of great interest that new types of antimicrobials be developed in light of the increase in antibiotic-resistant bacteria. Antimicrobial peptides can complement or, in selected cases, substitute for antibiotics and chemical preservatives. Many antimicrobial peptides exert their antimicrobial activity in a manner entirely different from antibiotics and preservatives. The optimal use of peptides as antimicrobials will require detailed insight at the molecular level into their mode of action and the structural features important for antimicrobial activity, specificity, and immunity. A major challenge for future research in the field of antimicrobial peptides will be the elucidation of these aspects.

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