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Cationic Peptides

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I. INTRODUCTION

It has been often observed that no new classes of antibiotics have been developed since the introduction of the first quinoline, nalidixic acid, in 1962. However, over the past decade scientists have discovered that one of nature's most persistent approaches against bacteria involves cationic peptides. For example, cationic peptides are the major mechanism of defense against microbes in insects and plants, a predominant local defense at host surfaces including the skins of amphibians and mucosa of mammals, and the major proteinaceous species of the dedicated antimicrobial defense cells of mammals—namely, neutrophils. These peptides have a variety of structures and functions that include antibacterial (Gram-positive and -negative), antifungal, antiviral, antiendotoxin, and anticancer activities. Thus, they present perhaps the most profound example of convergent evolution, in which a variety of different peptides have evolved to a common set of functions.

Cationic peptides were traditionally isolated from natural sources or synthesized by solid phase or solution phase chemistry. Moreover, they have recently been synthesized by recombinant DNA methods in bacteria (1), insect cells (2), and plants (3,4). The fact that cationic peptides are produced naturally by certain bacteria (e.g., see Chapter 17), as well as the newly discovered ability to synthesize virtually any peptide by recombinant means in bacteria (1), clearly merits the use of the term "antibiotic" for these compounds. Thus, cationic peptides represent not only the first new class of antibiotic in the past 30 years, but the world's first genetically engineering antibacterials.

II. OCCURRENCE OF CATIONIC PEPTIDES IN NATURE

Recently, we reviewed the natural cationic peptides in depth and identified 145 sequences that have been isolated from nature (5). Some of these are listed according to structural class in Tables 1 and 2. Cationic peptides are ubiquitous in nature; they have been identified in bacteria, fungi, plants, insects, crustaceans, amphibians, mammals, and humans.

Table 1 Examples of Cationic Peptides

Mammalian defensins (NP-1)	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR
β-Defensins (BNBD5)	EVVRNPQSCRWNMGVCIPISCPGNMRQIGTCFGPRVPCCR
Insect defensins (Sapecin)	ATCDLLSGTGINHSACAAHCLCRGNRGGYCNGKAVCVCRN
Tachyplesins (Tachyplesin)	RRWCFRVCYRGFCYRKCR
Thionins (Rabbitwood)	KSCCRNTWARNCYNVCRIPGTISREICAKKCDCKIISETTCPS-DYPK
Loops (Bactenicin)	RLCRIVVIRVCR
α-Helical (Cecropin A)	KWKFKKIEKMGRNIRDGIVKAGPAIEVIGSAKAI
Histidine-rich (Histadin 2)	MKFFVALILALMLSMTGADSHAKRHHGYKRKFHEKHHSHRGY-RSNYLYDN
Tryptophan-rich (Indolicidin)	ILPWKWPWWPWRR
Proline-rich (Bac 5)	PFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLRFP

Generally speaking, these compounds provide relatively nonspecific defenses against microbes (Table 3). Even those compounds elicited by bacteria are known to function as bacteriocins that kill other bacteria, presumably as a mechanism of competition for an ecological niche.

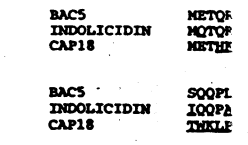
A. Mammals

A variety of peptides are involved in the mammalian oxygen-independent antimicrobial defense mechanism. Defensins are a family of small (29–35 amino acids) arginine- and cysteine-rich peptides that have been isolated from a variety of mammals, including rats, rabbits, and humans (6,7). Six human defensins have been identified, four of which, human neutrophil peptides (HNP-1,2,3,4), were purified from polymorphonuclear leukocytes and two of which, human defensins (HD-5 and 6), have been detected in the intestinal Paneth cells by *in situ* hybridization. Mouse defensins, cryptidins, are also found in the Paneth cells of the small intestine. All six human defensins share sequence homology that includes six cysteine residues forming three disulfide bridges. This results in a β-pleated sheet secondary structure. Defensins, although capable of killing a wide range of bacteria, fungi, and viruses, are more active against Gram-positive than Gram-negative bacteria. In addition to their permeabilization of biological membranes, these peptides also exhibit chemotactic and endocrine regulatory activities (8).

Human defensins are synthesized as 94- to 100-amino-acid preprodefensins that contain a conserved 19-amino-acid *N*-terminal signal sequence that targets the peptide to the endoplasmic reticulum. This is followed by an anionic propiece, proposed to balance the cationic charge of the defensin (9).

A subset of defensins, the β-defensins, have been isolated from bovine neutrophils (10). A unique consensus sequence distinguishes these defensins from those described above, although both contain the characteristic three disulfide bridges. Tracheal antimicrobial peptide (TAP) isolated from the bovine respiratory tract also contains the triple disulfide motif but is specifically expressed in the respiratory tract (11). This peptide is active against Gram-negative and -positive bacteria and yeast.

A distinct family of peptides, termed the cathelicidins, has been isolated from mammalian neutrophils; these include the bovine peptides bactenecin 5 (Bac5) (12) and indolicidin (13), the porcine PR-39 (14), and the rabbit peptide CAP18 (15). These



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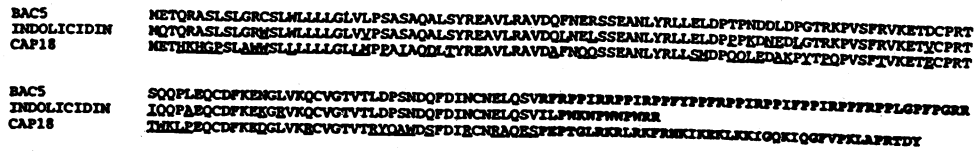

Figure 1 Alignment of Bac5, indolicidin, and CAP18 proregion sequences. Sequence variation from Bac5 is denoted by underlining in the indolicidin and CAP18 prosequences. Cleaved antimicrobial domains are indicated by bold lettering. (Reproduced by permission of Zanetti et al. (16).)

peptides contain a highly conserved propiece that is also homologous to the cysteine-rich protease inhibitor cathelin (16,17). The antimicrobial *N*-terminal region of these proforms is cleaved by elastase. An alignment of the deduced proforms of Bac5, indolicidin, and CAP18 is presented in Figure 1.

B. Amphibians

Frog skin and frog gastric mucosa are rich in peptides, and many of them have antimicrobial activity (18,19). One of the first antimicrobial peptides to be isolated from this source was bombinin from the species of frog *Bombina variegata* (20). This and subsequently isolated, related bombinins display a high level of antibacterial activity against staphylococci (21).

A family of amphipathic α-helical peptides, the magainins, has been identified in the African clawed frog (*Xenopus laevis*) (22). Magainin has a broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria (23–25), fungi (24), and protozoa (23,24).

These peptides have been well characterized, and the analysis of many synthetic analogs has developed an understanding of the components required for biological activity (22,26). The cloning of the cDNA for magainin and other related amphibian peptides (PGLa, PGO, and xenopsin) has revealed that all are produced as precursor molecules, the signal peptides of which share considerable homology (23,27–30).

Cationic peptides have also been isolated from other species of frogs. For example, cationic peptides termed brevinins have been isolated from *Rana brevipoda* and *Rana esculenta*—brevinin-1 and brevinin-1E, respectively (31,32). These 24-amino-acid peptides both possess single C-terminal disulfide bonds and two prolines. Also, dermaseptin has been isolated from the South American frog *Phyllomedusa sauvagii*. This peptide has no homology with other amphibian peptides, but due to its amphipathic nature, it permeabilizes membranes in a similar fashion (19).

C. Insects

Upon infection, insects can produce a wide range of antimicrobial peptides, which are synthesized in the fat body and/or haemocytes and secreted into the haemolymph. Such peptides include cecropins (33), and defensin-like peptides such as sapecin and phormicin (34,35). Cecropins are highly amphipathic peptides containing 31–39 residues that form voltage-dependent channels in lipid membranes (36). They were initially isolated from the silk moth *Hyalophora cecropia* (37) and have subsequently been isolated from the flesh fly (sarcotoxin I) and *Drosophila* (38,39). Cecropins are distinct from other insect cationic peptides in that they contain no cysteine residues and fail to lyse eukaryotic cells (33),

Table 2 Structural Classes of Cationic Peptides

Class of peptide	Structural motifs	Sources	Examples
Mammalian defensins	3 β-strands 3 disulfides	Rat, rabbit, guinea pig, human neutrophils, rabbit alveolar macrophages, human, mouse Paneth cells	MCP, NP, HNP, GNCP, rat NP, cryptidins
β-defensins	3 disulfides β-stranded	Bovine neutrophils, trachea	TAP, BNBD
Insect defensins	3 disulfides 2 β-strands 1 α-helix	Dragonfly, blowfly, flesh fly	Phormicin, sapecin, sarcotoxin, royalisin
Tachyplesins	2 disulfides 2 β-strands	Pig leukocytes, crabs, amaranth plants, maize, turnip	Protegrins, polyphemusins, tachyplesins, Ac-AMP, 1AFP2, MBIP-1
Thionins	3 disulfides structure unknown	Maize, radish, rabbitwood, barley lead, rape, crambe	Mj-AMP1, trionin, crambin
α-Helical	amphipathic α-helix	Fruit fly, bees, frogs, toads, cattle	Bombolitin, bombinin, cecropins, magainins, melittin, dermaseptin
Loops	1 disulfide structures unknown	Bovine neutrophils, pit viper	Bactenicin, toxin 1
Histidine-rich	structures unknown	Primates, humans	Histadins
Tryptophan-rich	poly-L-proline II	Bovine neutrophils	Indolicidin
Proline-rich	poly-L-proline II	Fruit fly, honey bee, bovine neutrophils	Drosocin, abaecin, apidaecin, Bac5, Bac7

although they retain activity against Gram-negative and -positive bacteria in micromolar concentrations (37). Interestingly, cecropin-like peptides have now been isolated from the pig intestine (40). This latter peptide, cecropin P1, however, differs from the insect forms by not containing an amidated C-terminus and also in its tertiary structure (41).

Defensins have also been isolated from a variety of insect species (34,35). They share an array of six cysteine residues resulting in a tertiary structure containing three disulfide bridges but forming a structure that is distinct from mammalian defensins (42). These peptides instead share amino acid sequence homology and tertiary structure homology with royalisin from bees and charybdotoxin and defensin from scorpions (43–45).

Sapaecin, an insect defensin isolated from the flesh fly *Sacrophaga peregrina*, consists of 40 amino acids including the conserved six cysteine residues (46) and is most active against Gram-positive bacteria (35).

A number of well-characterized novel antimicrobial peptides have been isolated from the honeybee (*Apis mellifera*). These include abaecin (47), the apidaecins (48), and hymenoptaecin (49). The apidaecins are a family of small (18 residues) proline-rich peptides, isolated from the haemolymph of the honeybee, which have activity against Gram-negative and plant-associated bacteria (48). Apidaecin precursors consist of cassettes of tandemly repeated sequences of the mature peptide preceded by dipeptides that are cleaved to produce the mature peptide (50). These precursors retain antibacterial activity, although the increasing dipeptide content reduces activity. Abaecin is a 34-amino-acid proline-rich peptide that has sequence homology with the apidaecins but has a different antibacterial spectrum and a delayed antibacterial effect (47). Hymenoptaecin, larger than the other bee-derived peptides at 93 residues, does not contain a high proline content nor the characteristic cysteine residues of defensins (49). This peptide has been shown to be active against the inner and outer membranes of Gram-negative bacteria (49).

D. Crustaceans

Tachyplesins (I, II, and III) are a class of antimicrobial peptides produced in the haemocytes of the horseshoe crabs *Tachypleus tridentatus*, *Tachypleus gigas*, and *Carcinoscorpius rotundicauda* (51–53). Two tachyplesin analogs, polyphemusin I and II, have also been isolated from the horseshoe crab *Limulus polyphemus* (52). Tachyplesins (17 amino acids) and polyphemusins (18 amino acids) contain four cysteine residues and subsequently form a rigid structure containing two disulfide linkages, which results in a stable structure resistant to low pH and high temperature (51). These peptides have activity against Gram-negative and -positive bacteria and fungi (51).

E. Microbes

Bacterial antibiotic proteins have been studied for many years since their initial discovery in the 1920s. Common among Gram-negative bacteria are the colicins; rarer are the peptide bacteriocins such as microcin B17. Among Gram-positive bacteria, peptide bacteriocins are the most commonly isolated. Of these, the cationic type A antibiotics such as nisin (54) and Pep 5 (55) isolated from *Lactococcus lactis* and *Staphylococcus epidermidis*, respectively, are the most characterized. These peptides contain such unusual amino acids as lanthionine, 3-methyllanthionine, and dehydrobutyrine (55,56). The mode of action of these peptides is by the formation of transient voltage-dependent pores in the cytoplasmic membrane (57,58). Such activity causes ion leakage from the cell and a breakdown in the electropotential across the cell membrane, resulting in death. Fungi, such as *Rhizomucor pusillus*, have also been shown to produce antibacterial peptides. *R. pusillus* produces sillucin, a defensin-like peptide active against Gram-positive bacteria (59).

F. Plants

Thionins are specific cationic peptides produced by plants in response to infection. For example, barley produces a leaf-specific thionin, BTH6 (60). In addition, other cysteine-rich basic peptides belonging to the superfamily of peptides that includes thionins and

mammalian and insect defensins have been isolated from the seeds of plants. These contain generally between 30 and 50 amino acids and between four and eight cysteine residues. Examples of such peptides include Ac-AMP (61) and Mj-AMP (62). Plant-derived peptides are most active against Gram-positive bacteria and fungi. IC_{50} (concentration inhibiting 50% of fungi) values for Ac-AMP against a broad spectrum of fungi are 2 to 10 μg/ml, and for Mj-AMP2, 0.5 to 20 μg/ml against 13 plant pathogenic fungi (61,62).

III. ANTIMICROBIAL ACTIVITIES

A. Antibacterial Activities

It is a little difficult to assess the relative activities of cationic peptides compared with those of other antibacterial agents, for two basic reasons. The first is that many investigators working on cationic peptides utilize nonstandard assays. The appropriate method of measuring antibacterial activities is to determine a minimal inhibitory concentration (MIC) by either the broth dilution method, in which 10^3–10^4 bacteria are inoculated into a row of tubes containing serial twofold dilutions of antibiotics (63), or the agar dilution procedure, which involves incorporation of dilutions of the antibiotic into plates and subsequent spotting of 10^3–10^4 organisms onto the surfaces of the plates (64). In contrast, cationic peptides are often tested by measuring zones of clearance on plates spread with bacteria after inoculation of peptides into wells cut into the agar or onto paper discs (65), or by measuring the concentration of peptide killing 50% of bacteria in killing assays. The former method suffers from diffusion limitations of peptides, whereas the latter suffers from an uncertain relationship to MIC. Furthermore, the activities of cationic peptides tend to be reduced in media of high ionic strength or with high divalent cation concentrations.

With the foregoing general comments, cationic peptides have just moderate antibacterial activities compared with those of conventional antibiotics (Table 4). Nevertheless, cationic peptides do have certain highly desirable activities. First, they tend to have broad-spectrum activity that can encompass both Gram-negative and Gram-positive bacteria, although different cationic peptides often preferentially affect one or the other. Second, their activities do not appear to be compromised by resistance mechanisms that commonly appear in the clinic. Thus, common resistance mechanisms, such as methicillin resistance in *Staphylococcus aureus*, intrinsic antibiotic resistance in

Table 3 Roles of Cationic Peptides in Nature

Peptide	Host	Function	Mechanism
Pep5	*Straphylococcus epidermidis*	Antibacterial	Membrane disruption
Rs-AFP	Radish seeds	Antifungal	Cause hyperbranching and swelling of hyphae
Cecropins	Silk moth	Antibacterial	Membrane disruption
Tachyplesins	Horseshoe crab	Antibacterial, antifungal	Membrane disruption
Magainins	African clawed frog	Antibacterial, antifungal, antiprotozoal	Membrane disruption
Defensins	Human	Antibacterial, antifungal, antiviral	Membrane disruption

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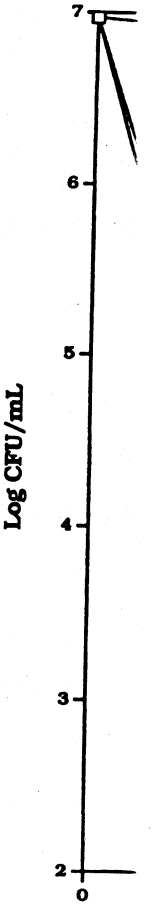

Figure 2 Killing of

Pseudomonas aeruginosa; β-lactam resistance due to chromosomal β-lactamase derepression in *Enterobacter cloacae* or plasmid-encoded TEM β-lactamase in *Escherichia coli*, and tetracycline efflux in *E. coli*, have no effect on the MIC of cationic peptides (66). Furthermore, they themselves do not tend to select resistant mutants, although some bacteria, such as *Burkholderia cepacia* tend to be naturally resistant.

Despite their modest MICs, cationic peptides can kill bacteria potently at or around the MIC, in contrast to most conventional antibiotics. Thus, a cecropin–melittin hybrid peptide can cause 3–4 orders of magnitude of killing of *P. aeruginosa* in 20 min at the MIC, whereas other potent antipseudomonal antibiotics generally cause less than one order of magnitude of killing at the analogous concentration (Figure 2).

The detailed mechanism of action of cationic peptides is described in Section IV.C. The most prominent effect on cells is the formation of channels in or disruptions of the cytoplasmic membrane. Thus, these molecules appear to kill by a physical method that takes advantage of the specific composition of bacterial membranes. In contrast, most

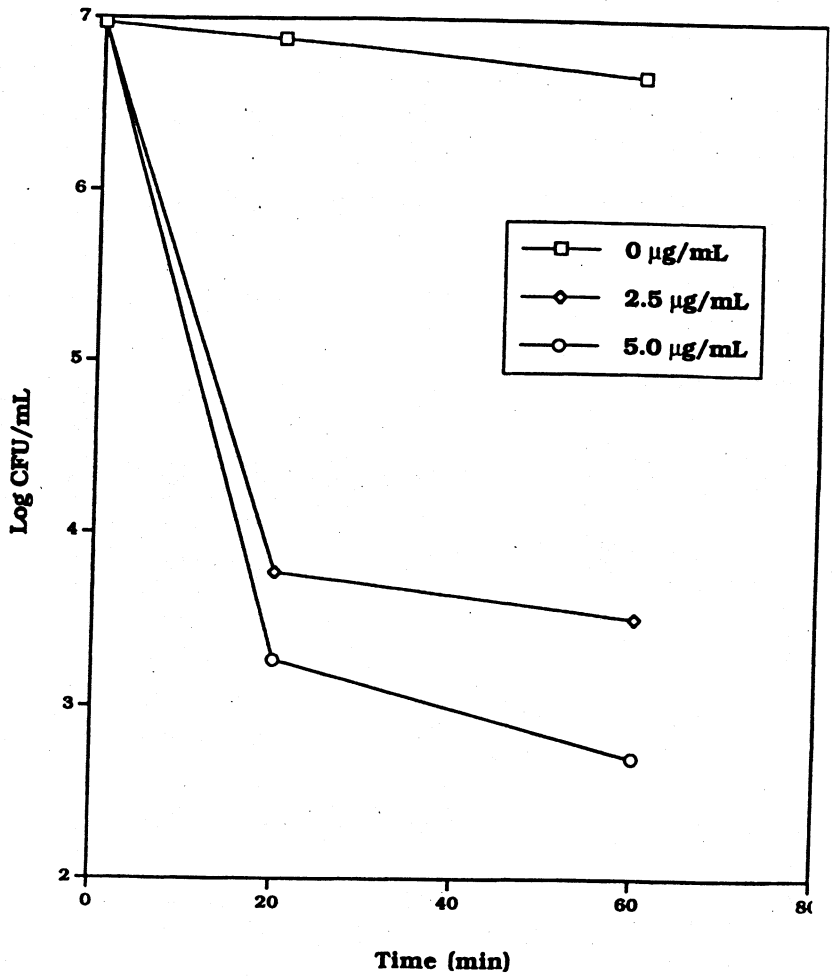

Figure 2 Killing of *P. aeruginosa* by the cationic peptide CEME. (From Ref. 67.)

conventional antibiotics are enzyme inhibitors that act on specific enzyme targets in bacteria (e.g., β-lactams acting on transpeptidases). This may explain many of the more desirable features of the cationic peptides, as described above, for example, (a) lack of resistance development, since it is difficult to fundamentally alter membrane composition, and (b) rapid killing, since the action is physical rather than catalytic.

Another feature of the mechanism of action that can be exploited is the ability of cationic peptides to break down the outer membrane barrier of Gram-negative bacteria. This barrier has been shown to limit the uptake of, and thus cellular susceptibility to, most conventional antibiotics, since its permeabilization by, for example, EDTA or specific mutations leads to reduced MICs for such antibiotics. In the same way, cationic peptides tend to be synergistic with certain conventional antibiotics, suggesting that they may be useful in the clinic in combination with such antibiotics. In keeping with these suggestions, Darveau et al. (68) demonstrated that magainin was synergistic with cefpirome in mouse protection experiments. The clinical implications of cationic peptides as antibiotic and antiendotoxic agents is discussed further in Sections II.B and IV.C.3.

B. Antiendotoxin Activities

Endotoxin is synonymous with lipopolysaccharide (LPS), a complex glycolipid that is an integral part of the outer membranes of Gram-negative bacteria (Figure 3). More specifically, endotoxin is the lipid A portion of LPS, which is the most membrane-proximal portion of the LPS making up the outer monolayer of the outer membrane. Endotoxin is a potent inducer of the cytokines interleukin 1 (IL1), tumor necrosis factor (TNF), and interleukin 8 (IL8). The presence of endotoxin in the body leads to a wide variety of physiological effects mediated in part by this vigorous cytokine response. These responses range from beneficial effects, such as fever response and tumor necrosis, to toxic effects,

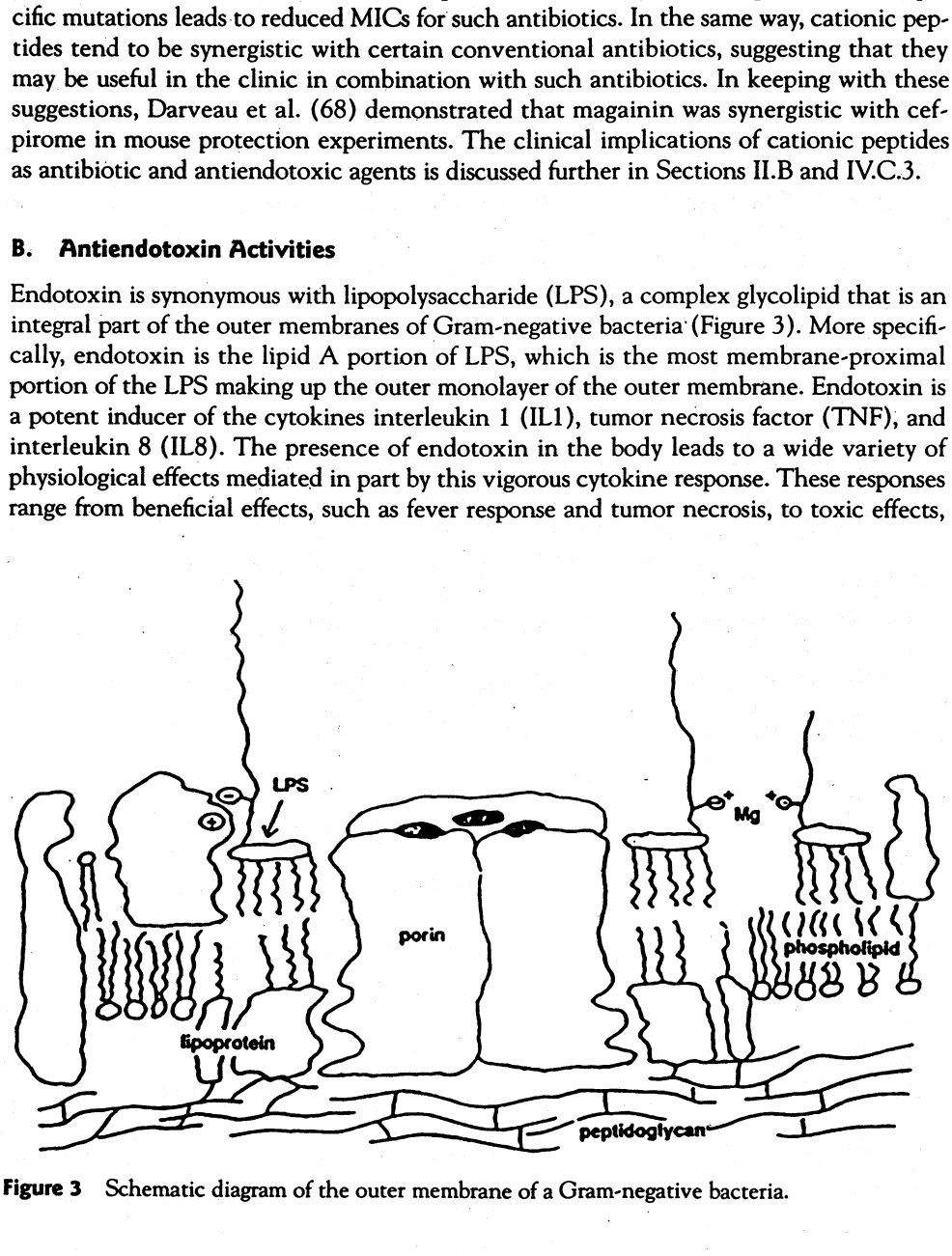

Figure 3 Schematic diagram of the outer membrane of a Gram-negative bacteria.

including toxic or septic shock. It has been observed in the clinic that patients with Gram-negative bacterial blood infections can die even under circumstances where antibiotic treatment clears the infection. It is generally accepted that high endotoxin levels play an important role in determining lethality. One of the complicating factors is the tendency of antibiotics to promote LPS release both through lysis of bacteria and nonlytic mechanisms (69). Thus, it is of great interest to develop either antibiotics that do not enhance the release of LPS or treatments that neutralize released LPS.

LPS molecules contain several phosphate moieties in addition to the unique acidic octasaccharride, 2-keto-3-deoxyoctanate. Thus, they are strongly negatively charged, a charge that is neutralized in part by divalent cations such as Mg^{2+} and Ca^{2+}. As described below, such anionic residues are the site of initial interaction of cationic peptides with bacterial outer membranes. Indeed, it has been clearly demonstrated that cationic peptides bind to bacterial LPS with an affinity that is at least three orders of magnitude higher than the divalent cations (1,71,72). Such binding prevents LPS from interacting with macrophages to elicit a TNF response both in vitro and in vivo (72,73). Consequently, cationic peptides are protective in a mouse endotoxic shock model (74). Thus, unlike other antibiotics, which promote endotoxin release and consequent endotoxic shock, cationic peptides neutralize endotoxin and prevent endotoxic shock.

C. Antifungal Activity

Mammalian defensins kill *Candida albicans* within minutes in vitro (75). The action of such peptides involves four distinct steps: primary binding, postbinding events, permeabilization, and secondary binding to internal macromolecules (76). Other cationic peptides having antifungal activity include the tachyplesins and polyphemusins, which have MIC values against C. *albicans* of 3.1 and 6.3 μg/ml, respectively (52). Not surprisingly, those peptides isolated from plants have a broad range of antifungal activity against plant pathogenic fungi. For example, Rs-AFP, the antifungal peptide isolated from radish seed, has MIC values as low as 0.3 μg/ml against certain plant pathogenic fungi (77). The plant peptides Mj-AMP and Ac-AMP cause a delay in growth of the fungal hyphae without changing mycelial morphology, whereas Rs-AFP causes a hyperbranching and swelling of the hyphae (61,62,77). However, these peptides show little or no activity against plant, insect, or human cells. Thionins cause a permeabilization of the plasmalemma around the hyphal tips (78).

D. Antiviral Activity

Several of the defensins have been found to neutralize herpes simplex virus (HSV) in tissue culture media (79). For example, rat NP-1 (50 μg/ml) caused direct viral neutralization, reducing HSV type 1 plaque forming units/ml by 90% in 60 min, and >99.9% of input viral titer was inactivated within 1 hr by 75 μg/ml guinea pig defensin at 37°C (80).

E. Other Properties

In addition to the killing of the microorganisms described above, certain cationic peptides have been associated with the killing of parasites. Killing of *Giardia lamblia* has been demonstrated with indolicidin, cryptidins 2 and 3, and NP-2 (81). These peptides reduced the viability of the protozoal trophozoites by three orders of magnitude in 2 hr. The binding and lysis of the cells appears to involve charge interactions, as NaCl, Ca^{2+}, and Mg^{2+}

all inhibited killing. In addition, magainin analogs disrupted the morphological integrity and motility of several parasites, including *Entamoeba histolytca* and *Trypanosoma cruzi* (82). The latter was killed by 100 μg/ml of the magainin analog magainin B.

Other properties may exist for cationic peptides within the host. For example, sapaecin, the insect defensin, has been found to stimulate cell proliferation of *Sarcophaga* embryo cells. This perhaps indicates a dual role of antimicrobial agent and developmental hormone for this peptide in the flesh fly (83). Indeed, Magainin Pharmaceuticals, Inc., Plymouth Meeting, PA has claimed to have available cationic peptides that promote reepithelialization of damaged corneas.

Much work has been carried out on the potential for cationic peptides as anticancer agents. This has been most extensively studied with the magainins. An ovarian cancer murine model (84) showed the elimination of 99% of tumor cells after two injections of a magainin analog. In this study there was only mild damage caused to surrounding tissue, which indicates a higher susceptibility of malignant cells to these compounds.

F. Immunogenicity, Toxicity, and Stability

There has been no detailed examination of immunogenicity of cationic peptides. However, the general consensus in the field is that they are weakly immunogenic or nonimmunogenic. This could be due to clonal deletion during development, because of the importance of peptides in nonspecific host defenses at mucosal surfaces, and their secretion by neutrophils at sites of inflammation, resulting in their recognition as "self" antigens.

Although many cationic peptides are antimicrobial to some extent, their propensity to be toxic to mammalian cells varies greatly. For example, Schluesener et al. (85) found that although indolicidin and, to a lesser extent, bactenecin are strongly cytotoxic to T lymphocytes, the defensins HNP-1, HNP-2, and HNP-3 did not affect the proliferation or viability of the T lymphocytes. On the other hand, the cationic peptides melittin and charyldotoxin are the potent toxins of bee and scorpion venom, respectively.

The issue of stability in vivo has not been addressed in detail. Clearly proteases, which are found in all body fluids, provide the potential for cleavage. One approach to overcoming such problems has involved synthesis of peptides with all D–amino acids; such peptides are not protease susceptible and often have equal activity to that of the L-form peptides (see Section IV.B).

IV. BIOCHEMICAL BASIS FOR ANTIBACTERIAL ACTIVITIES

A. Common Themes, Different Structures

All known cationic peptides share two properties: a high proportion of basic amino acids that are protonated and, thus, positively charged at neutral pH; and a high proportion of hydrophobic amino acids. These tend to distribute themselves through the three-dimensional structure of the peptide so as to create an amphipathic molecule having a hydrophilic, positively charged face and a hydrophobic face. However, the secondary and tertiary folding patterns of such peptides are quite diverse. For this reason, we consider the cationic peptides to be one of the finest arguments for convergent evolution, in which a variety of peptides have converged toward a common function, namely, defense against microbes.

The basic amino acids include arginine (pI = 10.8) and lysine (pI = 9.5), which are protonated and thus positively charged at pHs below their pI values. In addition, histidine has a pI of 7.6, rendering it only partially positively charged at neutral pH. However, at acidified sites in the body, including the upper gastrointestinal tract, the interior of phagocytic cell phagolysosomes, or some infection sites, histidine would be strongly positively charged. Nevertheless, with a few exceptions, histidines are relatively uncommon in cationic peptides. In contrast, cationic peptides usually contain four to nine positive residues that can comprise exclusively arginine (in defensins and thionins) or lysine (in cecropins or magainins) residues, or a mixture of the two. The acidic amino acids glutamate and aspartate are sometimes found in cationic peptides, but not more than two residues per peptide. The remaining (uncharged) residues often exclude several amino acids for a given peptide. Overall nonpolar (hydrophobic) residues usually exceed polar (hydrophilic) residues by a ratio of 2:1. Despite this high proportion of hydrophobic residues, the cationic peptides tend to be soluble in water, buffer, or acidified aqueous solutions due to their ability to fold and aggregate to mask their hydrophobic faces.

Despite their thematic similarities (i.e., their amphipathic nature), cationic peptides offer a range of secondary and tertiary folding patterns. The two most pronounced structural classes are the β-stranded and α-helical classes. The β-stranded class includes the defensins. The mammalian defensins have been crystallized (HNP-3) and studied by two-dimensional nuclear magnetic resonance (NMR) techniques (HNP-1, NP-2, NP-5) with rather similar results. They comprise two antiparallel β-strands with a short stretch of triple-stranded β-sheet (Figure 4). The β-strands are connected by short β-turn regions, and the entire structure is stabilized by three disulfide bridges. Despite some sequence variations, within a given class of defensins the positions of cysteines, the disulfide bonding patterns, and the positions of charged residues are strongly conserved. At least three classes of defensins exist, the mammalian defensins, β-defensins, and insect defensins (Table 2). Although not structurally well characterized, the plant thionins may make up another class of defensins. Only one other class has been examined structurally, namely, the insect defensins. These compounds have a different disulfide bonding array than do the mammalian defensins. The structure of the insect defensin, sapecin, has been defined by NMR and shown to contain two extended (β-stranded) regions, a short stretch of α-helix, and a flexible loop (87). However, the structurally characterized defensins all retain the characteristic hydrophobic surface and hydrophilic, positively charged surface. NMR evidence suggests that, in solution, defensins dimerize to mask the hydrophobic surface (88). It has also been demonstrated that tachyplesin adopts an amphipathic β-structure, in this case with two antiparallel β-strands stabilized by two disulfide bridges.

The second major structural class studied is the α-helical class. Interestingly, such structures tend to be rather disorganized in aqueous solution, but they become α-helical structured upon entering a membrane environment or exposure to nonpolar solvents (89,90). The predominant structures observed upon interaction with membranes are helix–bend–helix with a 9–16 amino acid amphipathic α-helix, a 2–4 residue bend, and a 11–14 amino acid amphipathic but more hydrophobic α-helix, as demonstrated by two-dimensional NMR of cecropins A and B, melittin, the magainins, and a synthetic cecropin–melittin hybrid (89,91–93). A small variation is provided by mammalian cecropin P1, which comprises an uninterrupted amphiphilic helix for 24 amino acids, bounded by 2–4 residues at the *N*- and C-termini.

Several cationic peptides are rich in proline, and specific peptides within this group have been demonstrated to adopt a poly-L-proline II helical structure (94–96). In the

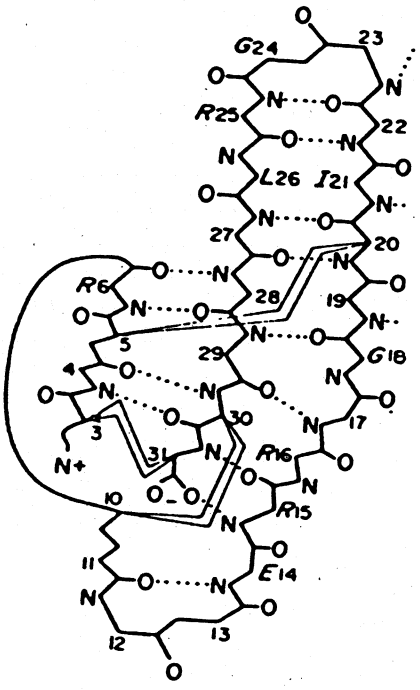

Figure 4 Model of the mammalian defensin structure. The triple-stranded antiparallel β-sheet structure of an HNP-3 monomer. The disulfide bonds are represented as "lightning bolts." Charged residues are indicated as R = arginine and E = glutamate. (Reproduced by copyright permission of Hill et al. (86). © American Association for the Advancement of Science.)

case of the proline/arginine-rich peptides, bactenecin 5 and PR-39, this structure is unaffected by the presence of lipid vesicles (95,96). However, in the case of the proline/tryptophan-rich peptide indolicidin, the assumption of this specific helical structure is greatly increased in the presence of negatively charged liposomes (94). Other peptides form loops due to single disulfide bonds, or they have extremely high histidine or tryptophan contents (Table 3). We anticipate that these will have a variety of structures.

B. Structure–Activity Relationships

The influence of substitutions or deletions of specific amino acids on the activity of the cationic peptides has been investigated in detail in several studies. The following principles seem to apply: (a) There is considerable specificity in how the changes in amino acid sequence influence activity (97,98). For example, introduction of a turn-promoting proline at positions 4 or 8 in the first α-helical segment of cecropins had a substantial effect on activity against *Micrococcus luteus*, lesser effects on activities against *Bacillus megaterium* and *P. aeruginosa*, and no effect on activity against *E. coli*. Furthermore, even conservative substitutions (changing selected amino acids to ones with similar physical properties) can substantially influence function. (b) For the α-helical peptides, changes that increase the tendency to form an α-helix in aqueous solution (i.e., prior to interacting with membranes) tend to increase activity (97–100). (c) There is no clear relationship

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between the numbers of positive charges and activity, and the position of specific positive charges is important (98). (d) Enantiomers (i.e., all D–amino acids vs. all L–amino acids) of the structured peptides have equal activity (101), showing that chirality is not important. (e) Decreased ability of cationic peptides to bind to bacteria or to lyse liposomes correlates to some extent with decreased MIC (99). However, this correlation is not absolute. For example, one can design peptides that bind extremely well to Gram-negative bacteria but have little or no antibiotic activity (66; Hancock REW and Gough M, unpublished data). Furthermore, it must be noted that some cationic peptides that are potent mediators of liposomal lysis are potent toxins but relatively weak antibacterial agents. (f) For the disulfide-bonded peptides, reduction of the cysteine disulfides destroys activity. (g) Finally, there is no absolute correlation between peptide size and antibacterial potency. For example, reduction in size of cecropin–melittin hybrids from 26 to 14 amino acids did not influence activity so long as these compounds maintained an α-helical structure (102).

It must be stressed, however, that we do not at present have a set of design rules that will create the perfect peptide antibiotic. For example, although amphipathicity and α-helicity favor activity, a perfectly amphipathic α-helix (Lys–Ala–Ala–Lys–Ala–Ala–Ala–Lys) was a potent hemolysin.

C. Interactions with Membranes

The primary mechanism of action of cationic peptides is probably through the generation of channels in membranes. These can range from ordered channels through to so-called multistate channels. It must be stressed that we do not know in detail the basis for membrane target selectivity. For example, although they both fall into the amphipathic α-helical class, moth cecropins are strongly antibacterial and demonstrate minimal eukaryotic selectivity (i.e., toxicity), whereas melittin from bee venom is a weak antibacterial compound but a potent toxin. The primary basis for selectivity has been reported to be the target lipid composition (see below).

A secondary mechanism of action is a detergent-like effect (103). However, it is unclear whether this merely represents the cooperative accumulation of multistate channels or gross multimerization of cationic peptides in the membrane, and whether this mechanism is relevant to bacterial cell killing, since it has only been demonstrated in nondefinitive experiments in eukaryotic cell lines and model liposomes (103). In contrast, the lysis of bacteria—often at concentrations exceeding the MIC—probably arises from the triggering of autolytic enzymes (104). With these caveats, it is worth considering how cationic peptides interact with membranes.

1. Model Systems

The process of interaction of the peptides with lipid layers can be modeled as shown in Figure 5. The peptides initially present in solution as aggregates are present in the form of dimers (e.g., defensins) (88) and/or conformers (e.g., cecropins are relatively unstructured in solution). Interaction with the negatively charged head groups of lipids occurs in a cooperative, rapid process involving progressive binding and alignment of positive charges of the peptide with lipid head groups (105–107). The extent of binding corresponds to the zeta potential of the lipids involved, leading one to conclude that it is electrostatic in nature (106,107). Lipid composition is important: binding to liposomes composed of negatively charged lipids is extremely fast, but binding to zwitterionic lipids is slower and can demonstrate negative cooperativity (107).

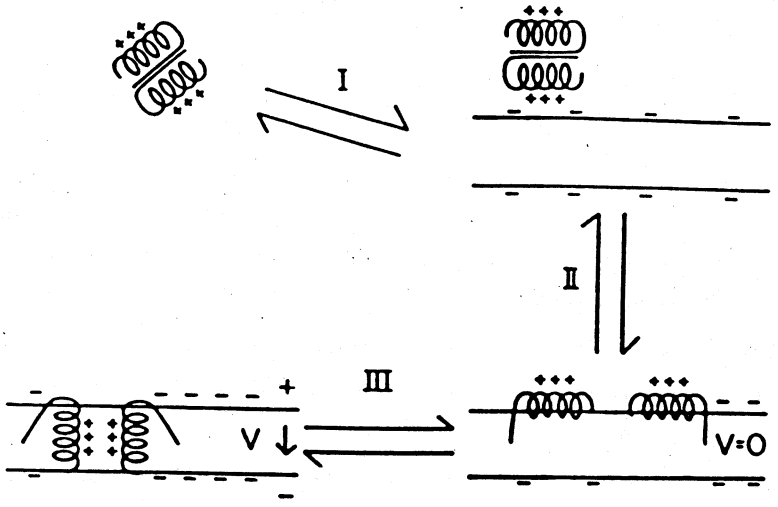

Figure 5 Tentative model for the interaction of cecropins with a lipid bilayer membrane. Aggregates adsorb to the bilayer–water interface by electrostatic forces (I). Only a dimer is sketched for the sake of simplicity, but larger aggregates are likely to occur. The next step (II) would be insertion of the hydrophobic segment into the membrane core. Upon application of voltage (positive on the side of the peptide addition), a major conformational rearrangement takes place (III), which results in channel formation. This rearrangement could be insertion of the positively charged amphipathic helix into the membrane or opening of preformed, closed channels. (Reproduced by copyright permission from Christensen et al. (36). © The National Academy of Sciences of the United States of America.)

It is uncertain whether at this point the permeability of the target lipid membrane changes. However, it seems credible that the phenomenon of leakiness (usually assessed by carbofluorescein leakage from liposomes) may occur in part at this stage. Furthermore, it is probable that the cationic peptide undergoes a change in conformation and aggregation state as a result of this interaction.

The next event is the insertion of the cationic peptide into the membrane. This occurs at a critical concentration of peptide that depends on the nature of the peptide and the target membrane, as well as on the fluidity of the membrane and the existence and size of the transmembrane electrical potential. Although insertion can occur into membranes with little or no transmembrane potential, it seems likely that the membrane potential of living cells (oriented interior negative) is always an important factor in peptide insertion. In addition, it has been demonstrated in planar lipid bilayer model membrane experiments (in which the "membrane potential" is provided as an applied voltage) that this potential must be oriented positive on the cis side (where the cationic peptides are added) and negative on the trans side of the membrane (toward which the cationic peptides move as they enter the membrane). This results in an observable increase in conductance as the peptides enter the membrane and form channels (36,94,108–111). Reversal of the voltage actually causes peptides to leave the membrane (36). It is relevant to the issue of toxicity that bacterial cytoplasmic membranes bear large transmembrane electrical potential gradients, $\Delta\psi$ (up to –140 mV), whereas eukaryotic membranes have gradients of only about –20 mV or less.

The process of insertion causes changes in phase and/or motion of the lipids of the target membrane (112). However, the lipid composition can dramatically influence the possibility of insertion, and positively charged phospholipids and cholesterol decreased the formation of membrane channels by cecropin by 5- to 60-fold (36). Indeed, this may explain, in addition to the difference in $\Delta\psi$, the selectivity of cationic antibacterial peptides for bacteria over eukaryotes, since the former lack cholesterol, which is abundant in eukaryotic membranes, whereas anionic phosphatidyl glycerol and cardiodipin, major components of bacterial membranes, represent excellent target lipids.

The process of insertion can also cause a conformational change in the cationic peptides (e.g., from unstructured to α-helical), for example, with melittin (113) and magainins (90,114). In many cases, the peptides are thought to end up spanning the membrane bilayer (41,102,113) in multimeric complexes. Other peptides are too short to span the bilayer and presumably must form aggregates to permit transmembrane channel formation (102,114,115).

Generally speaking, cationic peptides form multistate channels, and planar lipid bilayer experiments demonstrate a substantial range of channel sizes, with single-channel conductances (which reflect size) varying from 10 to 2000 pS (94,108–110,116) and lifetimes ranging from milliseconds to seconds (110). This behavior is similar to that observed for alamethicin (117), for which it has been proposed that application of a voltage induces alamethicin monomers to span the membrane, and that these monomers associate and disassociate with various rate constants, leading to aggregates of different sizes and lifetimes. Each aggregate contains a variable number of monomers arrayed like staves of a barrel around a central axis, and oriented with the hydrophilic portion of the monomer facing inward toward the channel interior and the hydrophobic face adjacent to the membrane interior. Thus, the size of the aggregates would decree the size of the channel. These channels are water filled and tend to be weakly selective for chloride over sodium ions.

In specific instances, for example, the cecropins, the channels formed are more defined (118). In this case, the actual channel forming unit has been modeled at atomic-level resolution. Two arrangements of six dimers have been proposed to account for the two discrete conductance increments (0.4 and 1.9 nS) reported by Christensen et al. (36).

2. *Bacterial Cytoplasmic Membranes*

As discussed above, cationic peptides can form channels in model bilayers. Thus, it seems likely that their primary antibacterial action is to disrupt the integrity of bacterial cytoplasmic membranes. This would have the effect of permitting leakage of ions and small metabolites, and destroying the ability of bacteria to maintain a transmembrane proton gradient (proton-motive force) with consequent loss of ability to generate adenosine triphosphate and transport substrates (see Ref. 119 for review of cytoplasmic membranes).

Bacteria maintain, across their cytoplasmic membranes, a proton-motive force of approximately –170 mV (120), comprising an electrical potential gradient $\Delta\psi$ (oriented interior negative) and a proton chemical gradient ΔpH (oriented interior alkaline). Treatment of cells with any of several different cationic peptides (e.g., magainins, nisin, or Pep5) leads to destruction of $\Delta\psi$ at concentrations approaching the MIC, as assessed using the cationic lipid-soluble probe triphenyl phosphonium (121,122). Evidence suggests that this decrease of proton-motive force occurs as a sigmoidal function of peptide

concentration, indicating that peptides act in a cooperative fashion on cytoplasmic membranes (121). Other data favoring the hypothesis that destruction of cytoplasmic membrane integrity is the primary basis of the activity of cationic peptides against bacteria include the demonstration that mastoporan and melittin cause K^+ leakage in Gram-positive bacteria (123).

3. *Bacterial Outer Membranes*

Only Gram-negative bacteria have outer membranes, and it is clear that the interaction of an antibiotic with outer membranes cannot directly lead to cell death. However, outer membranes are discussed separately here for two reasons. First, the cationic peptides include molecules that are rare among antibiotics in having better activities against Gram-negative than Gram-positive bacteria (normally, the influence of the outer membrane on penetration of antibiotics decreases activity). Second, the interaction of cationic peptides with the outer membranes of Gram-negative bacteria explains two of the pharmaceutically interesting properties of these molecules, namely, their "enhancer" and antiendotoxin properties.

Cationic peptides, like other polycationic antibiotics, traverse the outer membrane using a process termed self-promoted uptake (124); in contrast, small hydrophilic antibiotics such as β-lactams diffuse through the water-filled channels of porin proteins (125). Self-promoted uptake (Figure 6) involves the initial interaction of cationic peptides with the negatively charged, divalent-cation-binding sites of the surface glycolipid lipopolysaccharide (LPS). Since the cationic peptides have an affinity for LPS that is three orders of magnitude higher than the native divalent cations, Mg^{2+} or Ca^{2+} (70), they competitively displace these cations. This causes a distortion of outer membrane structure that has been visualized in the electron microscope as induction of outer membrane blebs (70), and a consequent permeabilization of the membrane to probe molecules, including lysozyme and the hydrophobic probe 1-*N*-phenyl-napthylamine (71,126). By analogy with other polycations, this distortion of the membrane is proposed to lead to enhanced ability of the cationic peptide to promote its own uptake (hence the term self-promoted uptake). The basic features of this uptake system have been demonstrated for interaction of both the α-helical (66) and β-structured cationic peptides (70).

The ability of cationic peptides to act in synergy with certain classical antibiotics (68) can be explained by their ability to disrupt outer membrane integrity, promoting the uptake of antibiotics across this barrier. Interestingly, the most potent cationic peptides do not have this "enhancer" activity for most antibiotics, presumably since they kill cells

Table 4 MICs of Selected Cationic Peptides Compared with Conventional Antibiotics

	MIC[a](μg/ml)				
Organism	CP-29[b]	CP-11c[c]	Gentamicin	Ceftazidime	Polymyxin
Pseudomonas aeruginosa	4	8	0.3	0.5	0.3
Escherichia coli	2	2	0.3	0.3	0.1
Staphylococcus aureus	16	8	2	2	>64
Candida albicans	32	8	>64	>64	>64

[a] MIC values were determined by the broth dilution method (63).
[b] CP-29 is a cecropin/mellitin hybrid cationic peptide (Hancock, Gough, and Farmer, unpublished data).
[c] CP-11c is an extended helix cationic peptide (Falla, T., Hancock, R. E. W., unpublished data).

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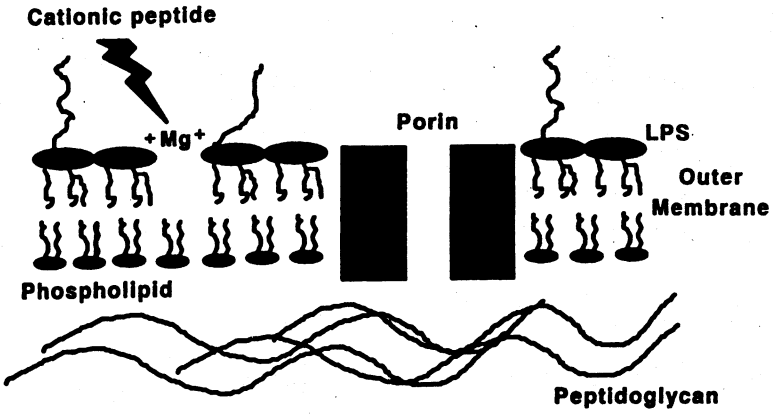

Figure 6 Schematic diagram of the self-promoted uptake model. Cationic antibiotics disrupt the Mg^{2+} cross-bridges, displace the Mg^{2+} ions, and cause perturbation of the lipid bilayer. Further disruption of the outer membrane results in uptake of normally excluded compounds.

at concentrations equal to their permeabilizing concentrations (66). This is analogous to the situation for the polycationic antibiotic polymyxin B, which is not an enhancer, whereas its deacylated derivative PMBN (which interacts weakly with cytoplasmic membranes but strongly with outer membranes) is a potent enhancer of antibiotic activity (127).

The antiendotoxin activity of cationic peptides is also related to the above uptake mechanism. Endotoxin is in fact LPS, or more precisely the lipid A portion of LPS. As mentioned above, cationic peptides bind to polyanionic LPS (70,128,129). The binding is of high affinity and cooperative (70). This binding can neutralize the ability of LPS to induce TNF in macrophage cell lines or in a murine model, and it reduces endotoxin mortality in galactosamine-sensitized mice (130,131).

V. PRODUCTION METHODS

A. Natural Sources

As described in Section II, cationic peptides are very widely distributed in nature. Recovery from these sources involves a wide range of methods. One effective procedure is extraction with 30% acetic acid, which tends to solubilize cationic peptides and precipitate many globular proteins. This is usually followed by a variety of chromatographic procedures often including reverse phase HPLC or FPLC as the final step in purification. However, purification from natural sources is rarely a practical alternative for commercial purposes, since yields tend to be relatively low. For example, a single rabbit will permit the recovery of only 200 mg of rabbit defensins. The one exception is the production of cationic lantibiotic peptides such as nisin from bacteria and commercial production of nisin by fermentation of *Lactococcus lactis* (see Chapter 15).

B. Protein Chemical

A very convenient laboratory-scale procedure for making peptides is the use of automated peptide synthesizers using t-boc or f-moc chemistry (132). However, the expense

of reagents and the limited capacities of these automated synthesizers has limited the scale and, thus, the industrial relevance of this method. An alternative is provided by solution phase chemistry, which is, unfortunately, less conductive to automation.

C. Recombinant Procedures

One potential advantage of the peptide nature of cationic peptides is their potential ability to be synthesized recombinantly, since they can be directly encoded by DNA. There are certainly some limitations to this, since nonnatural amino acids (e.g., in the antibiotics) and many modifications (e.g., carboxyl-terminal amidation) are difficult to introduce recombinantly. As with the protein chemical procedures, however, one is not limited to "natural" cationic peptides.

The first attempt to synthesize cationic peptides recombinantly appears in the patent literature (133). A sequence encoding cecropin was fused to a portion of the *araC* gene of *E. coli*. Although few details were provided, it is clear that this method was not optimized: although cecropin could be manufactured recombinantly, it had poor potency (i.e., 5 μg of recombinant cecropin gave a clearing zone diameter against *E. coli* of 5 mm, whereas 1 μg of authentic cecropin gave a clearing diameter of 7 mm). Furthermore, the patent claimed that virtually any fusion partner would work to support the production of cecropins, whereas it is now evident that this is not the case. Subsequently, it was demonstrated that cecropin could be synthesized as a fusion with a protein A–like, IgG-binding domain, using baculovirus vector in an insect cell line (2). After affinity purification of the fusion protein and cleavage of cecropin from its carrier using cyanogen bromide, the cecropin could be recovered in its amidated form. The yields were 600 μg/ml of haemolymph, of which 70% was amidated, indicating that this method may be cost prohibitive given the expense associated with animal cell culture.

Piers et al. (1) have developed a procedure for the synthesis of cationic peptides in bacteria. The main feature is inclusion of an anionic stabilizing fragment in the fusion protein to counteract the cationic peptide portion. This anionic fragment could be the carrier protein itself if the fusion protein was expressed in *S. aureus*, but for expression in *E. coli* an extra anionic sequence equivalent to the pre pro sequence from the gene for human defensin (which sequence stabilizes defensin during its synthesis in human cells; 134) is needed. Additional elements were the inclusion of a methionine residue immediately adjacent to the cationic peptide sequence, to permit removal of the cationic peptide by CNBr cleavage, and a carrier sequence that, when desired, could be tailored to enhance affinity purification of the resultant fusion. The general nature of the fusion protein is demonstrated in Figure 7. We believe this system offers significant advantages in the production of cationic peptides. An interesting side note is that the successful production of antibacterial cationic peptides by molecular genetic means makes these cationic peptides the first recombinant antibiotics.

VI. AN EXCITING FUTURE

Cationic peptides do not have as potent activities against the most susceptible bacteria as do other antibiotics. Furthermore, the spectrum of cationic peptides includes some of the most potent toxins (e.g., bee venom and scorpion toxin), so that toxicity will always be a closely observed issue. In addition, being peptides, they are potentially susceptible to host

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1. Piers KL, I
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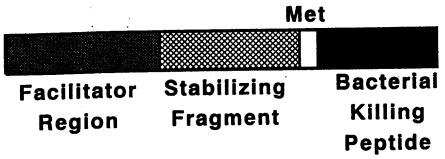

Figure 7 Schematic diagram of a protein A–cationic peptide fusion protein. Met represents the position of the methionine residue used to cleave the peptide with cyanogen bromide.

peptidases and proteases, and innovative approaches will have to be applied to overcome this problem (e.g., the use of D–amino acids). However, activity, toxicity, and pharmacology are issues with every compound used in medicine.

With these reservations, we believe that cationic peptides offer an exciting future. They represent a "natural" solution to infection, since they mimic the anti-infective defense systems of several eukaryotes. Their activities cover a far broader spectrum than do those of other antibiotics. Indeed, they offer the potential for organ-specific therapy directed against the major bacterial and fungal infections of a given body site. In addition, the most active cationic peptides have activities against some of the more refractory antibiotic-resistant pathogens (e.g., *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*) that are equivalent to those observed for the antibiotics tailored for use against those pathogens. Indeed, that they do not seem to induce antibiotic resistance and are effective against most bacteria resistant to conventional antibiotics are important features of cationic peptides. In addition, their potential to act in synergy with conventional antibiotics and to neutralize endotoxin released by these antibiotics makes them an attractive candidate for use in combination therapy.

ACKNOWLEDGMENT

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REFERENCES

1. Piers KL, Brown MH, Hancock REW. Recombinant DNA procedures for producing small antimicrobial cationic peptides in bacteria. Gene 1993; 134:7–13.
2. Hellers M, Gunne H, Steiner H. Expression of post-translational processing of prececropin A using a baculovirus vector. Eur J Biochem 1991; 199:435–439.
3. Jaynes JM, Nagpala P, Destefano-Bettran L, Huang JCH, Kim JH, Denny T, Cetiner S. Expression of a Cecropin B lytic peptide analog in transgenic tobacco confers enhanced resistance to bacterial wilt caused by *Pseudomonas solanacearum*. Plant Sci 1993; 43–53.
4. Mitra A, Zhang Z. Expression of a human lactoferrin cDNA in tobacco cells produces antibacterial protein(s). Plant Physiol 1994; 106:971–981.
5. Hancock REW, Falla T, Brown MM. Cationic bactericidal peptides. Poole RK, ed. Advances in Microbial Physiology, 2nd ed. London: Academic Press, 1995:137–175.
6. Couto MA, Harwig SSL, Cullor JS, Hughes JPÊ, Lehrer RI. eNAP, a novel cysteine-rich bactericidal peptide from equine leukocytes. Infect Immun 1992; 60:5042–5047.

7. Eisenhauer PB, Lehrer RI. Mouse neutrophils lack defensins. Infect Immun 1992; 60:3446–3447.
8. Lehrer RI, Ganz T, Selsted ME. Defensins: Natural peptide antibiotics from neutrophils. ASM News 1990; 56:315–318.
9. Michaelson D, Rayner J, Cuoto M, Ganz T. Cationic defensins arise from charge-neutralized propeptides: A mechanism for avoiding leukocyte autocytotoxicity. J Leukocyte Biol 1992; 51:634–639.
10. Selsted ME, Tang YQ, Morris WL, McGuire PA, Novotny MJ, Smith W, Henschen AH, Cullor JS. Purification, primary structures and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J Biol Chem 1993; 268:6641–6648.
11. Diamond G, Zasloff M, Eck H, Brasseur M, Maloy WL, Bevins CL. Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: Peptide isolation and cloning of a cDNA. Proc Natl Acad Sci USA 1991; 88:3952–3956.
12. Gennaro R, Skerlaray B, Romeo D. Purification, composition and activity of two bactenecins, antibacterial peptides of bovine neutrophils. Infect Immun 1989; 57:3142–3146.
13. Selsted ME, Novotny MJ, Morris WL, Tang YQ, Smith W, Cullor JS. Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils. J Biol Chem 1992; 267:4292–4295.
14. Agerberth B, Lee JY, Bergman T. Carlquist M, Boman HG, Mutt V, Jornvall H. Amino acid sequence of PR-39: Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides. Eur J Biochem 1991; 202:849–854.
15. Larrick JW, Morgan JG, Palings I, Hirata M, Yen MH. Complementary DNA sequence of rabbit CAP18—a unique lipopolysaccharide binding protein. Biochem Biophys Res Commun 1991; 179:170–175.
16. Zanetti M, Del Sal G, Storici P, Schneider C, Romeo D. The cDNA of the neutrophil antibiotic Bac5 predicts a pro-sequence homologous to a cysteine proteinase inhibitor that is common to other neutrophil antibiotics. J Biol Chem 1993; 268:522–526.
17. Levy O, Weis J, Zarember K, Ooi CE, Elsbach P. Antibacterial 15-kDa protein isoforms (p15s) are members of a novel family of leukocyte proteins. J Biol Chem 1993; 268:6058–6063.
18. Bevins CL, Zasloff M. Peptides from frog skin. Annu Rev Biochem 1990; 59:395–414.
19. Lazarus LH, Attila M. The toad, ugly and venomous, wears yet a precious jewel in his skin. Prog Neurobiol 41:473–507.
20. Csordas A, Michl H. Isolation and structure of a hemolytic polypeptide from the defensive secretion of European Bombina species. Monatsh Chem 1970; 101:182–189.
21. Simmaco M, Barra D, Chiarini F, Noviello L, Melchiorri P, Kreil G, Richter K. A family of bombinin-related peptides from the skin of *Bombina variegata*. Eur J Biochem 1991; 199:217–222.
22. Chen HC, Brown JH, Morell JL, Huang CM. Synthetic magainin analogues with improved antimicrobial activity. FEBS Lett 1988; 236:462–466.
23. Zasloff M. Magainins, a class of antimicrobial peptides from *Xenopus* skin: Isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci USA 1987; 84:5449–5453.
24. Zasloff M, Martin B, Chen HC. Antimicrobial activity of synthetic magainin peptides and several analogues. PNAS 1988; 85:910–913.
25. Levison M, Pitsaki PG, May PL, Johnson CC. The bactericidal activity of magainins against *Pseudomonas aeruginosa* and *Enterococcus faecium*. J Antimicrob Chemother 1993; 32:577–585.
26. Cuervo JH, Rodriquez B, Houghten RA. The Magainins: Sequence factors relevent to increased antimicrobial activity and decreased hemolytic activity. Pept Res 1988; 1:81–86.
27. Hoffmann W, Richter K, Kreil G. A novel peptide designated PYLa and its precursor as predicted from cloned mRNA of *Xenopus laevis*. EMBO J 1983; 2:711–714.
28. Moore KS, Be
bial peptides i
29. Sures L, Cripp
boxyl terminu
30. Terry AS, Pou
The cDNA se
this polypeptic
31. Morikawa N,
from the skin
189:184–190.
32. Simmaco M,
tion of the Eu
33. Steiner H, Hu
two antibacter
34. Lambert J, Ke
Hoffman J, Fo
dipteran *Phorr*
rabbit lung m
266.
35. Matsuyama K
the sapecin g
263:17117–17
36. Christensen B
and related m
Sci USA 198
37. Hultmark D,
structure of ce
Biochem 1982
38. Okada M, Na
peregrina. Bioc
39. Samakovlis C
Drosophila: P
9:2969–2976.
40. Lee J-Y, Bom
peptides from
1989; 86:915
41. Sipos D, And
cecropin P1 i
42. Hoffmann JA
1992; 13:411-
43. Bontems F, R
toxin: Comm
44. Cociancich S
and character
similarities wi
194:17–22.
45. Fujiwara S, Ir
terial protein
isin. J Biol Cl
46. Kuzuhara T, N
sapecin, an
107:514–518.

28. Moore KS, Bevins CL, Brasseur MM, Tomassini N, Turner K, Eck H, Zasloff M. Antimicrobial peptides in the stomach of *Xenopus laevis*. J Biol Chem 1991; 266:19851–19857.
29. Sures L, Crippa M. Xenopsin: the neurotensin-like octapeptide from *Xenopus* skin at the carboxyl terminus of its precursor. Proc Natl Acad Sci USA 1984; 81:380–384.
30. Terry AS, Poulter L, Williams DH, Nutkins JC, Giovannini MG, Moore CH, Gibson BW. The cDNA sequence for prepro-PGS (prepro-magainins) and aspects of the processing of this polypeptide. J Biol Chem 1988; 263:5745–5751.
31. Morikawa N, Hagiwara K and Nakajima T. Brevinin-1 and -2, unique antimicrobial peptides from the skin of the frog *Rana brevipoda porsa*. Biochem Biophys Res Commun 1992; 189:184–190.
32. Simmaco M, Mignogna G, Barra D, Bossa F. Novel antimicrobial peptides from skin secretion of the European frog *Rana esculenta*. FEBS Lett 1993; 324:159–161.
33. Steiner H, Hultmark D, Engström A, Bennich H, Boman HG. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature 1981; 292:246–248.
34. Lambert J, Keppi E, Dimarcq JL, Wicker C, Reichhart JM, Dunbar B, Lepage P, Van DA, Hoffman J, Fothergill J, Hoffman D. Insect immunity: Isolation from immune blood of the dipteran *Phormia terranovae* of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. Proc Natl Acad Sci USA 1989; 86:262–266.
35. Matsuyama K, Natori S. Molecular cloning of cDNA for sapacin and unique expression of the sapecin gene during the development of *Sacrophaga peregrina*. J Biol Chem 1988: 263:17117–17121.
36. Christensen B, Fink J, Merrifield RB, Mauzerall D. Channel forming properties of cecropins and related model compounds incorporated into planar lipid membranes. Proc Natl Acad Sci USA 1988; 85:5072–5076.
37. Hultmark D, Engström A, Bennich H, Kapur R, Boman HG. Insect immunity: Isolation and structure of cecropin D and four minor antibacterial components from *Cecropia pupae*. Eur J Biochem 1982; 127:207–217.
38. Okada M, Natori S. Ionophore activity of sarcotoxin I, a bactericidal protein of *Sarcophaga peregrina*. Biochem J 1985; 229:453–458.
39. Samakovlis C, Kimbrell DA, Kylsten P, Engström A, Hultmark D. The immune response in *Drosophila:* Pattern of cecropin expression and biological activity. EMBO J 1990; 9:2969–2976.
40. Lee J-Y, Boman A, Sun CX, Anderson M, Jornvall H, Mutt V, Boman HG. Antibacterial peptides from pig intestine: Isolation of a mammalian cecropin. Proc Natl Acad Sci USA 1989; 86:9159–9162.
41. Sipos D, Andersson M, Ehrenberg A. The structure of the mammalian antibacterial peptide cecropin P1 in solution, determined by proton-NMR. Eur J Biochem 1992; 209:163–169.
42. Hoffmann JA, Hetru C. Insect defensins: Inducible antibacterial peptides. Immun Today 1992; 13:411–415.
43. Bontems F, Roumestand C, Gilguin B, Menez A, Toma F. Refined structure of charybdotoxin: Common motifs in scorpion toxins and insect defensins. Science 1991; 254:1521.
44. Cociancich S, Goyffon M, Bontems F, Bulet P, Bouet F, Menez A, Hoffmann J. Purification and characterization of scorpion defensin, a 4KDa antibacterial peptide presenting structural similarities with insect defensins and scorpion toxins. Biochem Biophys Res Commun 1993; 194:17–22.
45. Fujiwara S, Imai J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of rayalisin. J Biol Chem 1990; 265:11333–11337.
46. Kuzuhara T, Nakajima Y, Matsuyama K, Natori S. Determination of the disulphide array in sapecin, an antibacterial peptide of *Sarcophaga peregrina* (flesh fly). J Biochem 1990; 107:514–518.

47. Casteels P. Possible applications of insect antibacterial peptides. Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). Res Immun 1990; 141:940–942.
48. Casteels P, Ampe C, Jacobs F, Vaeck M, Tempst P. Apidaecins: Antibacterial peptides from honeybees. EMBO J 1989; 8:2387–2391.
49. Casteels P, Ampe C, Jacobs F, Tempst P. Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (*Apis mellifera*). J Biol Chem 1993; 268:7044–7054.
50. Casteels-Josson K, Capaci T, Casteels P, Tempst P. Apidaecin multipeptide precursor structure: A putative mechanism for amplification of the insect antibacterial response. EMBO J 1993; 12:1569–1578.
51. Nakamura T, Furunaka H, Miyata T, Tokunaga F, Muta T, Iwanaga S, Niwa M, Takao T, Shimonishi Y. Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*): Isolation and chemical structure. J Biol Chem 1988; 263:16709–16713.
52. Miyata T, Tokunaga F, Yoneya T, Yoshikawa K, Iwanaga S, Niwa M, Takao T, Shimonishi Y. Antimicrobial peptides isolated from horseshoe crab hemocytes, tachyplesin II, and polyphemusins I and II: Chemical structures and biological activity. J Biochem 1989; 106:663–668.
53. Muta T, Fujimoto T, Nakajima H, Iwanaga S. Tachyplesins isolated from hemocytes of Southeast Asian horseshoe crabs (*Carcinoscorpius rotundicauda* and *Tachypleus gigas*): Identification of a new tachyplesin III, and a processing intermediate of its precursor. J Biochem 1990; 108:261–266.
54. Hurst A. Nisin. Adv Appl Microbiol 1981; 27:85–123.
55. Kaletta C, Entian KD, Kellner R, Jung G, Reis M, Sahl HG. Pep5, a new lantibiotic: Structural gene isolation and prepeptide sequence. Arch Microbiol 1989; 152:16–19.
56. Weil HP, Beck-Sickinger AG, Metzger J, Stevanovic S, Jung G, Josten M, Sahl HG. Biosynthesis of the lantibiotic Pep5: Isolation and characterization of a prepeptide containing dehydroamino acids. European J Biochem 1990; 194:217–223.
57. Sahl HG. Pore formation in bacterial membranes by cationic lantibiotics. Jung G, Sahl HG, eds. Nisin and Novel Lantibiotics. Leiden: ESCOM, 1991:347–348.
58. Benz R, Jung G, Sahl HG. Mechanism of channel-formation by lantibiotics in black lipid membranes. Jung G, Sahl HG, eds. Nisin and Novel Lantibiotics. Leiden: ESCOM, 1991:359–372.
59. Bradley WA, Somkuti GA. The primary structure of sillucin and antimicrobial peptide from *Mucor pusillus*. FEBS Lett 1979; 97:81–83.
60. Bohlmann H, Clausen S, Behnke S, Giese H, Hiller C, Reimann-Philipp U, Schrader G, Barkholt V, Apel K. Leaf-specific thionins of barley—a novel class of cell wall proteins toxic to plant pathogenic fungi and possibly involved in the defence mechanism of plants. EMBO J 1988; 7:1559–1565.
61. Broekaert WF, Marlen W, Terras FR, De Bolle MF, Proost P, Van Damme J, Dillen L, Claeys M, Rees SB, Vanderleyden J, et al. Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. Biochem 1992; 31:4308–4314.
62. Cammue BP, De Bolle MF, Terras FR, Proost P, Van Damme J, Rees SB, Vanderleyden J, Broekaert WF. Isolation and characterization of a novel class of plant antimicrobial peptides from *Mirabilis jalapa L.* seeds. J Biol Chem 1992; 267:2228–2233.
63. Amsterdam D. Susceptibility testing of antimicrobials in liquid media. Lorian V, ed. Antibiotics in Laboratory Medicine, 3rd ed. Baltimore: Williams and Wilkins, 1991:72–128.
64. Barry AL. Procedures and theoretical considerations for testing antimicrobial agents in agar media. Lorian V, ed. Antibiotics in Laboratory Medicine, 3rd ed. Baltimore: Williams and Wilkins, 1991:1–14.
65. Acar JF, Goldstein FW. Disk susceptibility test. Lorian V, ed. Antibiotics in Laboratory Medicine, 3rd ed. Baltimore: Williams and Wilkins, 1991:17–48.
66. Piers KL, I
and lipopo
modificatic
67. Piers K. Tl
outer mem
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68. Darveau R
Watkins C
and in vivc
69. Goto H, N
otics. Japar
70. Sawyer JG
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71. Piers KL, H
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72. Lin Y, Kol
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73. Rogy MA,
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74. Gough M
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76. Lehrer RI,
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77. Terras FR,
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78. Terras FR
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1993; 103:
79. Daher KA
defensins.
80. Eisenhaue
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81. Aley SB,
cryptidins
82. Huang CN
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83. Komano H
Sarcophage
84. Baker MA
peptides. (
85. Schluesen
autoimmu
86. Hill CP, Y
dimer: Me
87. Hanzawa
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66. Piers KL, Brown MH, Hancock REW. Improvement of outer membrane-permeabilization and lipopolysaccharide-binding activities of an antimicrobial cationic peptide by C-terminal modification. Antimicrob Agents Chemother 1994; 38:2311–2316.
67. Piers K. The bacterial production of antimicrobial cationic peptides and their effects on the outer membrane of Gram-negative bacteria. PhD thesis, University of British Columbia, 1993.
68. Darveau RP, Cunningham MD, Seachord CL, Cassiano-Clough L, Cosand WL, Blake J, Watkins CS. Beta-lactam antibiotics potentiate magainin 2 antimicrobial activity in vitro and in vivo. Antimicrob Agents Chemother 1991; 35:1153–1159.
69. Goto H, Nakamura S. Liberation of endotoxin from *Escherichia coli* by addition of antibiotics. Japan J Med 1980; 50:35–43.
70. Sawyer JG, Martin NL, Hancock REW. Interaction of macrophage cationic proteins with the outer membrane of *Pseudomonas aeruginosa*. Infect Immun 1988; 56:693–698.
71. Piers KL, Hancock REW. The interaction of a recombinant cecropin/melittin hybrid peptide with the outer membrane of *Pseudomonas aeruginosa*. Mol Microbiol 1994; 12:951–958.
72. Lin Y, Kohn FR, Kung AH, Ammons WS. Protective effect of a recombinant fragment of bactericidal/permeability increasing protein against carbohydrate dyshomeostasis and tumor necrosis factor-alpha elevation in rat endotoxaemia. Biochem Pharm 1994; 47:1553–1559.
73. Rogy MA, Moldawer LL, Oldenburg HS, Thompson WA, Montegut WJ, Stackpole SA, Kumar A, Pulladino MA, Marra MN, Lowry SE. Anti-endotoxin therapy in primate bacteremia with HA-1A and BPI. Ann Surg 1994; 220:77–85.
74. Gough MA, Hancock REW, Kelly NM. Anti-endotoxin activity of cationic peptide antibiotics. Infect Immun 1996; 64:4922–4927.
75. Selsted ME, Szklarek D, Ganz T, Lehrer RI. Activity of rabbit leukocyte peptides against *Candida albicans*. Infect Immun 1985; 49:202–206.
76. Lehrer RI, Szklarek D, Ganz T, Selsted ME. Correlation of binding of rabbit granulocyte peptides to *Candida albicans* with candidacidal activity. Infect Immun 1985; 49:207–211.
77. Terras FR, Schoofs HM, De Bolle MF, Van Leuven F, Rees SB, Vanderleyden J, Cammue BP, Broekaert WF. Analysis of two novel classes of antifungal proteins from radish (*Raphanus sativus L.*) seeds. J Biol Chem 1992; 267:15301–15309.
78. Terras FRG, Torrekens S, Van Leuven F, Osborn RW, Vanderleyden J, Cammune BPA, Broekaert WF. Synergistic enhancement of the antifungal activity of wheat and barley thionins by radish and oilseed rape 2S albumins and by barley trypsin inhibitors. Plant Physiol 1993; 103:1311–1319.
79. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. J Virol 1986; 60:1068–1074.
80. Eisenhauer PB, Harwig SS, Szlarek D, Ganz T, Selsted M, Lehrer RI. Purification and antimicrobial properties of three defensins from rat neutrophils. Infect Immun 1989; 57:2021–2027.
81. Aley SB, Zimmerman M, Hetsko M, Selsted ME, Gillin FD. Killing of *Giardia lamblia* by cryptidins and cationic neutrophil peptides. Infect Immun 1994; 62:5397–5403.
82. Huang CM, Chen HC, Zierdt CH. Magainin analogs effective against pathogenic protozoa. Antimicrob Agents Chemother 1990; 34:1824–1826.
83. Komano H, Homma K, Natori S. Involvement of sapecin in embryonic cell proliferation of *Sarcophage peregrina* (flesh fly). FEBS Lett 1991; 289:167–170.
84. Baker MA, Malog WL, Zasloff M, Jacob L. Anticancer efficacy of magainin 2 and analogue peptides. Cancer Res 1993; 58:3052–3057.
85. Schluesener HJ, Radermacher S, Melms A, Jung S. Leukocytic antimicrobial peptides kill autoimmune T cells. J Neuroimmun 1993; 47:199–201.
86. Hill CP, Yee J, Selsted ME, Eisenburg D. Crystal structure of defensin HNP-3, an amphiphilic dimer: Mechanisms of membrane permeabilization. Science 1991; 251:1481–1485.
87. Hanzawa H, Shimada I, Kuzuhara T, Komano H, Kohda D, Inagaki F, Natori S, Arata Y. 1H nuclear magnetic resonance study of the solution conformation of an antibacterial protein, sapecin. FEBS Lett 1990; 269:413–420.

88. Zhang X, Selsted ME, Pardi A. NMR studies of defensin antimicrobial petides. Resonance assignment and secondary structure determination of rabbit NP-2 and human HNP-1. Biochem 1992; 31:11348–11356.
89. Marion D, Zasloff M, Bax A. A two dimensional NMR study of the antimicrobial petide magainin 2. FEBS Lett 1988; 227:21–26.
90. Bechinger B, Zasloff M, Opella SJ. Structure and interactions of magainin antibiotic peptides in lipid bilayers: A solid-state nuclear magnetic resonance investigation. Biophys J 1992; 62:12–14.
91. Holak TA, Engström A, Kraulis PJ, Lindberg G, Bennich H, Jones TA, Gronenborn AM, Clore GM. The solution conformation of the antibacterial peptide cecropin A: A nuclear magnetic resonance and dynamical simulated annealing study. Biochem 1988; 27:7620–7629.
92. Bazzo R, Tappin MJ, Pastore A, Harvey S, Carver JA, Campbell ID. The structure of melittin A: 1H-NMR study in methanol. Eur J Biochem 1988; 173:139–146.
93. Sipos D, Chandrasekhar K, Arvidsson K, Engström A, Ehrenberg A. Two dimensional proton-NMR studies on a hybrid peptide between cecropin A and melittin. Resonance assignments and secondary structure. Eur J Biochem 1991; 199:285–291.
94. Falla TJ, Karunaratne DN, Hancock REW. Mode of action of the antimicrobial peptide indolicidin. J Biol Chem 1996; 271:19,298–19,303.
95. Cabiaux V, Agerberth B, Johansson J, Homblé F, Goormaghtigh E, Ruysschaert J-M. Secondary structure and membrane interaction of PR-39, a Pro+Arg-rich antibacterial peptide. Eur J Biochem 1994; 224:1019–1027.
96. Raj PA, Edgerton M. Functional domain and poly-L-proline conformation for candidacidal activity of bactenecin 5. FEBS Lett 1995; 368:526–530.
97. Andreu D, Merrifield RB, Steiner H, Boman HG. N-terminal analogues of cecropin A: Synthesis, antibacterial activity, and conformational properties. Biochem 1985; 24:1683–1688.
98. Blondelle SE, Houghten RA. Hemolytic and antimicrobial activities of the twenty-four individual omission analogues of melittin. Biochem 1991; 30:4671–4678.
99. Steiner H, Andreu D, Merrifield RB. Binding and action of cecropin and cecropin analogues: Antibacterial peptides from insects. Biochim Biophys Acta 1988; 939:260–266.
100. Frohlich DR, Wells MA. Peptide amphipathy: A new strategy in design of potential insecticides. Intl J Pept Res 1991; 37:2–6.
101. Wade D, Boman A, Wahlin B, Drain CM, Andreu D, Boman HG, Merrifield RB. All-D amino acid–containing channel-forming antibiotic peptides. Proc Natl Acad Sci USA 1990; 87:4761–4765.
102. Andreu D, Ubach J, Boman A, Wahlur B, Wade D, Merrifield RB, Boman HG. Shortened cecropin A-melittin hybrids. Significant size reduction retains potent antibiotic activity. FEBS Lett 1992; 296:190–194.
103. Bashford CL, Alder GM, Menestrina G, Micklem KJ, Murphy JJ, Pasternak CA. Membrane damage by hemolytic viruses, toxin, complement, and other cytotoxic agents. A common mechanism blocked by divalent cations. J Biol Chem 1986; 261:9300–9308.
104. Chitnis SJ, Prasad KSN, Bhargava PM. Isolation and characterization of autolysis-defective mutants of *Escherichia coli* that are resistant to the lytic activity of seminalplasmin. J Gen Microbiol 1990; 136:463–469.
105. Batenburg AM, Hibbeln JCL, de Kruijff B. Lipid specific penetration of melittin into phospholipid model membranes. Biochim Biophys Acta 1987; 903:155–165.
106. Matsuzaki K, Harada M, Funakoshi S, Fujii N, Miyajima K. Physicochemical determinants for the interactions of magainins 1 and 2 with acidic lipid bilayers. Biochim Biophys Acta 1991; 1063:162–170.
107. Sekharam KM, Bradrick TD, Georghiou S. Kinetics of melittin binding to phospholipid small unilamellar vesicles. Biochim Biophys Acta 1991; 1063:171–174.
108. Kagan BL,
dependent
USA 1990;
109. Hanke W, H
cally modif
1983; 727:1
110. Kordel M, H
age depend
170:84–88.
111. Cociancich
antibacteria
1993; 268:1
112. Vogel H, Ja
113. Smith R, S
ers: A solid-
114. Williams R
troscopy of
29:4490–44
115. Agawa Y, L
with phosp
amphiphili
266:20218–
116. Duclohier H
anion-perm
117. Boheim G.
Biol 1974; 1
118. Durell SR, H
phys J 1992
119. Cronan JE,
M, Umbarg
Biology. Wa
120. Bakker EP, H
by electroge
121. Juretic D, C
and analogu
olysis. FEBS
122. Westerhoff
brane-linke
123. Katsu T, Ku
of wasp ven
124. Nicas TI, H
ment in ad
and gentam
125. Hancock R
126. Hancock R
aeruginosa c
127. Vaara M. A
56:395–411
128. Rana FR, M
ainin 2 and
Biochem 19

108. Kagan BL, Selsted ME, Ganz T, Lehrer RI. Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc Natl Acad Sci USA 1990; 87:210–214.
109. Hanke W, Methfessel C, Wilmsen HU, Katz E, Jung G, Boheim G. Melittin and a chemically modified trichotoxin form alamethicin-type multistate pores. Biochim Biophys Acta 1983; 727:108–114.
110. Kordel M, Benz R, Sahl HG. Mode of action of the staphylococcin like peptide Pep5: Voltage dependent depolarization of bacterial and artificial membranes. J Bacteriol 1988; 170:84–88.
111. Cociancich S, Ghazi A, Hetru C, Hoffman JA, Letellier LJ. Insect defensin, an inducible antibacterial peptide, forms voltage dependent channels in *Micrococcus luteus*. J Biol Chem 1993; 268:19,239–19,245.
112. Vogel H, Jahnig F. The structure of melittin in membranes. Biophys J 1986; 50:573–582.
113. Smith R, Separovic F, Bennett FC, Cornell BA. Melittin-induced changes in lipid multilayers: A solid-state NMR study. Biophys J 1992; 63:469–474.
114. Williams RW, Starman R, Taylor KMP, Gable K, Beeler T, Zasloff M, Covell D. Raman spectroscopy of synthetic antimicrobial frog peptides magainin 2a and PGLa. Biochem 1990; 29:4490–4496.
115. Agawa Y, Lee S, Ono S, Aoyagi H, Ohno M, Tamguchi T, Anzai K, Kirino Y. Interaction with phospholipid bilayers, ion channel formation, and antimicrobial activity of basic amphiphilic alpha-helical model peptides of various chain lengths. J Biol Chem 1991; 266:20218–20222.
116. Duclohier H, Molle G, Spach G. Antimicrobial peptide magainin I from *Xenopus* skin forms anion-permeable channels in planar lipid bilayers. Biophys J 1989; 56:1017–1021.
117. Boheim G. Statistical analysis of alamethicin channels in black lipid membranes. J Membr Biol 1974; 19:277–303.
118. Durell SR, Raghunathan G, Guy HR. Modelling the ion channel structure of cecropin. Biophys J 1992; 63:1623–1631.
119. Cronan JE, Gennis RB, Maloy SR. In: Ingraham JL, Brookslow K, Magnasanik B, Schaechter M, Umbarger HE, eds. *Escherichia coli* and *Salmonella typhimurium:* Cellular and Molecular Biology. Washington, D.C. 5:31–55: ASM Press, 1987.
120. Bakker EP, Mangerich WE. Inconversion of components of the bacterial proton motive force by electrogenic potassium transport. J Bacteriol 1981; 147:820–826.
121. Juretic D, Chen HC, Brown JH, Morell JL, Hendler RW, Westerhoff HV. Magainin 2 amide and analogues: Antimicrobial activity, membrane depolarization and susceptibility to proteolysis. FEBS Lett 1989; 249:219.
122. Westerhoff HV, Juretic D, Hendler RW, Zasloff M. Magainins and the disruption of membrane-linked free-energy transduction. Proc Natl Acad Sci USA 1989; 86:6597–6601.
123. Katsu T, Kuroko M, Morikawa T, Sanchika K, Yamanaka H, Shinoda S, Fujita Y. Interaction of wasp venom mastoparan with biomembranes. Biochim Biophys Acta 1990; 1027:185–190.
124. Nicas TI, Hancock REW. Outer membrane protein HI of *Pseudomonas aeruginosa:* Involvement in adaptive and mutational resistance to ethylenediaminetetraacetate, polymixin B, and gentamicin. J Bacteriol 1980; 143:872.
125. Hancock REW. Bacterial outer membranes: Evolving concepts. ASM News 1991; 57:175–82.
126. Hancock REW, Wong PG. Compounds which increase the permeability of the *Pseudomonas aeruginosa* outer membrane. Antimicrob Agents Chemother 1984; 26:48–52.
127. Vaara M. Agents that increase the permeability of the outer membrane. Microbiol Rev 1992; 56:395–411.
128. Rana FR, Macias EA, Sultany CM, Modzradowski MC, Blazyk J. Interactions between magainin 2 and *Salmonella typhimurium* outer membranes: Effect of lipopolysaccharide structure. Biochem 1991; 30:5858–5866.

129. David SA, Mathan VI, Balaram P. Interaction of melittin with endotoxic lipid A. Biochim Biophys Acta 1992; 1123:269–274.
130. Fisher CJ Jr, Marra MN, Palardy JE, Marchbanks CR, Scott RW, Opal SM. Human neutrophil bactericidal/permeability-increasing protein reduces mortality rate from endotoxin challenge: A placebo controlled study. Crit Care Med 1994; 22:553–558.
131. Marra M, Thornton MB, Snable JL, Wilde CG, Scott RW. Endotoxin-binding and -neutralizing properties of recombinant bactericidal/permeability-increasing protein and monoclonal antibodies HA-1A and E5. Crit Care Med 1994; 22:559–565.
132. Fields GB, Tian Z, Barany G. In: Grant GA, ed. Synthetic Peptides: A User's Guide. 1992:77–150.
133. Lai J, Lee J-H, Lin Y-L, Ray D, Wilcox G. Method of producing cecropins by microbiological techniques. U.S. Patent 5,206,154; April 27, 1994.
134. Daher KA, Lehrer RI, Ganz T, Kronenberg M. Isolation and characterization of human defensin cDNA clones. Proc Natl Acad Sci USA 1988; 85:7327–7331.
135. Bodansky M, Bodansky A. The practice of peptide synthesis. 284p, Springer Verlag, Berlin, 1994.

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