Ubiquitous Natural Antibiotics

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Bacteria and fungi coexist with all animals and plants in close physical association. Indeed, health depends on the existence of natural flora that provides vitamins and stimulates the development of specific immune responses in higher vertebrates. However, when particular bacteria or fungi are introduced into a host in a niche that permits their growth as well as the elaboration of virulence factors, disease can result. Given the number and diversity of organisms with which we are in constant contact, this result is thankfully rare—likely because of our defense systems. One such system is that of nonspecific immunity, which can proceed along either oxidative or nonoxidative antimicrobial pathways.

These nonoxidative defense pathways comprise a wide variety of oligopeptides and proteins with potent antibacterial and antifungal activity. Such proteins occur in all animals and plants in which they have been sought. A recent meeting sponsored by the Ciba Foundation (18 to 20 January 1994) brought together scientists working on these antimicrobial proteins. Despite the wide variety of sources and identities of these peptides, a number of common themes emerged.

On the basis of sequence and structural information, these antimicrobial agents can be arranged in groups, as indicated in the table. The low molecular weight (3000 to 5000) peptides are processed from larger precursors. Some are linear molecules and some contain highly conserved disulfide bonds. In addition, there are families within these two groups. Among the linear molecules, the cecropins, which have been isolated from both insects and mammals (*1*, *2*), and the magainins (*3*) form well-defined groups on the basis of highly conserved sequence similarities. A specific motif characterized by proline and arginine occurs in the bactenecins Bac-5 and Bac-7 (*4*) and in cecropin PR-39 (*2*) from pig intestine. This motif may be important for the mechanism of action of these peptides, as discussed below.

The disulfide-containing peptides include three families, defined on the basis of the number of disulfide bonds as well as sequence similarity. Defensins are a common, highly conserved group of peptides (*5*).

With the exception of the serprocidins, the high molecular weight proteins are less easy to arrange in families. The serprocidins are related by virtue of their structural similarity to other serine proteases (*6*).

These molecules are widespread within the organism. The ability of phagocytes to kill ingested microbes has been recognized since the time of Metchnikoff in the late 19th century; it is from the granules of these cells that antimicrobial proteins were originally identified. More recently antimicrobial proteins have also been found in secretions such as seminal fluid, lymph, and serum. Most striking is the recent discovery that a variety of tissue surfaces either directly expose or possess cells that can exocytose antimicrobial peptides. These surface peptides include the magainins from frog skin and defensin-like peptides derived from both the granulated epithelial cells in the mammalian small intestine and the tracheal mucosa (*7*). Thus, it seems that most locations that are in contact with indigenous microorganisms are equipped to limit their inappropriate multiplication.

In addition to their antimicrobial activity, many antimicrobial proteins have functions that are not directly related to killing microorganisms. The serprocidins cathepsin G and proteinase 3 are proteases for connective tissue matrix components and are probably responsible for tissue damage during inflammation (*6*). Although protease activity per se is not required for microbe killing, many of the antimicrobial peptides are present in locations that are protease-rich. Proteolysis may allow the antimicrobial protein to gain easier access to the site of infection. Proteinase 3 is also a determinant of myeloid differentiation in the human leukemia HL-60 cell line (*6*). The relation among antimicrobial, proteolytic, and developmental activities of this protein is obscure but certainly deserves further attention. Coincidentally, sapecins (insect defensins) have also been implicated as growth factors for fly embryos (*8*). BPI, azurocidin, and the tachyplesins also bind lipopolysaccharide (LPS); this ability may facilitate their interactions with Gram-negative organisms, yet LPS binding alone is insufficient for antimicrobial activity because the major LPS-binding protein from mammalian serum, LBP, has no detectable antimicrobial activity (*6*, *9*, *10*). In addition to binding to LPS, BPI can neutralize LPS activity and thus must contribute to host defense against endotoxin.

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26. We thank D. Pappagianis for providing the basis for the figure.

Antimicrobial peptide	Source	Spectrum	Possible mechanism
	Low molecular weight		
Linear			
Cecropin	Moths, pigs, *Drosophila*	GP, GN	Lyse, form pores
Magainin	Frog skin	GP, GN, F	Form pores
Bactenecin	Bovine neutrophils	GN	IM, OM permeability
Disulfide-containing			
Defensins	Widespread	GN, GP, F	Form pores, IM, OM
Tachyplesins	Horseshoe crab	GN, GP, F	K^+ efflux
Protegrins	Pig leukocyte	GN, GP, F	?
	High molecular weight		
Attacins	Moths	GN	Lyse
BPI	Human, rabbit, bovine, neutrophils	GN	OM, IM permeability
Lysozyme	Widespread	GP	Peptidoglycan degradation
Serprocidins			
Proteinase 3	Human, monkey, neutrophils	GN, GP, F	?
Azurocidin	Human, monkey, bovine neutrophils	GN, GP, F	?
Cathepsin G	Human neutrophils	GN, GP, F	Inhibition of metabolism

Selected antimicrobial peptides. GP, Gram-positive; GN, Gram-negative; F, fungus; IM, inner membrane; and OM, outer membrane.

The crucial point needing clarification is the mechanism of killing by antimicrobial proteins. How do these peptides and proteins recognize such diverse microorganisms? What are the targets for the lethal events? What is the structural basis for antimicrobial activity? In general, more is understood about the low molecular weight species, particularly the defensins and the cecropins. Virtually all antimicrobial peptides carry a net positive charge; thus, the initial contact between the antimicrobial peptide and the target organism is electrostatic, because many bacterial surfaces are anionic. In addition, there is an electrochemical potential on the order of –200 mV across the cytoplasmic membrane and a smaller but significant negative potential across the outer membrane of Gram-negative organisms owing to the presence of fixed, high molecular weight anionic species in the periplasmic space. These potentials may contribute to entry of cationic peptides. Indeed, *Escherichia coli* can be protected from the lethal effects of defensins by transient dissipation of the potential across the inner membrane (*5*). Both the linear and disulfide-containing low molecular weight peptides seem to be able to form pores in model test systems and in some cases can increase the permeability of bacterial outer and inner membranes (*3*, *5*). The cecropins actually cause rapid lysis after a brief delay during which the peptide travels across the periplasmic space (*1*).

The mechanism of pore formation, however, differs substantially between the linear and disulfide-containing peptides. Most of the linear peptides can form amphipathic α-helices that are thought to self-assemble to form transmembrane channels with permeabilities exceeding the homeostatic capacities of the microorganism. A synthetic cecropin made of D-amino acids has properties indistinguishable from those of the native L-amino acid–containing molecule. Thus, cecropin activity does not require a stereospecific interaction with a target cell component (*11*). The proline-arginine–rich bactenecins and cecropin PR-39 must act differently, because the high concentrations of proline are incompatible with α-helix formation. Indeed, PR-39 does not have pore-forming activity but does inhibit macromolecular synthesis (*12*). The three-dimensional structure of the defensins has been determined both by crystallography and nuclear magnetic resonance (*13*). These molecules form dimers with a cationic six-stranded β-sheet structure containing a nonpolar region thought to insert in the lipid bilayer and form a channel that somehow increases inner and outer membrane permeability.

Among the higher molecular weight antimicrobial proteins, the mechanism by which lysozyme kills Gram-positive organisms is clear (peptidoglycan degradation), yet there is evidence that even heat-denatured lysozyme devoid of enzymatic activity retains antibacterial activity, perhaps by stimulating autolysins, a subset of peptidoglycan synthesizing-enzymes. Under certain conditions, autolysins cause bacteria to form faulty peptidoglycan and lyse. Careful examination of the interaction of BPI with *E. coli* reveals a two-step mode of action: The first step is reversible outer membrane permeabilization, and the second is irreversible inner membrane damage and death (*14*). There is little information concerning the mechanisms of action of the other proteins except that they cause macromolecular synthesis to cease, but whether this is itself the lethal event or a consequence of it is not yet established. A small but significant fraction of azurocidin's activity is retained by a peptide corresponding to residues 20 to 44, which contain a disulfide conserved among the serprocidins (*15*). It is tempting to speculate that specific small domains within these proteins contain the active site for antimicrobial activity. The availability of complementary DNA clones in expression systems will facilitate the structure-function analyses of the larger molecules.

Microorganisms have coevolved with animals; most often the relationship is mutually beneficial. In instances where there is competition, both the microorganisms and the hosts have had ample opportunity to develop offensive and defensive strategies. Perhaps pathogens have developed resistance to antimicrobial proteins, and hosts have in turn compensated by producing a variety of related proteins. In spite of these endogenous antibiotics, we still succumb to infectious diseases and rely on exogenous antibiotics to turn the tide in our favor. As the availability of effective exogenous antibiotics decreases as a result of the increased resistance of the pathogens, we should explore the innate antibiotics of plants and animals as models for new therapeutic agents.

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