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COMMENTARY

MAGAININS: A NEW FAMILY OF MEMBRANE-ACTIVE HOST DEFENSE PEPTIDES

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Discovery of magainins

The African clawed frog, *Xenopus laevis*, is commonly used as a source of oocytes for molecular biological and recombinant DNA experiments. It has been observed that following careful but nonsterile abdominal surgery to obtain the oocytes, these animals are remarkable in their ability to resist wound infections when returned to holding tanks that resemble their natural environment, pond water, which contains an impressive array of a wide range of potential microbiological pathogens. Generally, the surgical wounds heal within 4 weeks with no postoperative care and show no signs (macroscopically or microscopically) of pus, inflammation, or infection. This is surprising considering that amphibian vertebrates such as *Xenopus* have a competent immune system [1] which can mount a humoral and cell-mediated inflammatory response characteristic of higher vertebrates. This phenomenon led Zasloff [2] and coworkers [3] to search the amphibian skin for new factors defending this vertebrate from microbiological invasion. It was postulated that such factors, which would constitute the basis of a novel host defense system, may also be present in higher vertebrates. In higher organisms, one could speculate that the actions of these molecules would include protection of wet mucosal surfaces from invasion by pathogenic microorganisms.

The investigations of Zasloff and colleagues led to the discovery of a class of peptides which constitute a novel antimicrobial and host defense system with broad-spectrum activity.

Zasloff [2] demonstrated using *Xenopus* skin extracts, peritoneal and subdermal fluid, a broad-spectrum antibacterial activity of a low molecular weight novel substance. Further purification and chromatographic separation led to isolation of two 23 amino acid cationic peptides of identical sequence except at two positions. A cDNA obtained from a *Xenopus* skin library using partially degenerate oligonucleotides revealed that both peptides were the product of a common precursor [2]. Synthetic peptides of corresponding amino acid sequence had antimicrobial activity of identical potency and similar broad-spectrum activity compared to the isolated material [3]. These peptides were named Magainins 1 and 2. The identity in frog skin of similar peptides has also been shown independently [4].

Properties of magainin peptides

Broad-spectrum activity. The magainin peptides are active and bacteriocidal against a very broad range of Gram-positive and Gram-negative bacteria [2, 3, 5], including *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. The antimicrobial activity is essentially independent of pH within the range tested (5.8 to 8.0), but is dependent on concentration, suggestive of some type of cooperativity. Activity can also be affected by salt concentration in the medium, with higher activity at isotonic and lower salt concentration. Magainins 1 and 2 have similar but non-overlapping activity spectra [2]. Newly designed synthetic derivatives of the magainins also demonstrate significant antimicrobial activity [3, 5, 6]. The magainins also possess significant antifungal and antiparasitic activity [2, 3].

Alpha-helical structure. Initial modelling predicts that the magainins adopt an amphipatic alpha-helix with hydrophobic residues on one side of the alpha-helix and hydrophilic residues on the other [5, 7, 8]. The long axis of the alpha-helix either alone or combined with additional molecules would be of sufficient length to span a lipid membrane bilayer. Experimentally, the magainins have been found to form a random coil in aqueous solution, but adopt a rod-like structure consistent with helix formation upon addition of a small amount of organic cosolvent (2.3 mol% trifluoroethanol) [8]. Further computer and molecular modelling suggests that the rod-like monomers could form a transmembrane structure surrounding a hydrophilic, positively-charged pore. This model leads to an hypothesis of the mechanism of activity, that the magainins are membrane-active and function by altering ion conductances across membrane barriers.

Membrane ion channel function. Based on present evidence a major site of action for the magainins is the cell membrane. Magainin 2 has been shown by patch-clamp analysis to form voltage-dependent ion channels in synthetic phospholipid bilayers [9]. The concentration-dependence of the magainins for channel formation is complex, but suggestive of a multimeric active structure. In cytochrome *c* oxidase unilamellar liposomes, magainin was shown to dissipate membrane potential via aggregates composed

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of at least four molecules [10]. Membrane potential is also dissipated by magainin in respiring bacteria and in isolated rat liver mitochondria. Unlike detergents, the magainin membrane activity is reversible. Also for a number of magainin-like compounds, no significant hemolysis of erythrocytes is observed at concentrations of peptides 1–3 orders of magnitude higher than for these activities [2, 5]. Thus, it appears that the most likely mechanism of magainin antimicrobial and host defense activity is via ion channel formation which alters membrane potential and function. However, the precise channels involved and possible additional mechanisms remains to be elucidated.

Evidence for a magainin family of peptides

Further investigations have established that the magainins may be members of a family of related gene products with some similarities [11, 12]. To date, there are four peptides of the granular gland, aside from the magainins, that may belong to the "magainin-like peptide family". Other family members are xenopsin precursor factor [4, 13] (XPF, a 25 amino acid peptide derived from a precursor that also contains the neurotensin-like molecule xenopsin); caerulein precursor factor [14–18] (CPF, a 27 amino acid peptide from a precursor that also contains the cholecystokinin/gastrin homologue caerulein); PGLa [15, 19] (a 21 amino acid peptide with an amino terminus of glycine and a carboxy-terminus of leucine which is amidated); and levitide precursor factor [20] (LPF, a recently discovered 25 amino acid peptide whose precursor contains the neuropeptide levitide).

Despite similarities in biological activity, amphipathic alpha-helical secondary structure, and peptide precursor structure, there is sparse conservation in the primary sequence of the active peptides in this family. This suggests that the evolutionary selection exerted on the processed, active peptide is tolerant of many seemingly indiscriminant changes, or (more likely) that the primary sequence is also important for other unique biological activities for each family member that are not yet apparent. Also, significant conservation in the precursor suggests that these domains may be important in coordinating physiological control of the system.

To simplify matters for this review, the umbrella term magainins or "magainin family of molecules" refers to: Magainins 1 and 2; closely related chemical congeners possessing similar antimicrobial and probable host defense functions (e.g. PGLa, XPF, also originally described in frog skin); amphipathic or natural synthetic homologs which also produce ion channels in cell membranes resulting in antimicrobial or host defense activity.

Relationship of magainins to other antibacterial peptides and proteins

The presence of this type of antimicrobial peptide is not unique to the vertebrate world. Boman and colleagues [21–24] have studied the cecropins, 35–37 amino acid broad-spectrum antimicrobial peptides isolated from the Cecropia moth as well as synthetic homologs. The parent peptides are synthesized by the moth larvae in response to injury of the body wall [24]. Like the magainins, the cecropins are predicted to form amphipathic alpha-helices [22]. Recently, patch-clamp analysis of synthetic bilayers has demonstrated that cecropins form anion-selective channels in synthetic bilayers [25].

Similarly, Natori and coworkers [26–33] found that the larvae of the flesh fly, *Sarcophagia peregrina*, produces several amphipathic alpha-helical peptides in the hemolymph in response to injury [34]. These peptides, named sarcotoxins, were shown to be membrane active [28, 30]. Sarcotoxin IA is composed of 39 amino acids. It was suggested that these molecules dissipate the membrane potential in bacteria and thereby interfere with ATP synthesis [28]. Interestingly, it was found that the cholesterol content of synthetic phospholipid liposomes markedly diminishes the membrane interactions with sarcotoxins [35]. These investigators proposed that the selective toxicity of sarcotoxins towards bacteria may be explained, in part, by the fact that bacterial membranes do not contain cholesterol, increasing their susceptibility to these peptides. A similar mechanism may contribute to the selectivity of the magainins.

The presence of amphipathic antimicrobial peptides in the insect world and through the evolutionary tree to lower vertebrates demonstrates impressive conservation. This observation greatly strengthens the plausibility that these or similar molecules will be found further in higher organisms including mammals. The cecropins and sarcotoxins are about twice the size of Magainin 2, have a narrower biological spectrum of action, and will probably require rDNA synthesis to produce significant supplies. Other peptides of bacterial origin are already used as pharmaceuticals.

Gramicidin A is a linear 15 amino acid polypeptide isolated from bacteria which is composed of hydrophobic amino acids with alterating L- and D-configurations. Gramicidin forms transmembrane pores or ion channels [36, 37]. The active form of the molecule is a dimer. Gramacidin is not a transporter, but can actually form an ion channel structure in membranes. It has antibiotic activity against Gram-positive bacteria and is among the simplest molecules that form ion channels through lipid membranes. In mitochondria, ion fluxes induced by gramicidin inhibit oxidative phosphorylation. Gramacidin membrane channels are selective for monovalent cations such as alkali metals Ag^{+}, NH_4^{+}, H^{38+}. Crystal structure analysis is being directed to the study of the molecular forms important in ion channel or pore formation [36]. The mixture of natural and unnatural amino acids in gramacidin has complicated any search for improved derivatives.

The polymyxin antibiotics are a complex of cationic, basic, detergent peptides of bacterial origin. They have activity against Gram-negative organisms and, apparently, rapidly disrupt membranes and increase cell wall permeability. Like gramacidin, their structure has not been compatible with the synthesis of better derivatives.

Mammalian host defense peptides and proteins. Knowledge of the molecular biology of endogenous mammalian host defense functions is developing quickly (Table 1). Cell injury by complement occurs by way of a "killer molecule" referred to as the

Table 1. Membrane channel forming antibiotic proteins and peptides

Compound	Properties
Membrane attack complex of complement	• 20 proteins/1.7 million mol wt • Transmembrane channels
C9-RP (related protein)	• Cytotoxic lymphocytes/killer cells • Resemble C9 complement • 70,000 mol wt/requires Ca^{2+} • Perforins • Cell/cell contact
Bactericidal permeability increasing proteins	• PMN granules • 60,000 mol wt • Gram-negative spectrum • Intracellular action • Inactive in serum • Bind endotoxin (LPS)
Defensins	• 33 amino acid peptide; 4000 mol wt • Complex structure; disulfides • Serum/electrolyte inhibits • Cationic
Magainins	• 23 amino acid peptide • Amphibian/mammalian in origin • Broad spectrum (Gram-negative and Gram-positive bacteria, fungi, and parasites)

membrane attack complex (MAC). Composed of about twenty proteins with a molecular weight of about 1.7 million [39], the MAC forms transmembrane channels that vary in size as a function of the number of C9 molecules.

Cytotoxic lymphocytes, or their granular contents, produce discrete functional pores in target cells which may involve lysis of malignant cells, infected cells, or microorganisms. The active molecule is named C9RP or C9 Related Protein, because of its functional and immunological resemblance to the C9 component of complement [40]. The molecular weight is about 70,000, but killing requires cell–cell contact and is calcium dependent. The murine lymphocyte granule cytolysins are known as perforins [40].

There are proteins of phagocytes in granules that are antimicrobial. Bactericidal permeability increasing proteins (BPI) can be found in polymorphonuclear leukocytes (PMNs) including human [40]. Their molecular weight is about 60,000 and they are particularly active against Gram-negative organisms. BPI appear to primarily function intracellularly on ingested organisms and are inactive in serum.

Defensins are a family of peptides with a molecular weight of about 4000 and are cationic [40]. They have been isolated from macrophages, granulocytes, and human neutrophils. They are comprised of about 33 amino acids with a number of disulfide bonds, suggesting a complex structure. Serum and the electrolyte composition of extracellular fluid inhibit cytotoxic effects on target cells, and thus these agents are thought to be active intracellularly following phagocytosis. Progress is being made on the genetics of the defensins with emerging evidence of organ selective expression [41].

Also, mammalian antibiotic peptides of "professional" phagocytic cells such as neutrophils and macrophages may participate in the development of microbial resistance and virulence. For example, *Salmonella typhimurium*, a facultative intracellular parasite, has a gene responsible for resistance to defensins and magainins. Mutants of these organisms, which do not survive well intracellularly, lack this gene and are sensitive to defensins and magainins. This expression of virulence may have important links to susceptibility or resistance to endogenous host defense peptides [42]. The mechanism(s) for the resistance of certain microorganisms to endogenous antibiotic peptides has not been elucidated and merits study.

Among the common denominators for the abovementioned cytolysins and cytotoxins is surface membrane activity on target cells associated with alterations in membrane structure and function. While of obvious biological importance, their large size, complex structure, intracellular location, or rapid inactivation have put limits on these molecules as potential therapeutic candidates. In contrast, the magainin family of molecules appears to represent among the smallest and simplest class of host defense molecules endogenous to vertebrates or mammals thus far discovered. These properties make it possible for the first time to potentially design, synthesize and pharmacologically test a large number of therapeutic candidate host defense drugs.

Perspective on amphibian derived peptides

The presence of the magainin family of molecules in amphibian skin is of particular interest because of the rich history of this source of molecules. Amphibian skin peptides have proven to be powerful leads for advancing the understanding and discovery of physiologically important gastrointestinal and neurological peptides [43]. Several peptide families have fit into what Erspamer and Melchiorri have coined the "skin–gut–brain triangle" [43]. Examples are

many and include: caerulein (skin), cholecystokinin/gastrin (gut), CCK-8 (brain); xenopsin (skin), neurotensin (gut and brain); bombesin (skin), gastrin inhibitory polypeptide (gut); bombesin-like peptides (brain); tachykinins (skin), and substance P (gut and brain).

It is probable that the magainin family of molecules or closely related substances exists not only in amphibia, but also in mammals, including humans. Recently Wolff and colleagues [44] found that antibodies which recognized magainins also selectively recognised and bound to human salivary glands, suggesting the presence of a magainin-like molecule in that tissue. Thus, the magainin class of molecules appears to be a new family of endogenous and possibly "natural" host defense compounds, which represent a promising avenue for both further mechanistic studies and therapeutic application. In addition, the resemblance of magainin-like molecules to the amphipathic and helical peptides being utilized in approaches to channel engineering [45, 46] suggests a valuable role for magainins as tools for studying pore or channel formation in biological membranes.

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