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REVIEW ARTICLE

Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology

Sergio H. Marshall*
Laboratorio de Genética e Inmunología Molecular
Instituto de Biología
Facultad de Ciencias Básicas y Matemáticas
Pontificia Universidad Católica de Valparaíso
Avenida Brasil 2950, Valparaíso, Chile
Tel: 56 32 273373
Fax: 56 32 596703
E-mail: smarshal@ucv.cl

Gloria Arenas
Laboratorio de Genética e Inmunología Molecular
Instituto de Biología
Facultad de Ciencias Básicas y Matemáticas
Pontificia Universidad Católica de Valparaíso
Avenida Brasil 2950, Valparaíso, Chile
Tel: 56 32 273205
Fax: 56 32 596703
E-mail: garenas@ucv.cl

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A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Among them, cationic peptides are the most widespread. Interestingly, the variety and diversity of these peptides seem to be much wider than suspected. In fact, novel classes of peptides with varying chemical propertiescontinue to be isolated from different vertebrate and invertebrate species, as well as from bacteria. To the early characterized peptides, mostly cationic in nature, anionic peptides, aromatic dipeptides, processed forms of oxygen-binding proteins and processed forms of natural structural and functional proteins can now be added, just to name a few. In spite of the astonishing diversity in structure and chemical nature displayed by these molecules, all of them present antimicrobial activity, a condition that has led researchers to consider them as "natural antibiotics" and as such a new and innovative alternative to chemical antibiotics with a promising future as biotechnological tools. A resulting new generation of anti microbial peptides (AMPs) with higher specific activity and wider microbe-range of action could be constructed, and hopefully endogenously expressed in genetically-modified organisms.

The continuous use of antibiotics has resulted in multi-resistant bacterial strains all over the world and as expected, hospitals have become breeding grounds for human-associated micro organisms (Mainous and Pomeroy, 2001). Nonetheless, the same time-bomb effect is slowly developing with animal-associated pathogens in commercially driven activities, such as aquaculture and confined poultry breeding, where the indiscriminate use of antibiotics is perceivedasessential for industries survival. Consequently, there is an urgent need to search for alternatives to synthetic antibiotics. The discovery of two classes of antimicrobial peptides, non-ribosomally synthesized (Hancock and Chapple, 1999) - present in bacteria - lower eukaryotes and plants - and ribosomally-synthesized peptides, of wider distribution (Boman, 1995; Broekaert et al. 1997; Hancock and Lehrer, 1998; Hoffmann et al. 1999; Thevissen et al. 1999; Zasloff, 2002; Ezekowitz and Hoffmann, 2003), provided a new therapeutic strategy to fight micro organisms. Recent studies show that several cationic and non-cationic peptides expressed in many vertebrate, invertebrate and bacterial species (Lüders et al. 2003) act synergistically to improve immune responses**.**

The knowledge acquired in the past two decades and the discovery of new groups of antimicrobial peptides make natural antibiotics the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections (De Lucca, 2000; Hancock, 2000; Welling et al. 2000; Selitrennikoff, 2001). In addition, the wide spectrum of

*Corresponding author

(Tossi and Sandri, 2002; Zasloff, 2002). The resulting most important groups are the following:

From Eukaryotes

Cationic peptides: This is the largest group and the first to be reported, being widely distributed in animals and plants. So far, more than a thousand of such peptides have been characterized and over 50 % of them have been isolated from insects (Bulet et al. 1999; Andreu and Rivas, 1998; http://www.bbcm.univ.trieste.it/~tossi/antimic.html). On the basis of their structural features, cationic peptides can be divided as well into three different classes: (1) linear peptides forming-helical structures; (2) cysteine-rich open-ended peptides containing single or several disulfide bridges; and (3) molecules rich in specific amino acids such as proline, glycine, histidine and tryptophan.

Important subfamilies of cationic peptides include:

- *Cecropins:* This is a family of 3 - 4 kDa linear amphipatic peptides described in the haemolymph of insects in the early 1980s (Hultmark et al. 1980; Andreu and Rivas, 1998; Boman 1998; Zheng and Zheng , 2002). These molecules are devoid of cysteine residues and contain two distinctive helical segments: a strongly basic N-terminal domain and a long hydrophobic C-terminal helix, linked by a short hinge. Shortly thereafter, other linear amphipatic peptides such as the magainins isolated from *Xenopus* skin, were isolated from vertebratesand included in the same group (Zasloff, 1987; Bechinger et al. 1993; Simmaco et al. 1998). These were the first molecules used to evaluate their biomedical applications (Hancock, 2000; http://www.genaera.com; http://www.inimexpharma.com; http://biotech.deep13.com/Alpha/alpha.html; http://www.geniconsciences.com/).

- *Defensins:* This isa highly complex group of 4-kDa open-ended cysteine-rich peptides arranged with different structural motifs. They have been mostly isolated from mollusc, acari, arachnids**,** insects, mammals and plants. Defensins are arranged in families, based on their structural differences. Invertebrates (Hubert et al. 1996; Andreu and Rivas, 1998; Dimarcq et al. 1998; Bulet et al. 1999; Mitta et al. 1999; Silva et al. 2000; Nakajima et al. 2001) and plant (Broekaert et al. 1997; García-Olmedo et al. 1998; Segura et al. 1998; Liu et al. 2000) defensins are characterized by three and four disulfide bridges, respectively. They show a common structure comprising an ά-helix linked to a β-sheet by two disulfide bridges, distinctive structure known as the CSab motif.In mammals, ά - and β-defensins are characterized by an antiparallel -β sheet structure, stabilized by three disulfide bridges (Zasloff, 2002). Some of them naturally exist as cyclic molecules such as the theta-defensins (Tang et al. 1999; Lehrer and Ganz, 2002). It has been difficult to determine whether all molecules are homologous or have independently evolved similar features, but evidences are in favour of a distant relationship. The best evidence of this relationship is structural, particularly from their overall three-dimensional structure and from the spacing of half-cystine residues involved in intra-chain disulfide bonds.

- *Thionins:* These are antimicrobial, and generally basic, plant peptides with a molecular weight of 5000 Da, which contain 6 or 8 conserved cysteine residues. Their *in vitro* toxicity against plant pathogenic bacteria and fungi indicates a role in the resistance of plants (Bohlmann, 1999). Ligatoxin B, a new basic thionin containing 46 amino acid residues has been recently isolated from the mistletoe *Phoradendron liga* (Li et al. 2002). Similarities observed by structural comparison of the helix–turn–helix (HTH) motifs of the thionins and the HTH DNA-binding proteins, led the authors to propose that thionins might represent a new group of DNA-binding proteins.

- *Amino acid-enriched class:* This is a distinctive class of antibacterial and antifungal cationic peptides, enriched in specific amino acids, with distinctive features depending on the organism from which they are isolated . Those proline- and glycine-rich are mostly from insects and active against Gram-negative bacteria (Bulet et al. 1999; Otvos, 2000); while cysteine-rich peptides, not related to defensins, represent the most diverse family among arthropods (Dimarcq et al. 1998). On the other hand those enriched in histidine are particularly basic, mostly from mammals (Pollock et al. 1984). Among them, histatin recovered from saliva from humans and primates and primarily directed against fungal pathogens, outstands for its distinctive mechanism of action which does not involve channel formation in the fungal cytoplasmic membrane but rather translocates efficiently into the cell and targets the mitochondrion (Tsai and Bobek, 1998). Those enriched in histidine and glycine are quite large, also affecting fungal pathogens and a distinctive feature is that their residues are arranged in approximately regular but different structural repeats (Tossi and Sandri, 2002). Finally, only two peptides enriched in tryptophan residues have been described, both derived from porcine cathelicidin precursors (Schibli et al. 2002). The outstanding feature though, is broad spectrum of activity including hundreds of Gram-positive and negative clinical isolates in addition of fungi and even the enveloped HIV virus (Gennaro and Zanetti, 2000).

- *Histone derived compounds:* This is a family of cationic helical peptides corresponding to cleaved forms of histones originally isolated from toad – (buforin) (Park et al. 1996) and fish epithelia (parasin)