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A review on antimicrobial peptides: Structure, function and classification

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Abstract
Antimicrobial peptides (AMPs) are oligopeptides with five to hundred of amino acids. AMPs have a wide range of inhibitory effects against bacteria, fungi, parasites and viruses. The emergence of antibiotic-resistant microorganisms and the increasing of concerns about the use of antibiotics resulted in the development of AMPs, which have a good application prospect in medicine, food, animal husbandry, agriculture and aquaculture. Here we summarize size, charge of AMPs, their modes of action, and the biological role. In addition, we discuss the synthesis, location inside the cell and expression and molecular structure of AMPs.

Keywords: antimicrobial peptides, Structure, function, classification

Introduction
Proteins are informatory bio-molecular devices present in nature. They can receive or send information and have certain catastrophic functions such as divergence, recruitment and mixing/matching of domains. Divergence is change in sequence and structure, lead to change in specificity and nature of the reaction catalyst. One protein adapted without any structural change for second function is called recruitment. Antimicrobial peptides (AMPs), also called host defence peptides (HDPs) are part of the innate immune response found among all classes of life. Fundamental differences exist between prokaryotic and eukaryotic cells that may represent targets for antimicrobial peptides (Groenink *et al.*,1999) [8].

Biological Role and Classification of AMPs
According to Hancock, 2000 [9], from prokaryotes to humans, AMPs are produced by all life forms, and are evolutionary conserved in the genome. AMPs constitute important components of the innate immunity in higher organisms, and protect the host against infections. On contrary, bacteria produce AMPs for its own protection from other bacteria competing for the existance in same ecological environment (Hassan *et al*., 2012) [12]. Many AMPs are effective against both Gram-positive and Gram-negative bacteria as well as fungi, viruses, and unicellular protozoa therefore exhibit an extraordinarily broad range of antimicrobial activity (Reddy *et al*., 2004; Marr *et al*., 2006) [23, 18].
Apart from direct antimicrobial activity, these AMPs also have the ability to modulate the innate immune responses of the host also and promote pathogen clearance indirectly. But the widespread distribution and abundance of AMPs in all multicellular organisms underscores their critical role in innate immunity. Their importance is further demonstrated by the increased infection susceptibility of mice in which gene encoding for mouse analog of the human AMP LL-37 was knocked out. Similarly, atopic dermatitis in humans with reduced AMP production (Ong *et al*., 2002) [21].

Synthesis and location inside cell and expression:
AMPs in nature are produced by both ribosomal translation of mRNA and by nonribosomal peptide synthesis. In all species, AMPs are synthesised ribosomally which are genetically encoded while in bacteria nonribosomally synthesis also occurs. Peptides of nonribosomal origin are known for several years and many are used as antibiotics (e.g., polymyxins and gramicidin S), while the role of ribosomally synthesized AMPs are mainly recognized for their critical role in innate immunity and for their therapeutic potential (Hancock, 2000) [9].
In mammals, AMPs are found mainly within granules of neutrophils and also secreted from epithelial cells covering skin and mucosal surfaces.

Many times it is also observed that AMPs are encoded in clusters in the genome and co-expressed which results in accumulation of multiple AMPs at a single site. These require proteolytic cleavage to become active as many AMPs are produced as inactive precursors. Therefore, their regulation depends not only on their own expression but also on the abundance of appropriate proteases. In multicellular organisms, their expression is both constitutive and inducible. AMPs which are constitutively expressed are stored at high concentrations in granules as inactive precursors and released locally at infection and inflammation sites, whereas the expression of others is induced in response to pathogen-associated molecular patterns (PAMPs) or cytokines (Lai and Gallo, 2009) [15].

Size, charge and classification:
Today several databases are there for natural AMPs, covering more than 2000 peptides AMPs are mostly short, consisting of 10–50 amino acids, display an overall positive charge ranging from +2 to +11, and contain an approximate proportion (typically 50%) of hydrophobic residues. Classification of AMPs is mainly based on their secondary structure into α-helical, β-sheet, or peptides with extended/random-coil structure, and mostly belonging to α and β categories (Wang, 2015) [27].

A) α-helical
In aqueous solution, α-helical peptides are unstructured, but when come in contact with a biological membrane these adopt an amphipathic helical structure. Two of the most studied peptides in this group are: (i) LL-37, which is produced as an inactive precursor in the 18-kDa human cathelicidin antimicrobial protein (hCAP18), present in neutrophils and epithelial cells (Lai and Gallo, 2009) [15], and (ii) human lactoferricin, which is derived by proteolytic cleavage of the antimicrobial and immunomodulatory iron-binding glycoprotein lactoferrin, present in milk and exocrine secretions (Legrand *et al*., 2005) [16].

B) β-sheet
β-sheet peptides are stabilized by disulphide bonds and are organized to create an amphipathic molecule. As these possess rigid structure, so are more ordered in aqueous solution and do not undergo as drastic conformational change as helical peptides upon membrane interaction. The best-studied β-sheet peptides are the defensins constitute a large group of AMPs, which are produced as inactive precursors in neutrophils, macrophages, and epithelial cells (Yeaman and Yount, 2003) [28].

C) Extended/random-coil peptides
A small portion of the natural AMPs belong to the third class of extended/random-coil peptides these contain a high content of arginine, proline, tryptophan, and/or histidine residues and lack secondary structure. Similarly to other AMPs, many of the extended peptides fold into amphipathic structures after contact with a membrane. One of the best studied peptides in this group is indolicidin, produced by bovine leukocytes.
On the basis of structural homology motifs, a different classification of families of antimicrobial peptides can be generated that reflects the relationship between family members. In humans, peptide antibiotics of three families have been identified: the defensins, cathelicidins, and histatins (Table 1).

Table 1: Different types of antimicrobial peptides (Kagan *et al*., 1994)

Peptide Family	Features
Defensins	These are greatly definitive and demonstrate bactericidal, antifungal and antiviral activity and are primarily found in cells and tissues of host defence. According to the position of the cysteins, defensins have been classified as α, β- and θ-defensins.
Cathelicidins	These are structurally diverse and located at carboxyl terminus. They are synthesized and stored in cells as two-domain proteins and on demand break into cathelin protein or antimicrobial peptide.
Histatins	They are small, cationic, histidine-rich peptides. They are secreted by the oral salivary gland (parotid, submandibular and sublingual glands) and show a potent bactericidal and also fungicidal activity. Therefore play an important role in maintaining oral health by limiting infections in the oral cavity.

Mechanism of Action
Antimicrobial peptides (AMPs) are evolutionary developed weapons of multicellular organisms (Zasloff, 2002) [29]. They are widely distributed in plant and animal kingdoms (vertebrates and invertebrates) as a tool for self defence and offense. A variety of host defence peptides, e.g., Defensins, cathelicidins, cecropins, histatins and magainins have been discovered by the pioneer studies involved in isolation of these peptides. These days antimicrobial peptides are fascinating novel antibiotic molecules when antibiotic resistance has become a very serious problem globally. Advantages of peptides include accessibility, practicality and simplicity. AMPs are mostly located in the epithelial and non-epithelial cells of mucosal layers in body where they aid to maintain natural barriers and protect against microbial intervention.
The AMPs activity begins when they come in contact with the target organism electrostatically. AMPs bind to the cell membrane and and causing the alteration in structure after which they enter the cell and interact with the targets. They initiate the activity of autolytic enzymes and cease the cell wall biosynthesis, synthesis of DNA, RNA and protein. They possess immunomodulation functions; cause clearance of infection, promote wound healing, and modulate the responses of dendritic cells and cells of the adaptive immune response (Hancock and Sahl, 2006) [10].
There are many studies available on antimicrobial peptides killing mechanism. Some AMPs have been destined to act intracellularly (Brogden, 2005) [2], while mostly function primarily by disrupting bacterial cell membranes. Bacterial cell membranes consist of negatively charged phospholipids which give rise to an electrostatic attraction to the highly cationic AMPs. On the other hand, eukaryotic membranes having predominantly neutral phospholipids, are usually less susceptible to the destruction by AMPs. In addition, the presence of cholesterol in eukaryotic membranes also increase the resistance against membrane disruption by AMPs. Upon association with the membrane, unstructured peptides become structured and thinning the bacterial membrane starts proceeding to disrupt the membrane through one of three broadly defined methods. The barrel stave method involves peptide insertion into the membrane parallel to the lipid

bilayer normal, the toroidal pore method induces bending of the lipid bilayer resulting in pores in the membrane where lipids tilt in such a way that the lipid head groups define the surface of the pore, and the micellization model results in the degradation of membranes through the formation of lipid encompassed peptides (Huang *et al.*, 2004) [13]. There are other mechanisms, generally categorized as carpet mechanism, that destabilize the membrane structure to cause cell death. Their amino acids compositions, cationic charges, amphipathicity and size allow them to attract and attach to bacterial lipid bilayer to form pores by 'carpet', 'barrel-stave' or 'toroidal-pore' models (Table 2) (Brogden, 2005) [2].

Table 2: Different models of antimicrobial peptide mechanism of action against microbes

Model	Description
Carpet model	Attracted or attached peptides aggregates and penetrate into the membrane bilayer. The hydrophilic peptide domain form the interior region of the pore and hydrophobic peptide domain align with the lipid core region.
Barrel-Stave Model	Peptides position themselves around the cell membrane for binding and this leads to conversion to a bilayer membrane with peptide aggregation. In this way, hydrophobic peptides attach to the lipid side, whereas hydrophilic peptides attach to the interior part of the cell membrane.
Toroidal Model	In this, peptide helices put into the membrane and induce the lipid monolayers to bend constantly through the pore so that water core is lined by equally inserted peptides and the lipid head groups

Beta defensins

Defensins are small cationic peptides, present in a wide range of species across the animal and plant kingdoms. Characterized by a conserved six cysteine signature, three sub-classes of defensins have been identified as α, β, and θ (Ganz, 2004) [6]. α-defensins arose from β-defensins in some mammals and both the classes are structurally separated by the bridging pattern between the three pairs of intramolecular disulphide bonds made by the six cysteines. The specific C1-C5, C2-C4, and C3-C6 cysteine pairing, which is conserved across all β-defensins, indicates that the disulfide bonds are essential to the function of the molecule. The disulfide bonds maintain peptide stability by conferring resistance to proteolysis and may also be important for the cell signaling and immunomodulatory functions of these molecules (Bruhn *et al*., 2009) [3]. θ-defensins have been recently acquired primate specific peptide, formed by the merging of α and β classes of defensins (Lehrer, 2004) [17]. In mammals, due to multiple gene duplication and sequence diversification a large family of β-defensin peptides with diverse amino acid sequence and virtually identical tertiary structures based on their characteristic intramolecular disulphide bonds has been evolved (Bauer *et al*., 2001) [1].

Molecular Structure

β-defensins are translated from characteristic two exon gene structures, out of these, first one encodes a pre-pro-peptide while the second one encodes the mature peptide containing the six cysteine motif (Ganz, 2003) [5]. Sequencing of many more vertebrate genomes has facilitated a comparative genomics approach for characterizing the β-defensin gene repertoire, and species-specific clades have been identified. Currently, number of β-defensin genes vary from 14 in chicken to 29 in pigs, 38 in dog, 33 in chimp, and 48 in mice and humans, but the final numbers will be subjected to change as more genomes will be correctly annotated and copy number variation (CNV) will be recorded. Comparative immunological analyses have also identified specific amino acid sites under positive selection so there may arise some additional functional divergence between species. With lapse of time the classic α-helical region disappeared from all bovine β-defensins suggests a divergence in the mechanisms of action which may have contributed to the expansion identified in the ruminant clade (Tu *et al*., 2015) [26]. The implications of the apparent loss of α-defensins from the genomes of some livestock species and the expansion of the β-defensin family in a species-specific manner are only now becoming apparent, and could potentially be harnessed to improve animal health (Meade *et al*., 2014) [26].

Expression of β-Defensins

Different β-defensins are present in different epithelial and mucosal tissues which are either constitutively expressed or can be induced in response to external stimuli (Mathews *et al*., 1999) [19]. Their anatomical distribution in the body clearly reflects they have the ability to neutralize different pathogens and are more abundant at sites prone to the microbial infections they are specific for. That is why, hBD2 is strongly expressed in lung (Kao *et al*., 2003) [14]; hBD4 is highly expressed in the stomach and testes (García *et al*., 2001) [7], and hBD3 in the skin and tonsillar tissue (Harder *et al*., 2001) [11]. hBD1–hBD4 are expressed in the respiratory tract, with constitutive expression of hBD1 and inducible expression of hBD2–hBD4 in response to inflammation or infection. In keratinocytes, there is constitutive mRNA expression of hBD1; conversely hBD2 expression is induced by lipopolysaccharides (LPS) or other bacterial epitopes in combination with interleukin-1β, released by resident monocyte-derived cells. hBD3 and hBD4 are inducible by stimulation with tumor necrosis factor (TNF), toll-like receptor ligands, interferon (IFN)-γ, or phorbolmyristate acetates (Selsted & Ouellette, 2005) [24]. hBD3 is also induced in response to local release of surface-bound epidermal growth factor receptor (EGFR) ligands via activation of metalloproteinases (Sorensen, 2005; Doss *et al*., 2010) [4].

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