Small but Potent Killers

Barat Ali Fakheri and Mitra Jabbari

Department of Plant Breeding & Biotechnology, Faculty of Agriculture, University of Zabol

Corresponding author: Mitra Jabbari

ABSTRACT: The antimicrobial peptides (AMPs) are biologically active molecules produced by wide variety of organisms as an essential component of their innate immune response. Animals and plants have coexisted with microbes throughout their evolution, sometimes to their mutual benefit, often in open warfare. The cells of insects and animals produce various antimicrobial substances that act as endogenous antibiotics or disinfectants. The primary role of the AMPs is host defense by exerting cytotoxicity on the invading pathogenic microorganisms, and they also serve as immune modulators in higher organisms. Although hundreds of antimicrobial peptides have now been characterized as having widely diverse sequences, these peptides have been classified into relatively few conformational paradigms. In this article, attempts have been made to describe antimicrobial peptide's structure and diversity, types and characteristics of antimicrobial peptides, classify antimicrobial peptides according to their secondary structure and the modes of action by which antimicrobial peptides kill bacteria. We mention methods used to determine the mechanisms of antimicrobial peptide in plants and animals and factors that are related to the selectivity property of AMPs and Also describe bacterial resistance and regulation of expression of AMPs. Finally provides list of database and bioinformatics tools required for research.

Keywords: Antimicrobial peptides (AMPs), Mode of action, Innate immunity, Selectivity property, Bioinformatics tools

INTRODUCTION

Environment is fully laden with microorganisms such as bacteria, viruses, fungi, paramecium etc. which may parasitize multicellular organisms. In order to thrive in this situation, the multicellular organisms depend on their well developed host-defense mechanism, which is made of broadly three levels of interacting systems (Sarika, 2012). The first interaction of microorganism with host occurs at body surface and epithelial surfaces like skin, secretions of skin, moist surfaces of the eyes, nose, lungs, mouth, digestive tract, and mucous membrane which is also known as first line of defense mechanism (Sarika, 2012). The second level includes phagocytic white blood cells, antimicrobial proteins and inflammatory response. The third level of defense is the acquired immunity, where key role is played by lymphocytes (B cells and T cells) (Sarika, 2012).

A new approach is needed, as we explore novel types of antimicrobials for which the pathogens that currently plague us have not yet developed resistance. Perhaps scientists have overlooked one of nature’s most potent molecules in the arsenal against invasive organisms (Jay Hardy, 2013). Consider that the cornea of the eye of an animal is almost always free of signs of infection. The insect flourishes without lymphocytes or antibodies. A plant seed germinates successfully in the midst of soil microbes. How is this accomplished? Both animals and plants possess potent, broad-spectrum antimicrobial peptides (AMPs), which they use to fend off a wide range of microbes, including bacteria, fungi, viruses and protozoa (Jay Hardy, 2013). The antimicrobial peptides (AMPs) are biologically active molecules produced by wide variety of organisms as an essential component of their innate immune response (Pushpanathan, 2013).
Tossi timicrobial peptides are a unique and diverse class of molecules that are essential for the host defense system (Hoffmann, 1999; Liu, 2000; Saether, 1995). The cells of insects and animals produce various antimicrobial substances that act as endogenous antibiotics or disinfectants (Lehrer and Ganz, 1999).

The primary role of the AMPs is host defense by exerting cytotoxicity on the invading pathogenic microorganisms, and they also serve as immune modulators in higher organisms (Zanetti, 2004). AMPs are considered as a promising and potential drug candidate for the future due to their broad range of activity, lesser toxicity, and decreased resistance development by the target cells (Hancock and Rozek, 2002). The AMPs were found to exist in a wide range of secondary structures such as α-helices, β-strands with one or more disulfide bridges, loop and extended structures. The existences of such diverse structural forms of AMPs are highly essential for their broad spectrum antimicrobial activity (Hancock, 2001).

Antimicrobial peptides have been recognized in prokaryotic cells since 1939 when antimicrobial substances, named gramicidins, were isolated from Bacillus brevis and were found to exhibit activity both in vitro and in vivo against a wide range of Gram-positive bacteria (David, 2013; Dubos, 1939 I, 1939 II). Gramicidins were later shown to successfully treat infected wounds on Guinea-pig skin, indicating their therapeutic potential for clinical use (Gause and Brazhnikova, 1944), and were the first AMPs to be commercially manufactured as antibiotics (David, 2013; Van Epps, 2006).

In the case of humans and other living creatures, which are constantly exposed to the threat of microbial infection, it had long been known that protection against these infections was provided by the adaptive immune system (David, 2013). However, this left the question as to why plants and insects, which lack an adaptive immune system, also remain free from infections for most of the time. The answer to this question is now known to be that similarly to prokaryotes, eukaryotes also produce AMPs and, historically, some sources attribute the discovery of euukaryotic AMPs to early work on plants (Stec, 2006) when in 1896 it was shown that a substance lethal to bread yeast was present in wheat flour (David, 2013; Jago and Jago, 1926). Purothionin from wheat flour (Triticum aestivum) is reported to be the first plant antibacterial peptide having the ability to inhibit growth of phytopathogens like Pseudomonas solanacearum, Xanthomonas campestris and Corynebacterium michiganense (Sarika, 2012). Forty years later, number of additional plant peptides with antibacterial activity have been characterized, represented by thionins (or defensins), cyclotides, glycine-rich proteins, snakins, 2S albumins, and hevein-type proteins (Daly, 2009; Koltay et al, 2005; Pelegrini and Franco, 2005; Selitrennikoff, 2001).

Although hundreds of antimicrobial peptides have been characterized as having widely diverse sequences, these peptides have been classified into relatively few conformational paradigms (Yeaman and Yount, 2003). Macrophages, neutrophils, epithelial cells, hemocytes, fat body, reproductive tract, etc. are the various sources of AMPs (Sarika, 2012). Presently, antimicrobial peptides are being used in development of techniques to reduce crop losses and production of novel antibiotics for the treatment of wide range of human infections (Pelegrini and Franco, 2005; Selitrennikoff, 2001; Terras, 1995).

However, still it is not clear about mechanism of antimicrobial peptides pathogen interaction which is responsible for the host-defense immune system (Hoffmann, 1999; Liu, 2000; Saether, 1995). In this article, attempts have been made to describe antimicrobial peptide's structure and diversity, types and characteristics of antimicrobial peptides, classify antimicrobial peptides according to their secondary structure and the modes of action by which antimicrobial peptides kill bacteria. Mention methods used to determine the mechanisms of antimicrobial peptide in plants and animals and factors that are related to the selectivity property of AMPs. Also describe Bacterial resistance and Regulation of expression of AMPs. Finally provides list of database and Bioinformatics tools required for research.

**STRUCTURE AND DIVERSITY**

More than 500 antimicrobial peptides have been discovered so far from animals as well as plants (a good collection of sequences, besides a wealth of other useful information, can be found at http://www.bbcm.univ.trieste.it/~tossi/pag1.htm) (Zhao, 2003). Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure (Yeaman and Yount, 2003). Antimicrobial peptides are generally between 12 and 50 amino acids. These peptides include two or more positively charged residues provided by arginine, lysine or, in acidic environments, histidine, and a large proportion (generally >50%) of hydrophobic residues (Sitaram and Nagaraj, 2002; Papagianni, 2003; Dürer, 2006). The secondary structures of these molecules follow 4 themes, including i) α-helical, ii) β-stranded due to the presence of 2 or more disulfide bonds, iii) β-hairpin or loop due to the presence of a single disulfide bond
and/or cyclization of the peptide chain, and iv) extended (Dhople, 2006). Many of these peptides are unstructured in free solution, and fold into their final configuration upon partitioning into biological membranes. It contains hydrophilic amino acid residues aligned along one side and hydrophobic amino acid residues aligned along the opposite side of a helical molecule (Yeaman and Yount, 2003). This amphipathicity of the antimicrobial peptides allows them to partition into the membrane lipid bilayer. The ability to associate with membranes is a definitive feature of antimicrobial peptides (Hancock and Patrzykat, 2002) although membrane permeabilisation is not necessary. These peptides have a variety of antimicrobial activities ranging from membrane permeabilization to action on a range of cytoplasmic targets (Table 1).

Table 1. Types and characteristics of antimicrobial peptides

<table>
<thead>
<tr>
<th>Type</th>
<th>Characteristic</th>
<th>AMPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anionic peptides</td>
<td>Rich in glutamic and aspartic acids</td>
<td>Maximin H5 from amphibians, Dermcidin from humans</td>
</tr>
<tr>
<td>Linear cationic α-helical peptides</td>
<td>Lack in cysteine</td>
<td>Cecropins, andropin, moricin, ceratotoxin and melittin from insects, Magainin, dermaseptin, bombinin, brevinin-1,esculentins and buforin II from amphibians, CAP18 from rabbits, LL37 from humans</td>
</tr>
<tr>
<td>Cationic peptide enriched for specific amino acid</td>
<td>Rich in proline, arginine, phenylalanine, glycine, tryptophan</td>
<td>Abaecin, apidaecins from honeybees, prophenin from pigs, indolicidin from cattle</td>
</tr>
<tr>
<td>Anionic and cationic peptides that contain cysteine and form disulfide bonds</td>
<td>Contain 1–3 disulfide bonds</td>
<td>1 bond:brevinins, 2 bonds:protegrin from pig, tachyplesins from horseshoe crabs, 3 bonds:defensins from humans, more than 3:drosomycin in fruit flies</td>
</tr>
</tbody>
</table>

The diversity of antimicrobial peptides probably reflects the species' adaption to the unique microbial environments that characterize the niche occupied, including the microbes associated with acceptable food sources (Boman, 2000; Simmaco, 1998). The diversity of antimicrobial peptides discovered is so great that it is difficult to categorize them except broadly on the basis of their secondary structure (Van't Hof, 2001; Epand and Vogel, 1999). It is common to classify antimicrobial peptides into four groups according to their secondary structure (Zhao, 2003).

1- Linear peptides with an α-helical structure (Fig. 1, B)

One of the larger and better studied classes of antimicrobial peptides is those forming cationic amphipathic helices, e.g. magainin, cecropin A, temporins, as well as a number of de novo designed antimicrobial peptides (Zhao, 2003; Mangoni, 2000; Boman, 1995). These peptides adopt disordered structures in aqueous solution while fold into an α-helical conformation upon interaction with hydrophobic solvents or lipid surfaces. α-Helical peptides are often found to be amphipathic and can either absorb onto the membrane surface or insert into the membrane as a cluster of helical bundles (Zhao, 2003).

The majority of the cytotoxic amphipathic helical peptides are cationic and they do exhibit selective toxicity for microbes. There are also hydrophobic or slightly anionic α-helical peptides (Zhao, 2003). Peptides that are not cationic exhibit less selectivity towards microbes compared with mammalian cells. An example of a well-studied hydrophobic and negatively charged cytotoxic peptide is alamethicin (Zhao, 2003; Duclohier and Wroblewski, 2001; Koltay, 2005). This helical peptide forms clusters of helices that traverse the bilayer and surround an aqueous pore transporting ions (Sansom, 1993).

Figure 1. Molecular models of the different structural classes of antimicrobial peptides [42,137]. These models are based on two-dimensional nuclear magnetic resonance spectroscopy of the peptides in aqueous solution for human β-defensin-2 or a membrane mimetic condition for other peptides. (A) human β-defensin-2, which forms a triple-stranded β-sheet structure (containing a small α-helical segment at the N-terminus) stabilized by three cysteine disulphide bridges. (B) The amphipathic α-
helical structure of magainin 2. (C) The β-turn loop structure of bovine bactenecin. (D) The extended boat-shaped structure of bovine indolicidin.

**2- conformationally more restrained peptides, predominantly consisting of β- strands connected by intramolecular disulfide bridges (Fig. 1, A)**

In contrast to the linear α-helical peptides, β-sheet peptides are cyclic peptides constrained either by disulfide bonds, as in the case of human β-defensin-2 (Hancock, 2001), tachyplesins (Matsuzaki, 1999), protegrins (Harwig, 1995), and lactoferricin (Jones, 1994), or by cyclization of the peptide backbone (Zhao, 2003), as in the case of gramicidin S (Prenner, 1999), polymyxin B (Zaltas, 2000), and tyrocidines (Bu, 2002). They largely exist in the β-sheet conformation in aqueous solution that may be further stabilized upon interactions with lipid surfaces (Zhao, 2003).

The critical parameter associated with the antimicrobial action of this peptide appears to be the maintenance of a certain hydrophilic-hydrophobic balance (Zhao, 2003; Rao, 1999).

**3- linear peptides with an extended structure, characterized by overrepresentation of one or more amino acids (Fig. 1, D)**

Certain antimicrobial peptides have an unusual amino acid composition, having a sequence that is rich in one or more specific amino acids (Zhao, 2003). Tryptophan is generally not an abundant amino acid residue in peptides or proteins. Examples of antimicrobial peptides that are rich in Trp include tritrippcin (VRRFPWWFPFLRR) (Lawyer, 1996) and indolicidin (ILPWKWPWWPWRR-amide) (Zhao, 2003; Selsted et al, 1992). Indolicidin was reported to adopt a turn conformation which greatly enhances its membrane activity (Ladokhin, 1999). Indolicidin has been shown to permeabilize the outer membrane of E. coli (Falla, 1996; Subbalakshmi and Sitaram, 1998) to form channels, as revealed by conductance measurements with planar bilayers (Zhao, 2003; Wu, 1999; Falla, 1996). This peptide incorporates in a highly cooperative manner within the acyl chain region of the membrane (Ha, 2000) and its hemolytic activity is associated with the concentration required for its self-association (Zhao, 2003; Ahmad, 1995).

**4- peptides containing a looped structure (Fig. 1, C)**

In contrast to other antimicrobial peptides, proline arginine rich peptides cannot form amphipathic structures due to the incompatibility of high concentration of proline residues in such structures and have been proposed to adopt a polyproline helical typeII structure (Zhao, 2003; Cabiaux, 1994).

Lantibiotics contain small ring structures enclosed by a thioether bond and their structure and properties have recently been reviewed (Montville and Chen, 1998).

**MODE OF ACTION**

Many antimicrobial peptides bind in a similar manner to negatively charged membranes and permeate them, resulting in the formation of a pathway for ions and solutes (Prenner, 1999). Before reaching the phospholipid membrane peptides must transverse the negatively charged outer wall of Gram-negative bacteria containing LPS or through the outer cell wall of Gram-positive bacteria containing acidic polysaccharides. Hancock and coworkers described this process as a ‘self promoted uptake’ with respect to Gram-negative microorganisms (Hancock, 1997). In this mechanism, the peptides initially interact with the surface LPS, competitively displacing the divalent polyanionic cations and partly neutralize LPS. This causes disruption of the outer membrane and peptides pass through the disrupted outer membrane and reach the negatively charged phospholipid cytoplasmic membrane. The membrane active properties of such peptides have been extensively studied using model membranes (Prenner, 1999).

The amphipathic peptides can partition into cytoplasmic membrane through hydrophobic and electrostatic interactions, causing stress in the lipid bilayer. When the unfavorable energy reaches a threshold, the membrane barrier property is lost, which is the basis of the antimicrobial action of these peptides. The modes of action by which antimicrobial peptides kill bacteria are varied (Nguyen, 2011), and may differ for different bacterial species (O’Driscoll, 2013).

The cytoplasmic membrane is a frequent target, but peptides may also interfere with DNA and protein synthesis, protein folding, and cell wall synthesis (Nguyen, 2011). The initial contact between the peptide and the target organism is electrostatic, as most bacterial surfaces are anionic, or hydrophobic, such as in the antimicrobial peptide Piscidin. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers to form pores by ‘barrel-stave’, ‘carpet’ or ‘toroidal-pore’ mechanisms.
Alternately, they may penetrate into the cell to bind intracellular molecules which are crucial to cell living (Brogden, 2005). Intracellular binding models includes inhibition of cell wall synthesis, alteration of the cytoplasmic membrane, activation of autolysin, inhibition of DNA, RNA, and protein synthesis, and inhibition of certain enzymes. However, in many cases, the exact mechanism of killing is not known. One emerging technique for the study of such mechanisms is dual polarisation interferometry (Hirst, 2010; Tzong, 2010). In contrast to many conventional antibiotics these peptides appear to be bactericidal instead of bacteriostatic (Reddy, 2004). In general the antimicrobial activity of these peptides is determined by measuring the minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits bacterial growth (Amsterdam, 1996).

![Figure 2. The modes of action by Antimicrobial peptides](image)

Several methods have been used (Table 2) to determine the mechanisms of antimicrobial peptide activity (Brogden, 2005; O'Driscoll, 2013). In particular, solid-state NMR studies have provided an atomic-level resolution explanation of membrane disruption by antimicrobial peptides (Wildman, 2003; Hallock, 2003).

**BIOLOGICAL ACTIVITY**

Many antimicrobial peptides not only kill bacteria, they are cytotoxic also to fungi (Fehlbaum, 1996; Kieffer, 2003), protozoa (Arrighi, 2002), malignant cells (Baker, 1993; Cruciani, 1991; Lindholm, 2002), and even enveloped viruses like HIV, herpes simplex virus, and vesicular stomatitis virus (Robinson et al, 1998; Tamamura, 1998). The defining characteristic of the above targets of antimicrobial peptides is their possession of a distinct cell membrane. Antimicrobial peptides exhibit selectivity against different microorganisms, of which the molecular basis is not completely understood (Zhao, 2003).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>to visualize the effects of antimicrobial peptides on microbial cells</td>
</tr>
<tr>
<td>Atomic emission spectroscopy</td>
<td>to detect loss of intracellular potassium (an indication that bacterial membrane integrity has been compromised)</td>
</tr>
<tr>
<td>Fluorescent dyes</td>
<td>to measure ability of antimicrobial peptides to permeabilize membrane vesicles</td>
</tr>
<tr>
<td>Channel formation</td>
<td>to assess the formation and stability of an antimicrobial-peptide-induced pore</td>
</tr>
<tr>
<td>Circular dichroism and orientated circular dichroism</td>
<td>to measure the orientation and secondary structure of an antimicrobial peptide bound to a lipid bilayer</td>
</tr>
<tr>
<td>Dual Polarization Interferometry</td>
<td>to measure the different mechanisms of antimicrobial peptides</td>
</tr>
<tr>
<td>Solid-state NMR spectroscopy</td>
<td>to measure the secondary structure, orientation and penetration of antimicrobial peptides into lipid bilayers in the biologically relevant LIQUID-CRYSTALLINE STATE</td>
</tr>
<tr>
<td>Neutron and X-ray diffraction</td>
<td>to measure the diffraction patterns of peptide-induced pores within membranes in oriented multilayers or liquids</td>
</tr>
</tbody>
</table>

Peptides that preferentially kill fungi have also been described, e.g. drosomycin (Meister, 1997) and plant antimicrobial peptides (Tailor, 1997). Normal human cells are relatively resistant, but it should be mentioned that certain cationic antimicrobial peptides (Zhao, 2003), such as melittin from bees (Perez-Paya, 1994), mastoparan from wasps (Delatorre, 2001), charybdotoxin from scorpions (Tenenholz, 2000), and temporin L from frogs (Rinaldi,
2002) are potent toxins. Tachyplesin is an antimicrobial peptide present in leukocytes of the horse crab (Tachypleus tridentatus) (Hirakura, 2002).

A synthetic tachyplesin conjugated to the integrin binding sequence RGD showed that it inhibited the proliferation of both cultured tumor and endothelia cells by disrupting their membranes and inducing apoptosis (Zhao, 2003; Chen, 2001). Cyclotides, members of macrocyclic cystine-knotted peptides exhibit antimicrobial, anticancer, and antiviral activities (Lindholm, 2002; Tam, 1999). The activity profile of cyclotides differed significantly from those of antitumor drugs in clinical use, which may indicate a new mode of anticancer action (Lindholm, 2002; Zhao, 2003). Other antimicrobial peptides, such as defensins (Kagan, 1990), cecropin (Moore, 1994), lactoferricin (Vogel, 2002), and lactoferrin (Yoo, 1998) also have similar antitumor activity. Tachyplesins and polyphemusins are active against vesicular stomatitis virus, influenza A virus, and HIV (Tamamura, 1996). Melittins (Wachinger, 1998), cecropins (Wachinger et al, 1998), and indolicidin (Robinson, 1998) also display anti-HIV activity (Zhao, 2003).

**SELECTIVITY**

In the competition of bacterial cells and host cells with the antimicrobial peptides, antimicrobial peptides will preferentially interact with the bacterial cell to the mammalian cells, which enables them to kill microorganisms without being significantly toxic to mammalian cells (Matsuzaki, 2008). Selectivity is a very important feature of the antimicrobial peptides and it can guarantee their function as antibiotics in host defense systems. There are some factors that are closely related to the selectivity property of antimicrobial peptides, among which the cationic property contributes most. Since the surface of the bacterial membranes is more negatively charged than mammalian cells, antimicrobial peptides will show different affinities towards the bacterial membranes and mammalian cell membranes (Hancock, 2006).

In addition, there are also other factors that will affect the selectivity. It's well known that cholesterol is normally widely distributed in the mammalian cell membranes as a membrane stabilizing agents but absent in bacterial cell membranes; and the presence of these cholesterols will also generally reduce the activities of the antimicrobial peptides, due either to stabilization of the lipid bilayer or to interactions between cholesterol and the peptide. So the cholesterol in mammalian cells will protect the cells from attack by the antimicrobial peptides (Zasloff, 2002).

Besides, the transmembrane potential is well-known to affect peptide-lipid interactions (Matsuzaki, 1995). There's an inside-negative transmembrane potential existing from the outer leaflet to the inner leaflet of the cell membranes and this inside-negative transmembrane potential will facilitate membrane permeabilization probably by facilitating the insertion of positively charged peptides into membranes.

By comparison, the transmembrane potential of bacterial cells is more negative than that of normal mammalian cells, so bacterial membrane will be prone to be attacked by the positively charged antimicrobial peptides. Similarly, it is also believed that increasing ionic strength (Zasloff, 2002), which in general reduces the activity of most antimicrobial peptides, contributes partially to the selectivity of the antimicrobial peptides by weakening the electrostatic interactions required for the initial interaction.

Figure 3. Molecular basis of cell selectivity of antimicrobial peptides
The cell membranes of bacteria are rich in acidic phospholipids, such as phosphatidylglycerol and cardiolipin (Matsuzaki, 2008; Chou, 2008). These phospholipid headgroups are heavily negatively charged. Therefore, the outmost leaflets of the bilayer which is exposed to the outside of the bacterial membranes are more attractive to the attack of the positively charged antimicrobial peptides. So the interaction between the positive charges of antimicrobial peptides and the negatively charged bacterial membranes is mainly the electrostatic interactions, which is the major driving force for cellular association.

In addition, since antimicrobial peptides form structures with a positively charged face as well as a hydrophobic face, there are also some hydrophobic interactions between the hydrophobic regions of the antimicrobial peptides and the zwitterionic phospholipids (electrically neutral) surface of the bacterial membranes, which act only as a minor effect in this case.

In contrast, the outer part of the membranes of plants and mammals is mainly composed of lipids without any net charges since most of the lipids with negatively charged headgroups are principally sequestered into the inner leaflet of the plasma membranes (Hancock, 2006). Thus in the case of mammalian cells, the outer surfaces of the membranes are usually made of zwitterionic phosphatidylcholine and sphingomyelin, even though a small portion of the membrane's outer surfaces contain some negatively charged gangliosides. Therefore, the hydrophobic interaction between the hydrophobic face of amphipathic antimicrobial peptides and the zwitterionic phospholipids on the cell surface of mammalian cell membranes plays a major role in the formation of peptide-cell binding (Tennesen, 2005). However, the hydrophobic interaction is relatively weak when compared to the electrostatic interaction, thus, the antimicrobial peptides will preferentially interact with bacterial membranes.

Dual polarisation interferometry has been used in vitro to study and quantify the association to headgroup, insertion into the bilayer, pore formation and eventual disruption of the membrane (Lanlan, 2009; Tzong, 2007). A lot of efforts have been tried to control the cell selectivities. For example, Katsumi tried to modify and optimize the physicochemical parameters of the peptides to control the selectivities, including net charge, helicity, hydrophobicity per residue (H), hydrophobic moment (μ) and the angle subtended by the positively charged polar helix face (Φ) (Matsuzaki, 1995). Besides, other methods like the introduction of D-amino acids and fluorinated amino acids in the hydrophobic face is believed to break the secondary structures and thus to reduce hydrophobic interaction that’s necessary for interaction with mammalian cells.

Wan LZ, (2006), also found that Pro→Nlys substitution in Pro-containing β-turn antimicrobial peptides is a promising strategy for the design of new short bacterial cell-selective antimicrobial peptides with intracellular mechanisms of action. Nadezhda V, (2007), suggested that direct attachment of magainin to the substrate surface decreased nonspecific cell binding as well as led to improved detection limit for bacterial cells such as Salmonella and E. coli.

**BACTERIAL RESISTANCE**

Bacteria use various resistance strategies to avoid antimicrobial peptide killing (Brogden, 2005). Some microorganisms alter net surface charges. Staphylococcus aureus transports D-alanine from the cytoplasm to the surface teichoic acid which reduces the net negative charge by introducing basic amino groups (Peschel, 1999). S. aureus also modifies its anionic membranes via MprF with L-lysine, increasing the positive net charge (Peschel, 1999). The interaction of antimicrobial peptides with membrane targets can be limited by capsule polysaccharide of Klebsiella pneumoniae (Campos, 2004).

Alterations occur in Lipid A. *Salmonella* species reduce the fluidity of their outer membrane by increasing hydrophobic interactions between an increased number of Lipid A acyl tails by adding myristate to Lipid A with 2-hydroxymyristate and forming hepta-acylated Lipid A by adding palmitate. The increased hydrophobic moment is thought to retard or abolish antimicrobial peptide insertion and pore formation. The residues undergo alteration in membrane proteins. In some Gram-negative bacteria, alteration in the production of outer membrane proteins correlates with resistance to killing by antimicrobial peptides (China, 1994).

Nontypeable Hemophilus influenzae transports AMPs into the interior of the cell, where they are degraded. And H. influenzae remodels its membranes to make it appear as if the bacterium has already been successfully attacked by AMPs, protecting it from being attacked by more AMPs (Catherine, 2011). ATP-binding cassette transporters import antimicrobial peptides and the resistance-nodulation cell-division efflux pump exports antimicrobial peptides (Nikaido, 1996). Both transporters have been associated with antimicrobial peptide resistance. Bacteria produce proteolytic enzymes, which may degrade antimicrobial peptides leading to their resistance (Whitelock, 1996).
While these examples show that resistance can evolve naturally, there is increasing concern that using pharmaceutical copies of antimicrobial peptides can make resistance happen more often and faster. In some cases, resistance to these peptides used as a pharmaceutical to treat medical problems can lead to resistance, not only to the medical application of the peptides, but to the body's own use (Habets and Brockhurst, 2012), of those peptides. Further research is needed to determine if this will lead to greater harm than benefit from the use of certain antimicrobial peptides.

**REGULATION OF EXPRESSION**

The large majority of antimicrobial peptides synthesized by multicellular organisms are encoded by the genome. Insects and mammals typically express multiple antimicrobial peptides (Zhao, 2003). For example, at least ten sheep genes encode antimicrobial peptides, including eight cathelicidins and two β-defensins (Huttner, 1998). The bovine genome contains genes for at least eleven cathelicidins (Scocchi, 1997) and over twenty β-defensins (Ryan, 1998).

The antimicrobial peptides are produced mainly through regular processes of gene transcription and ribosomal translation, often followed by further proteolytic processing. Non-ribosomal biosynthetic antimicrobial peptides will not be addressed in detail and recent advances in this field are covered by a number of good review articles (Sablon, 2000; Zhao, 2003; Jack and Jung, 2000; Moffitt and Neilan, 2000). Magainins are synthesized as long preproproteins containing six copies of the peptide. Proteolytic processing leads to the release of the individual magainin peptides (Ketchem and Cross, 1993). The 35-37 residue insect cecropins are synthesized as preproproteins of 62 amino acids and processing involves several protease activities (Gudmundsson, 1991).

Interestingly, in many cationic antimicrobial peptides such as the temporins, the negative charge of the carboxyl terminus is removed by an amidation process (Simmaco, 1996). Recently, it was found that several antimicrobial peptides are released by cleavage of intact proteins that may have no or limited antibacterial activity themselves (Zhao, 2003). This has been demonstrated first for the milk protein lactoferrin (Bellamy, 1992). Proteolytic cleavage of the intact bovine protein by pepsin under acidic conditions releases a 25 residue peptide, lactoferrin B, which shows a significantly increased bacteriostatic potency compared to the intact protein (Bellamy, 1992).

In plants, the production of inducible antimicrobial proteins is upregulated via similar signaling pathways (Zhao, 2003). For instance, microbial products induce an intracellular proteolytic cascade in potato cells leading to binding of nuclear factors PBF-1 and PBR-2 to an elicitor-response element (ERE) on the DNA (Subramaniam, 1997). Again, differential responses against different types of microbes are observed (Cardinale, 2000) and several fungal (Keller, 1999; Nürnberger, 1994) and bacterial (Gomez-Gomez, 1999) response elicitors have been identified. Like animal cells, plant cells are able to communicate microbial infection to neighbor cells using plant hormones as functional analogues of interleukins (Zhao, 2003). In plants two different hormone-dependent signaling pathways have been identified that lead to differential responses to distinct microorganisms. The salicylic acid signaling pathway induces antibacterial responses while the cis-jasmonic acid/ethylene signaling pathway induces antifungal responses (Pieterse and Van Loon, 1999; Thomma, 1998).

**BIOINFORMATICS**

Several bioinformatic databases exist to catalogue antimicrobial peptides related to different plants, animals, fungal, shrimp etc. such as:
- AMSdb (http://www.bbcm.units.it/~tossi/amxsd.html) (Tossi and Sandri, 2002)
- Peptaibol (http://peptaibol.cryst.bbk.ac.uk/home.shtml) (Whitmore and Wallace, 2004)
- CAMP (http://www.bicnirrh.res.in/antimicrobial/) (Thomas, 2010)
- ANTIMIC (http://research.i2r.astar.edu.sg/Templar/DB/ANTIMIC/) (Brahmachary, 2004)
- Penbase (http://penbase.imunaqua.com/) (Gueguen, 2005)
- AMPer (http://www.cnbi2.com/cgi- bin/amp.pl) (Fjell, 2007)
- BioPD (http://biopd.bjmu.edu.cn/help.asp) etc (Sarika, 2012)

The Antimicrobial peptide databases may be divided into two categories on the basis of the source of peptides it contains, specific database and general database.

Additionally these databases have various tools for antimicrobial peptides analysis and prediction. For example CAMP has various tools like AMP prediction, feature calculator, BLAST search, clustalW, VAST, PRATT, Helical wheel etc (Wagh, 2013)
APD2 (http://aps.unmc.edu/AP/main.php) contains information which are based on extensive literature search and providing interactive interfaces for peptide query, prediction and design with a provision to view statistical data for a select group of peptide or all the peptides in the database (Sarika, 2012). It has flexible search queries and also provide interface for studying structural and functional relationship of AMPs (Wang and Wang Z. 2009).

The PenBase (http://penbase.immunaquaa.com) antimicrobial peptides database contains information related to penaeidin family and can be searched with respect to name, biochemical properties and references (Gueguen, 2005). It also integrates BLAST and other alignment tools to retrieve the information related to a particular AMP from the external source (Sarika, 2012).

AMPer (http://www.cnbi2.com/cgi-bin/amp.pl) is a publicly available database in which peptides are classified with accuracy up to 99% using Hidden Markov Models (HMM) (Fjell, 2007). AMPer has 146 models developed for mature peptides and 40 developed for propeptides for individual AMP classes through iterative scanning of the Swiss-Prot database for previously unknown gene-coded AMPs (Sarika, 2012).

PhytAMP (http://phytamp pfba-lab.org.), contains valuable information like taxonomic, microbiological and physicochemical data for plant AMPs (Sarika, 2012). It has a simple query based browser interface for extraction and prediction of structural/functional relationships and target organisms (Hammami, 2009).

CONCLUSION

Antimicrobial peptides are a vital component of the innate immune response and are found among all classes of plant and animal life. AMPs consist of small proteins with potent broad-spectrum antimicrobial activity. AMPs alter the host immune response through receptor-dependent interactions and have been shown to be important in such diverse functions as angiogenesis, wound healing, and chemotaxis. It appears that AMPs work hand in hand with other defense mechanisms in the human body. AMPs have been demonstrated to kill Gram negative and Gram positive bacteria (including strains that are resistant to conventional antibiotics), mycobacteria (including Mycobacterium tuberculosis), enveloped viruses, fungi and even transformed or cancerous cells. Unlike the majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators. Although some organisms, such as the human gut commensals, Lactobacillus spp. and Fusobacterium nucleatum, appear to be resistant to AMPs, this resistance cannot be transferred as we have seen with other antimicrobials.

These peptides are excellent candidates for development as novel therapeutic agents and complements to conventional antibiotic therapy because they generally have a broad range of activity, are bactericidal (Reddy, 2004) as opposed to bacteriostatic and require a short contact time to induce killing. A number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), cancer (Hoskin and Ramamoorthy, 2008), and skin and wound infections (O'Driscoll, 2013). Pexiganan has been shown to be useful to treat infection related diabetic foot ulcer.

A major limitation to the therapeutic potential is the possibility of bacteria developing resistance to the peptides, and particularly if that produces a resistance to the body's own immune system use of those peptides. That is, providing a lot of the peptides as a therapeutic agent makes it easier for resistance to evolve; unlike antibiotic resistance, however, resistance to antimicrobial peptides mimicking those produced by humans can make the bacteria more resistant to the body's own immune system rather than just the antibiotic (Habets and Brockhurst, 2012).

Antimicrobial peptides have been successively incorporated into topical therapeutics. A major challenge associated with systemic delivery of an antimicrobial peptides is their susceptibility to proteolytic degradation. That is, the peptides are quickly broken down when introduced in the bloodstream. The large number of related biological databases is available and one may use bioinformatics in identification, prediction and function as well as structural analysis of AMPs. The application of AMPs have shown very promising results in production of animal/plant and agricultural produce and therefore the latest tools and technology for production of AMPs with specific activity and wide microbe range of action can be effectively utilized to develop genetically modified disease resistance varieties/breeds through biotechnology and genetic engineering

REFERENCES


Hancock, Robert EW, Rozek A. 2002."Role of membranes in the activities of antimicrobial cationic peptides.," FEMS Microbiology Letters 206 (2): 143–149.


Jay Hardy, CLS, SM (ASCP) Santa Maria, CA

Jay Hardy, CLS, SM (ASCP) Santa Maria, CA


