



Antimicrobial Peptides in Drug Development

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Abstract

Antimicrobial peptides also known as HDP host defense peptides, have been considered as source of antibiotics in future due to their broader activities and mechanism of action different than conventional antibiotics. AMP can kill both gram positive and gram negative bacteria as well as fungi and viruses. AMP can also target cancerous cells. Some AMPs also function as immune modulators. They have certain limitations e.g susceptibility to proteases, toxicity and high cost. In order to minimize these limitation certain efforts have been carried. For example to avoid degradation of peptide by proteases peptide mimics or certain unusual amino acids are introduced into peptides. For economical production short peptides have been designed retaining anti microbial activities.

Keywords

Antimicrobial peptides (AMPs); Antibiotics; SAR structure activity relationship

Introduction

AMPs are polypeptides produced by multicellular organisms. These are produced to protect the host from activity of certain microbes. As AMP provide defense against microbes they are also known as host defense peptides HDPs. AMPs play key role in developing innate immunity [1-4]. AMPs re composed of linear amino acid chain ranging in length up to 50 aminoacids. AMPs have typically cationic amphipathic properties. One side of AMPs is positively charged and other side is hydrophobic in nature. Arginine along with lysine provides positive charge to AMPs [1,2,5,6].

AMPs show activity against a broader range of micro organisms including Gram positive and Gram negative bacteria as well as fungi and viruses [1]. AMPs are effective for bacteria that are resistant to traditional antibiotics i.e MDR bacteria multidrug resistance bacteria [7-9]. Moreover there is less chance that bacteria would get resistant to AMPs. Resistance in bacteria against antibiotics is achieved by inhibition of drug target interaction and modification of drug binding site in target proteins. Genetic pattern of microbes can also be altered by a complex system called sensor transducer response. For example bacteria can alter their gene expression in the presence of AMPs [10]. AMPs possess low propensity to develop resistance due to their unique mode of action. AMPs act directly act on membrane of pathogen. AMPs develop peptide-lipid interactions by their positive side and hydrophobic side [3,11-16].

This characteristic mechanism called membranolytic effect enable AMPs to avoid common resistance mechanism of general antibiotics. AMPs thus receiving great attention as alternative of conventional antibiotics.

Structure

AMPs are divided into sub groups on the basis of their amino acid in peptide and their structure. These are usually consist of 12-50 amino acid. These AMPs include arginine and lysine as positive charged amino acids and a large proportion of hydrophobic residues. AMPs show antimicrobial activity by targeting membranes or action to cytoplasm (Table 1).

Immunomodulation

In addition to kill bacteria directly AMPs induce innate immunity. It may involve

- Clearance of infection
- Ability to change host-gene expression
- Inhibiting production of cytokine induced by lipopolysaccharide.
- Modulating the response shown by dendritic cells and the cells of adaptive immune response

Animal models indicate that host defence peptides play key role in clearance and prevention of infection (Figure 1).

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Type	Characteristic	AMPs
Anionic peptides	Due to aspartic and glutamic acid	Maximin H5 obtained from amphibians Dermcidin obtained from humans
Linear cationic α -helical peptides	Deficient of cysteine	Cecropins and melittin extracted from insects Magainin, dermaseptin and buforin II extracted from amphibians CAP18 extracted from rabbits LL37 extracted from humans
Cationic peptide enriched for specific amino acid	rich in proline, arginine, phenylalanine, glycine, tryptophan	Abaecin and apidaecins obtained from honeybees Prophenin obtained from pig indolicidin obtained from cattle
Anionic and cationic peptides that contain cysteine and form disulfide bonds	contain 1~3 disulfide bond	1 bond:brevinins, 2 bonds:protegrin obtained from pig tachyplesins obtained from horseshoe crabs 3 bonds:defensins obtained from humans more than 3:drosomycin in fruit flies

Table 1: AMPs show antimicrobial activity by targeting membranes or action to cytoplasm

1	Antiviral peptides	These peptides kill viruses by integrating into their envelope or host cell membrane both DNA and RNA viruses can be targeted by AMPs	For example NP1 its and AMP obtained from rabbit neutrophils prevent Caski cell lines by HSV2 herpes simplex virus 2.
2	Antibacterial peptides	These are peptides of most interest. These peptides target the bacterial cell either by targeting their membranes or may disrupt lipid bilayer	For example nisin can be used against MRSA, methicillin resistant <i>Staphylococcus aureus</i> . Nisin block the cell wall synthesis
3	Antifungal peptides	These peptides act against fungi either by forming pores in their cell membrane or by direct action on cell wall of fungi.	For example indolicin
4	Antiparasitic peptides	It's a smaller group of peptides	First antiparastic peptide reported is magainin

Table 2: Differentiation of peptides based on mode of action and target AMPs

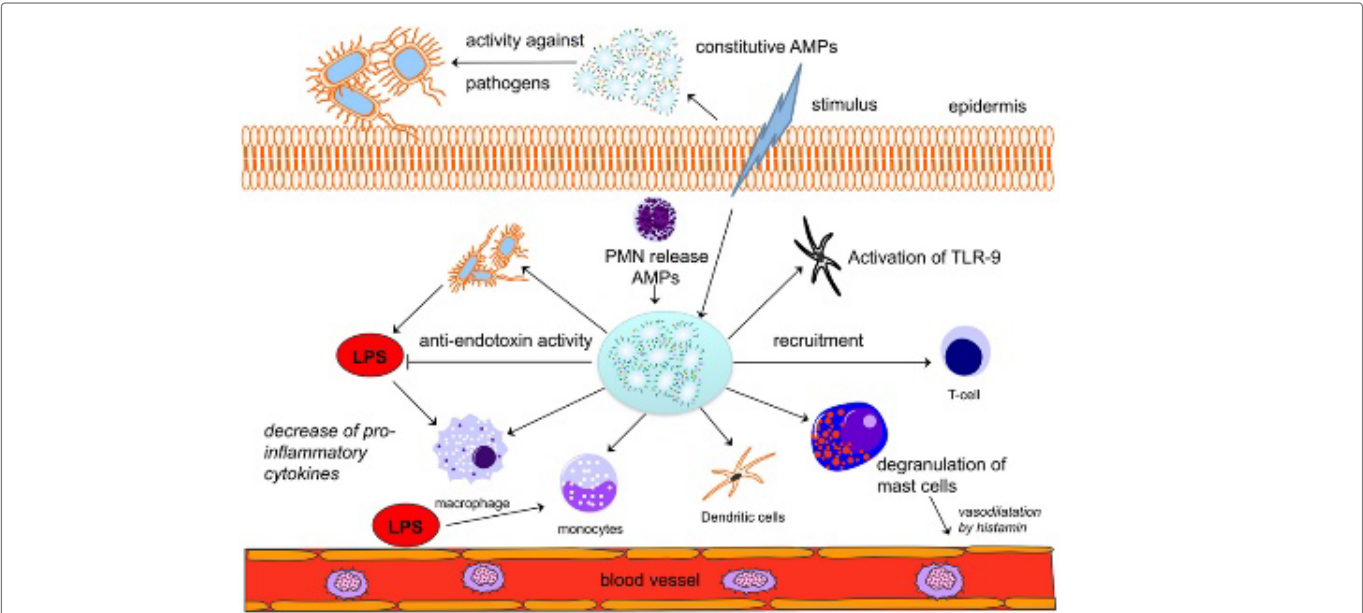


Figure 1: Antimicrobial peptides play a central role in innate and adaptive immunity. A given stimulus by bacteria leads to the release of constitutively expressed AMPs in different cells (here: epidermis). AMPs are released by neutrophils and will activate and recruit macrophages, monocytes, dendritic cells, and T-cells. A direct anti-endotoxin effect of AMPs may decrease the activation of immune cells and thus lead to a decrease in pro-inflammatory cytokine release.
Source; https://www.researchgate.net/figure/Antimicrobial-peptides-play-a-central-role-in-innate-and-adaptive-immunity-A-given_fig2_281622331

Structure activity relationships (SAR) of AMPs

AMPs are of four types β -sheet, extended, α -helical and loop peptides [1-3,6]. The α -helical are well studied in structure activity relationships. It includes magainin, cecropin, and pexiganan. This group of peptides usually lose its structure in aqueous medium. These AMPs form barrel like bundles in bacterial cell membranes and these transmembrane aggregate line amphipathic pores. Many α -helical AMPs can target bacterial membranes by forming carpet like aggregates of peptides. Some α -helical form toroidal pores to distort the bacterial membranes [1-7,9,11].

The β -sheet AMPs are stabilized by S-S bridges and usually form rigid structure. These include β -defensins, and protegrin. Most of β -sheet AMPs disrupt bacterial membranes by tilting or inserting into lipid bilayer to form toroidal pores and hydrophobic regions of peptides interact with polar head groups of membranes [3].

The extended AMPs contain some amino acids in more amount such as proline, arginine, tryptophan and histidine. Indolicidin is a tryptophan and proline rich peptide that falls in extended type category. Bac5 and Bac7 are proline and arginine rich peptides these peptides target the cell membrane [17,18]. Many extended AMPs are not effective against membranes of microbes but they can show antimicrobial activity by interacting with bacterial proteins inside [3]. Some extended AMPs e.g. indolicidin show membrane activity and act directly on membranes of pathogens.

The loop AMPs form a loop formation with the help of 1 sulphide bonds. For example bactenecin [17,18].

Understanding structure activity relationships SAR of AMPs is crucial for development of novel peptides that have better properties than natural peptides. Pexiganan also called as MSI-78, a synthetic derivative of magainin is one of the best understood peptide in terms of drug development [19,20]. Pexiganan is currently on clinical trials for the treatment of diabetic foot ulcer infections [4,20,21]. 3D structure of pexiganan determined by NMR (nuclear magnetic resonance) revealed that peptide forms dimeric anti parallel structure in the presence of membrane mimetics [22-30]. Structure of peptide and the orientation of peptide in membrane plays key role in understanding the mode of action of peptide [22].

Antimicrobial peptides as therapeutic agents

AMPs got attention to study as new generation antibiotics because of their broad spectrum activity against microbes and MDR bacteria. Since the isolation of magainins from frog skin there have been many attempts to synthesize antibiotics from natural AMPs [31]. Despite the efforts currently there is no AMP agent approved by FDA [4,32,33-37]. Although AMPs have certain advantages over conventional antibiotics they have certain limitations. The natural AMPs are liable depending upon surrounding conditions such as presence of proteases, change in pH and potential toxicity [2]. Moreover high cost of peptide production makes it less economical. In order to overcome these problems many methods have been presented. For instance introduction of unusual amino acids in peptides to prevent its degradation by proteases. Stability of peptides can also be improved by introduction of amide or acetyl groups at terminal regions of peptides. Use of efficient drug delivery vehicles such as liposomes can reduce the potential toxicity [38,39]. High production cost is one of the major issues for peptide production roughly cost estimated is \$50-\$400 per 1 gram of amino acid [7]. In order to sort the cost issue short peptides can be synthesized that retain the potential to act as antimicrobial agent. There have been several examples of peptide engineering to reduce the peptide size with retained anti microbial activity. For example thegaegurin 5 (GGN5N11) can retain the potential to kill microbes just by a single substitution of tryptophan with an amphipathic [29,30].

Classification of AMPs

Based on mode of action and target AMPs can be divided into following categories (Table 2)

Important Physiochemical Properties of AMPs

Following are important properties of AMPs

- Solubility
- Hydrophobicity
- Amphipathicity
- Net Charge
- Helicity
- Length

AMP Modifications

Most of AMPs are produced directly in their active form in order to enhance their stability certain modifications can be done. These are as follow

- Modification of AMPs with Covalent Bonds: Covalent bond modification can affect the structure and function of AMP. Even a small change in S-S bond can influence the antimicrobial activity of AMP.
- Modification of AMPs by Changing Amino Acid Content: By changing the amino acid content we can change the ability of AMP to interact with microbial cell wall.
- Modification of AMPs by Amidation: Kim *et al.* in 2011 developed this method of introducing unusual group into AMP to enhance its antimicrobial activity.
- Modification of AMPs with Unnatural Amino Acids: Introduction of unnatural amino acids in AMP can provide better folding properties.
- Modification of AMPs with Computer-Assisted Methods: Various bioinformatics tools can be used for this purpose.

Small Peptides in Drug Development

hLF1-11 (Human Lactoferrin 1-11): Lactoferrin is an iron binding glycoprotein that plays a role in innate defense system. LF can not only bind Fe^{3+} but target the bacterial membrane that enable LF to act against microbes [40-43]. The synthetic hLF-11 (GRRRRSVQWCA) is LF derivative. It shows activity against microbes including Gram positive as well as Gram negative bacteria. The synthetic peptide is also effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Acinetobacter baumannii* strains [43-45]. It is also effective against fluconazole-resistant *C. albicans* when used in combination with fluconazole [46].

3D structure of several LF derived peptides in membrane mimetic environment have been studied by NMR spectroscopy [47-50]. The confirmation of hLF1-11 varied depending upon environmental conditions [43]. Molecular dynamics MD results suggested that this peptide should be categorized as loop peptide.

The safety of hLF1-11 have been tested in haematopoietic stem cell transplantation (HSCT) recipients and healthy persons [43,51]. Intra venous injection of hLF1-11 were safe and tolerable in both healthy and HSCT recipient. While some adverse events were reported.

(CKPV)2 Peptide (α -MSH Derivative, also Named CZEN-002): The α -melanocyte stimulating hormone (SYSMEHFRWGKPV) is a neuro peptide showing antimicrobial and anti inflammatory activity [52-54]. It is studied that α -MSH peptides are effective against *Candida albicans* and their mode of action is different from other natural AMPs. This activity of peptide is caused by increased cyclic adenosine monophosphate (cAMP) in the *C. albicans* cells [55], whereas most of AMPs kill microbes by direct interaction with their membranes. It is suggested that cAMP-mediated modulation is essential for gene expression in *C. albicans* and the cAMP activating effect of α -MSH interferes with the cAMP-mediated signaling pathway [56,57].

The C-terminal tripeptide (α -MSH11-13; KPV) also has

antiinflammatory and antimicrobial activities same as shown by α -MSH [55,58]. The synthetic peptide (CKPV)2, also called as CZEN-002, was designed by modifying KPV (α -MSH11-13) peptide. This peptide is classified as loop peptide, it is a dimeric octamer consisting of two KPV units connected by cysteine-cysteine linker. The CKPV2 peptide show effective candidacidal activity against *C. krusei* and *C. glabrata* that are MDR strains [59-62]. This peptide also exerts anti inflammatory as well as candidacidal effect [60-62]. CKPV2 due to smaller size and simple sequence is regarded as effective agent for development of candidacidal and anti-inflammatory drugs. CKPV2 is being currently studied in clinical trials for treatment of vulvovaginal candidiasis [4]. CKPV2 adopts a symmetric dimer with an extended backbone structure, which resembles the α -MSH peptide [63]. The overall conformation of CKPV2 showed a β -turn like fold, which may be related to the higher activity of CKPV2 than KPV monomer [59].

Conclusions

The serious problem caused by MDR bacteria can be overcome by use of these AMPs. These are new generation antibiotics. There are certain problems with AMPs that can be covered by modification of peptides.

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