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ANTIMICROBIAL PEPTIDES SYNTHESIS AND MECHANISM OF ACTION

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Abstract

The increase of multidrug resistance bacteria (MDR) is a serious problem for health specialists and all the people in the world. Of the reasons for this problem are the misuse of antibiotics and the limited number of antibiotics as compared to the different human diseases. Antimicrobial peptides (AMPs) are new and hopefully an excellent group of antimicrobial drugs due to their potency and efficacy. These peptides may provide different treatment to traditional antibiotics, which can face resistance of microbial or may act synergistically with known antibiotics against a different strain of bacteria. Ultrashort cationic and conjugated antimicrobial peptides (USAMPs) consisting of less than 10 amino acids are a new group of drugs that qualify for development as new antimicrobial drugs. This is due to their unique mechanism of action that is a result of their variance in regards to peptide size and the arrangement of the amino acids.

Keywords: *antimicrobial peptide, antibiotics, cinnamic acid, amino acids.*

Introduction

Antimicrobial peptides (AMPs) are new and hopefully an excellent group of antimicrobial drugs due to their potency and efficacy. These peptides may provide different treatment to traditional antibiotics, which can face resistance of microbial or may act synergistically with known antibiotics against a different strain of bacteria [1].

Antimicrobial peptides

AMPs can be produced by various bacteria strains to improve their survival quality in their environment. Zeya et al (1966) discovered the first AMPs termed Defensing which was produced from human neutrophils. Up to date, there are more than 2500 AMPs that have potent antimicrobial activity [2].

AMPs are slightly had small in size, with different lengths and short sequences (10–50 amino acids). AMPs carry charge ranging from (+3 to +9) and have 30% hydrophobic residues with an amphipathic. These structural features that let them cause pore in the target membranes and consequently cause cell death [3].

To understand AMPs and their mode of action, it is important to know sequence-structure relationships. Poor understanding of the structure formation of AMPs made the classification of AMPs very difficult. Recently, the AMPs have been classified according to secondary structures. The four-major distinctive groups are alpha helices, beta strands, loop structures, and extended [4]. .

Generally, AMPs interact with the negative charge phospholipid head groups of bacterial membrane followed by insertion into lipid bilayer which leads to cell lysis. Various types of interaction between AMPs and bacterial membranes have been proposed including the barrel-stave pore model, toroidal pore model, and carpet model [5].

AMPs are considered an excellent alternative to conventional antibiotics due to their various advantages including their broad-spectrum and fast killing mechanism against a huge range of microorganisms. Additionally, they exhibit potential activity against multi-drug resistance bacteria. Furthermore, they have a low probability to induce microbial resistance due to high structural diversity which leads to unspecific interaction to components of bacteria [6].

Despite this advantage, many obstacles limit the development of AMPs. Of these, AMPs have no specific mode of action which would affect badly the mammalian cell membrane. Also, they are inactivated by biological fluids, sensitive to proteases, and the cost of their manufacturing is high [7].

Ultrashort antimicrobial peptides (USAMPs)

The synthetic conventional antimicrobial peptides have major problems which include; relatively large size which have a complex disulfide pattern, high manufacturing costs, high hemolytic activity, in addition to toxicity to the human cells due to their relative high hydrophobicity and instability toward protease enzymes in serum and plasma. These problems make the clinical development of these peptides very difficult [8] . These concerns can be overcome by developing ultrashort antimicrobial peptides (USAMPs) through a good selection of amino acids to be involved in the designs. These USAMPs have several advantages including cost-effectiveness as they are not hard to synthesize chemically and can be purified easily. Moreover, due to their relatively low hydrophobicity, they are supposed to have low toxicity towards human cells and red blood cells. These peptides have good selectivity toward bacterial cells because of the desirable interactions between the negative head groups on bacterial cell surfaces and the cationic side chains of the peptides [9].

Published data is showed that the antibacterial activity of these USAMPs is mostly due to their charge and hydrophobic parts. Additionally, to prevent cell toxicity, there should be a good balance between these bulky hydrophobic parts and cationic parts. Designing USAMPs needs careful selection of the charged and hydrophobic moiety that should be represented by specific amino acids with specific features. The tryptophan amino acid, which is mainly used in a large number of gene-encoded antimicrobial peptides, is one of the most important residues used to represent hydrophobic moiety. In general, tyrosine amino acid has been reported to show a high preference for the water-phospholipid interface and can be incorporated to represent hydrophobic moieties. The charged moieties can be represented by ornithine or arginine amino acids. Ornithine is an unusual and not coded amino acid, so the use of it can increase the stability

of peptide against protease. The activity of arginine against bacteria is mainly referred to as the hydrogen bond formation and electro-statistical interactions between the cationic side chain of arginine and the negatively charged surface of bacteria [10].

The use of cinnamic acid and its derivatives in addition to fatty acids, alcohols, and glyceryl esters, fatty amines have been reported to have some antimicrobial activity. It has been proposed to combine these chemicals with AMPs as a new technique to develop new potent antimicrobial peptides. These new antimicrobial agents can be achieved through the combination of the N-terminal acyl substituent (i.e. cinnamic acid or its derivatives that have an inherent activity against bacteria) and optimizing the peptidyl scaffold, whereby the modification of the N-terminal substituent would lead to better modulating the activity spectrum against bacteria [11].

There are few studies done on USAMPs, some of which used the solid phase technique. Laverty et al and his team designed and synthesized eight ultrashort and amino terminal-modified tetrapeptides. The antibacterial activities of these new designs against Gram-positive and Gram-negative bacteria and their toxicity toward red blood cells were studied. Some of the synthesized peptides have potent antibacterial activity against various bacterial strains with a negligible hemolytic effect at high dose levels (1000 μg/mL). The amino terminal of the tetrapeptides was also modified using p-hydroxycinnamic acid (pHCA), cinnamicacid (CIN), acetic anhydride (Ac), and 3-(4-hydroxyphenyl) propionic acid (HPPA). All the modified peptides showed a large increase in the antibacterial potency as compared to the control peptides, where the antibacterial activity of (pHCA-Orn-Orn-Trp-Trp-NH_2) was eightfold higher than (NH_2-Orn-Orn-Trp-Trp-NH_2). Additionally, the significance of C-terminal amide for antibacterial activity was observed via reporting a high MIC value (sixty fourfold) of (pHCA-OrnOrn-Trp-Trp-COOH) as compared to (pHCA-OrnOrn-Trp-Trp-NH_2) [12].

In another study, linear and non-hemolytic tetrapeptides were designed and synthesized by Lau et al Those peptides tested against various strains of methicillin-resistant staphylococcus aureus (MRSA) bacteria. This study has shown that having at least two cationic arginine residues is critical for antibacterial activity. This study was the first to use L-4-phenyl-phenylalanine residues to represent hydrophobic moiety as an alternative to tryptophan to increase membrane-insertion properties. The short peptide length against MRSA show low effect and revealed that there should be at least four residues (two arginines and two 4-phenyl-phenylalanines) [13].

In a different study, Rodriguez and his team designed a series of C-amidated ultrashort peptides (5-11 amino acids). These peptides mainly consist of aromatic amino acids and arginine. The antibacterial effect of these peptides was investigated against various strains of Gram-positive and Gram-negative bacteria and their hemolytic activity against mammalian erythrocytes. Furthermore, the outcomes of tryptophan replacement with tyrosine on the antibacterial activity were observed. As reported, the ultra-short peptides displayed potent antibacterial activity against all tested bacterial strains and were generally non-hemolytic. When tryptophan was replaced with tyrosine, a reduction in the antibacterial activity was observed. This demonstrates the essential role of the aromatic residue's size for bacterial membrane disruption, where tryptophan is bulkier than tyrosine and provides an extra antimicrobial activity [13].

Laverty et al described studied the microbiological and toxicological activity of ultrashort cationic antimicrobial peptides conjugated with saturated fatty acids (C6, C8, C10, C12, C14, and C16) for their antimicrobial activity. These ultrashort cationic lipopeptides showed an excellent and wide-spectrum antimicrobial activity against clinically isolated pathogens, including resistance-type organisms in both planktonic and biofilm-forming bacterial. Furthermore, it is observed that the number of carbon atoms and the degree of hydrophobicity of the N-acyl substituent is key determinants that can control the antimicrobial activity of lipopeptides. It was observed that increasing the number of carbons of the N-acyl substituent of lipopeptides led to increasing antibacterial activity until C12 after that the antibacterial activity gradually decreases. Nevertheless, lipopeptides conjugated with dodecyl derivative (C12) displayed the highest antimicrobial potency; it caused the highest biological toxicity and

hemolytic activity. These data indicated that thereis no relationship between N-acyl substituent hydrophobicity and antimicrobial activity. Furthermore, the conjugation strategy of the peptide with lipid moieties has a significant effect on increasing biological toxicity in general [14].

Functions of Antimicrobial Peptides

AMPs are considered multifunctional agents. Besides its potential therapeutic activity against large groups of Gram-positive and Gram-negative bacteria strains (including the MDR strains), the time killing is within minutes which raises their antimicrobial potency. Moreover, AMPs display a significant activity against fungi (ex. Filamentous fungi), parasites (ex. Protozoan and metazoan), cancer cells, and enveloped viruses. Due to their broad-spectrum activity, they have been termed "natural antibiotics". Their activity is mainly due to the direct interaction between the cell membranes of bacterial cells that carry negative charges and the positive residues of peptides. This interaction causes membrane lysis and increases membrane permeability. This is followed by rapid bacterial cell death [15].

Mechanisms of AMPs Action

There are mainly two mechanisms of action for the AMPs: Some of these peptides can create pores within cell membranes then these peptides stay over the cell and act on intracellular targeting including immune-modulatory actions. On the other hand, the antibacterial activity of some peptides is governed by cell membrane disrupting mode of action and hence cell lysis and death.

Reaction with Bacterial Membranes

The ability of AMPs to interact with cell membranes resulting in transient pores formation and leading to cell lysis is the classical mode of action that is featured by membrane permeabilization. The interaction between these AMPs and the targeted cell membrane is assisted by the positively charges domains of AMPs and the microbial negatively charged membrane leading to cell membrane penetration. Mainly, these negative charges were found in the head groups of a phospholipid bilayer. As a consequence of this interaction, the pH gradient and transmembrane potential are collapsed. Based on the previous studies, the activity of membrane-active AMPs is primarily determined according to the nature of peptide-membrane interactions [16].

The possible mode of action of AMPs is described by four different commonly used models (Fig. 1).

The Destruction of the DNA and RNA

AMPs can target multiple objects intracellularly as proteins, DNA, and RNA in addition to inhibiting their synthesis. Additionally, they can defect the cell wall synthesis process and interfere with bacterial cytokinesis.

These studies confirm the importance of AMPs soon concerning having a multi-targeting action regarding cell membrane translocation, DNA and RNA binding, and DNA condensation in addition to their toxic activity against vital enzymes [17].

The Antimicrobial Synergism between Ultra-Short Peptides and Antibiotics

Due to the dramatic increase in the rates of resistance among various pathogens (microbial species) toward conventional antimicrobial agents, universal studies are condensed on new antimicrobial approach regiment.

Recently, the combination synergistic approach is supposed to delay or eliminate the evolution of drug resistance, decrease the individual drug dosages, and hence, diminish the side effects. Many studies assessed the outcomes of HDPs or new synthesized AMPs and conventional antibiotics in combinations against various multidrug-resistant bacteria (MDRB) .These studies reported synergistic activities or an antibacterial activity improvement of many combinations. As AMPs are considered as membrane targeting agents that distort bacterial cell membranes via pore formation mechanisms and lead to an increase in the permeability of cell membrane, this consequently would lead to an increase of antibiotics entrée into the cell to accomplish the damage process more efficiently and rapidly.

Almaaytah et al reported that the combination of MelitAP-27, a hybrid antimicrobial peptide designed, with different types of antibiotics including (levofloxacin, rifampicin, chloramphenicol, and erythromycin) displayed a synergistic activity against planktonic and biofilm of many MDRB such as MRSA and P. aeruginosa. The HDPs Nisin showed synergistic activity against MRSA (planktonic and

biofilm) in combination with daptomycin and the azithromycin antibiotics [18].

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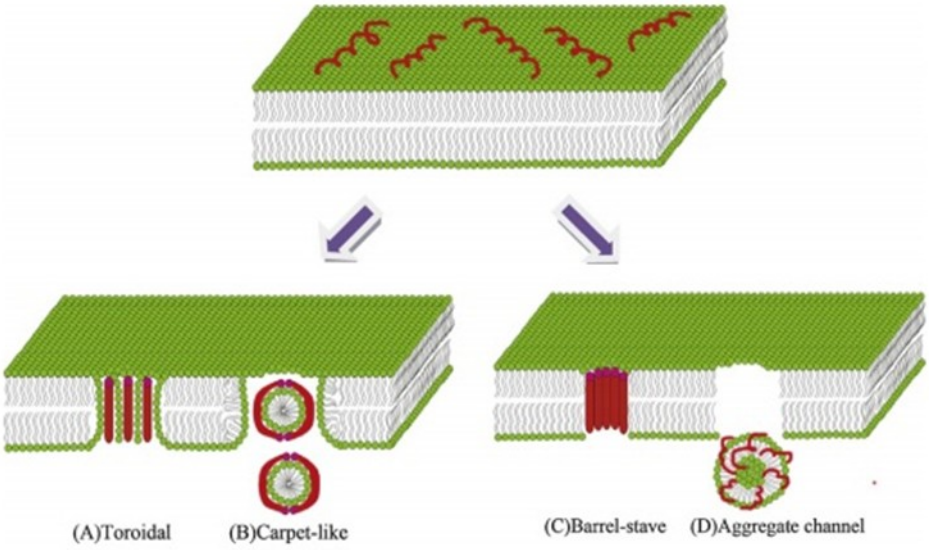

Figure 1. Current models of membrane disruption by AMPs**:** In the first step (top), the membrane surface attracts unstructured peptides via electrostatic interactions between the positive charge residues of peptides and anionic phospholipid heads of the membrane. Binding leads to a change in the conformation of the peptide into a structured form. The amphipathic peptide-lipid bilayer interactions including hydrophobic and hydrophilic nature govern pores formation after reaching threshold concentration. According to the Toroidal model (A), pores are formed as a consequence of electrostatic interaction between phospholipid head groups and positive charge portions of the peptide. These pores are lined with both peptide and lipid. In Carpet model (B), in a detergent-like mode of action, the interaction of the peptide with membrane lipids lead to form micelles structures and increase the disruption of the membrane. In the third one, barrel stave model (C), a peptide-lined pore is formed following spanning the entire bilayer. In the last mode of action, aggregate channel model (D); the membrane is disrupted as a consequence of unstructured aggregates forming.