A strategy for controlling the pathogenic bacteria: Antimicrobial peptides

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The emergence of multidrug-resistant microorganisms has resulted in an urgent need for developing new antimicrobials. Antimicrobial peptides (AMPs) are evolutionarily conserved components of innate immune system of all eukaryotic organisms, which protect hosts and some AMPs is widely applied as a preservative against bacteria. Many of these peptides have potential activities over antibiotics that include a broad spectrum against Gram-positive and Gram negative bacteria, fungi, protozoa, virus and tumor cells and selective cytotoxicity for hosts and do not easily induce resistance. AMPs target intracellular components to inhibit cellular functions, penetrate and damage cell membrane, leading to bacterial cell death. Recently, some AMPs have been reported to exert antibacterial property via different mechanism including eukaryotic apoptotic phenotypes. During the AMPs-induced apoptosis-like death, intracellular reactive oxygen species accumulation, phosphatidylserine externalization, and DNA damage are revealed in the bacterial cells. Additionally, caspase-like protein(s), RecA, were activated in apoptosis-like death response. Understanding their mechanism of action on bacterial cells will facilitate new approaches to develop therapeutical application.

Keywords Antimicrobial peptide; Mechanism of action; Reactive oxygen species; RecA

1. Introduction

Bacterial cells might still have the potential of microbial contamination to excrete toxic or food-spoiling metabolites, thereby affecting food safety [1]. Therefore, metabolically active bacteria are a very important aspect to consider in food production and for clinical applications. Bacterial infections have existed for a long time, and humans have tried to treat diseases caused by pathogenic bacterial strains, by developing antibiotics [2]. During the past two decades, antibiotic resistance to bacteria has grown into a major issue to public health, restoring infectious diseases to the list of leading causes of death worldwide [3]. Classifying the bacteria as Gram positive and negative is grated by protection to the outside is granted by two structures [4]. Gram positive bacteria have a thick peptidoglycan layer but lack the outer membrane [5]; their membranes are negatively charged due to the presence of peptidoglycan and to a lesser extent cardiolipins, [6]. In contrast, Gram negative bacteria consists a thin peptidoglycan layer between the cytoplasmic membrane (inner membrane) and the outer membrane [5, 7]. Outer membrane protects bacteria from detrimental agents, being composed of an interior leaflet of phospholipids and an exterior leaflet of lipopolysaccharide molecules that approximately cover three quarters of the surface and the remaining quarter is composed of membrane proteins. [7, 8].

As a reaction to this health crisis, several efforts are underway to discover and develop new natural and synthetic antimicrobial agents. One promising antibiotic class is antimicrobial peptides (AMPs), which is an important part of the innate immune system in all domains of life [3, 9]. AMPs is a component of the first line of defense for every living organism against invasion by pathogens [10]. AMPs are composed of less than 100 amino acids residues [11, 12] commonly used for cellular delivery and are often rich in cationic and hydrophobic residues, arranged in different groups depending on the amino acid composition, size, and conformatin [9, 13]. Although they categorized into several groups on the basis of their structures or sequences as α-helical, cysteine-rich, glycine-rich and proline-rich peptides [14], AMPs commonly possess certain characteristics such as cationic charge and amphipathicity [15]. Most AMPs are generated from post-translational processing of larger precursors [16] and present a broad spectrum of antimicrobial activity against parasite, bacteria, fungi, and viruses including multidrug-resistant microorganisms, and do not easily induce resistance compared to conventional antibiotics [9, 11, 12]. In this chapter, exploiting the potential of AMPs should facilitate the development of better antimicrobial strategies which could efficiently control the bacterial infection.

2. Disturbance of integrity of cell membrane

The mechanisms of AMPs are complex, above all, the classic action mechanism of AMPs involves their ability to interact with cell membrane [9, 16]. The microbe membrane is the main barrier that limits the distribution and entry of antibiotics [16]. Damage to the cell cytoplasmic membrane might cause loss of structural and functional integrity of the membrane [16]. The overall negative charge found on the membrane of bacteria has an important role in the preferential binding of some peptides to those microorganisms. Bacterial membranes possess high amounts of negatively charged phospholipids on the outer leaflet [17]. Those molecules that confer a charge to the bacterial surface were selected as targets for cationic AMPs [9]. AMPs are usually positively charged and adopt amphipathic α-helical or β sheet

structures. These properties suitable for a selective membrane-interacting activity, leading to a lethal disruption of the microbial envelope and can insert into lipid bilayers, eventually forming pores, as is their ability to target the anionic microbial membrane [17,18]. Therefore, cationic AMPs selectively target and disrupt anionic membranes of bacterial cells over neutral cell membranes from humans [17]. Therefore, it is generally accepted that most AMPs bind to microbial cell membrane or wall with electrostatic and/or hydrophobic interaction, and cause membrane disruption [19].

In order to further characterize the bacterial membrane permeability of peptides, the efflux amount of potassium ions was confirmed [16]. The release of the intracellular potassium ion provided the evidence that AMP probably acted on the plasma membrane by increasing permeabilization and causing ion leakage from the cell [16]. AMP showed its permeabilization on bacterial mimic membrane, and it increased the plasma membrane permeability and intracellular potassium ions leakage from cells. [16]. Also, AMPs can interact with microorganisms by electrostatic forces between their positive amino acid residues and negative charges exposed on cell surfaces [9]. Many anionic bacterial membrane exposed to AMPs depolarize as the dysfunction of membrane. The electrostatic interactions between cationic AMP and negatively charged membrane surfaces are commonly reported to explain the specific affinity for particular bacterial strains [17]. A dramatic depolarization was exhibited of the plasma membrane, increased cell membrane permeabilization and formed a pore on the cell membrane and finally lysed the cells to death [16, 20]. If pores are formed by peptides in the cell membrane, leakage of intracellular content caused, leading to cell death [1]. Most reports suggest that AMPs induce bacterial cell death mainly by damaging cell membrane integrity and forming membrane pores, leading to the outflow of intracellular contents [1].

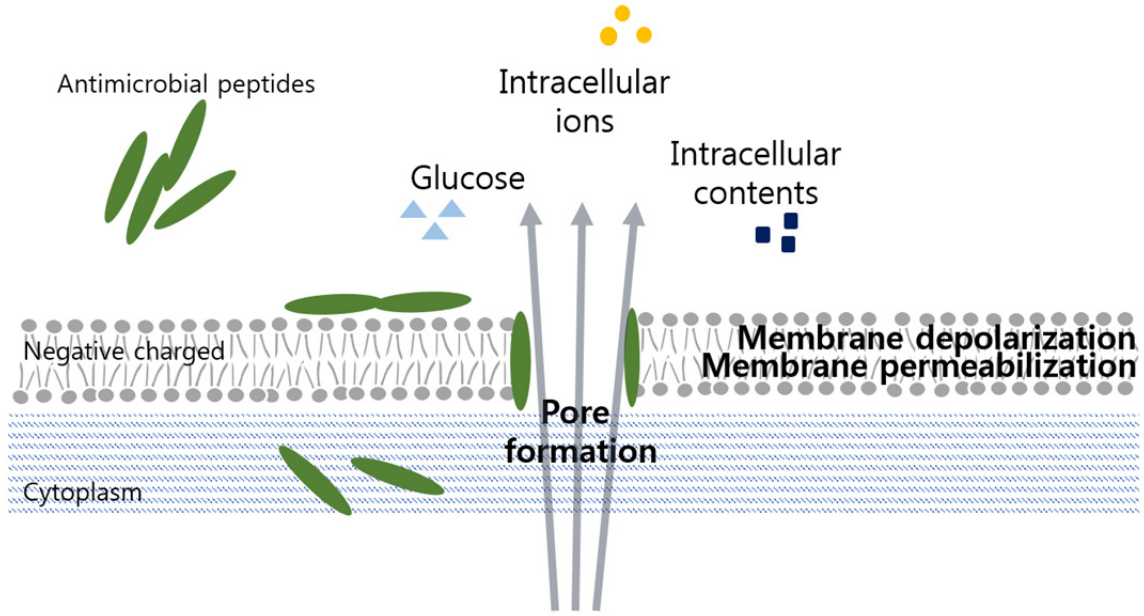

Fig. 1. Membrane disruption leading to leakage of intracellular components was caused by antimicrobial peptides.

3. Inhibition cellular functions

Cell death is the result of multiple inhibitory activity [16]. In contrast to most AMPs that have the bacterial outer and inner membranes as main target, some other AMPs [18]. Bacterial cell membranes are penetrated into the bacteria without damaging the membrane, targeting intracellular molecules without disruption of the membrane [9, 18] and the peptide reaches the inner structures of the cell such as nucleic acids and proteins, damaging critical intracellular targets and interfering with intracellular functions and normal metabolism [1, 9, 21]. AMPs also affect functions of other cell components such as DNA, RNA, and protein as well as enzyme activity without significantly permeabilizing the cell membrane [1, 10, 16, 19]. Several AMPs affect microbial viability by mechanisms involving interaction with intracellular targets and their mode of action is assumed to be mediated by translocation across the plasma membrane in a nonlethal manner [18]. These peptides act on its bacterial targets leading to the translocation of peptide molecules across the membrane [16, 18]. AMPs interaction with DNA can inhibit or hamper gene expression which is an effective way to shut down and block normal enzyme and receptor synthesis, disrupting the materials needed for the life cycle of bacteria that leads to death [16].

Some AMPs specific subunits of the ribosome or ribosome-associated proteins. They bind to the 70S ribosome strongly, thus making the ribosome the preferred target [14, 18]. The path of the peptide overlaps with the binding sites of multiple classes of antibiotics, thus suggesting that it inhibits the bacterial ribosome through a concerted mode of

action. This would probably prevent the transition to the elongation phase of protein synthesis and result in the accumulation of 70S ribosomal particles [14]. Likewise, AMPs inhibits the activity of enzymes produced by bacteria, such as trypsin-like proteinase and heat shock proteins [1] or inhibits the synthesis of macromolecules [1]. Moreover, some evidence suggests that most AMPs act on intracellular targets in bacteria by inhibiting the synthesis of DNA, RNA, and protein, as well as enzyme activity, killing the bacteria [1].

4. ROS and oxidative stress

In growing cells aerobically, there are generated reactive byproducts of cellular respiration, such as hydrogen peroxide, superoxide anion radical, and the highly reactive hydroxyl radicals by cellular respiration (presence of redox cycling compounds), synthesized by enzyme systems [22, 23]. Reactive oxygen species (ROS) have a role in cell signaling, including gene expression, apoptosis, and the activation of cell signaling cascades. It should be noted that ROS can serve as both intra- and intercellular messengers [23]. The biological targets are lipids, proteins and nucleic acids and much of the damage is caused by hydroxyl radicals generated from hydrogen peroxide via the fenton reaction, which requires iron (or another divalent metal ion) and a source of reducing equivalents (possibly NADH) to regenerate the metal. Polyunsaturated fatty acids in membranes are major targets during oxidative stress and lipid peroxidation was initiated. Its primary effect is a decrease in membrane fluidity, which alters membrane properties and can disrupt membrane bound proteins significantly. This effect is degraded to a variety of products and damage proteins [23].

Membrane oxidation occurs when cells are exposed to various levels of oxidative stress. Lipids and proteins can, for instance, react with ROS to generate compounds with altered structures and properties [13]. Oxidized lipids and proteins consequently do not behave in the lipid bilayer as their nonoxidized precursors do, and they often contribute to increasing the permeability of the membrane [13]. Oxidation of membrane lipids results in their hydroxylation, peroxidation, and carbonylation. Although the extent of the effect of these changes in the lipid membrane has not been fully explored, the newly created functional groups within the lipid membrane could provide enough driving force to change the biophysical properties of the membrane and introduce stress [13]. In principle, untargeted oxidation is also not desirable for cell killing applications because killing should be restricted to disease-causing cells and should not affect healthy cells [13]. DNA, also a main target, was attacked both the base and the sugar moieties producing single- and double-strand breaks in the backbone, adducts of base and sugar groups, and cross-links to other molecules, lesions that block replication [23]. An AMP-mediated ROS cascade is expected to stimulate automatically, since it will cause secondary macromolecular damage and thereby stimulate additional ROS generation. Hence, the cascade must be controlled to avoid runaway death due to minor, transient stress [24].

5. Interaction with DNA damage/ DNA associated pathway

DNA damage is a part of the bacterial cell death mechanism through specific oxidation of guanine and incorporation of oxidized guanine into synthesized RNAs [25]. This process is followed by formation of double stranded DNA breaks, DNA fragmentation, and cell death [26]. Cationic AMPs seem reasonable that it could be electrostatically attracted by the nucleoid, where the translation and the transcription take place or that a highly cationic peptide could bind the negatively charged nucleic acids [18]. AMPs also translocated to the inner leaflet of the cytoplasmic membrane and targeted intracellular DNA after penetrating the cell membranes [16]. In the intensively studied case of exposure to antibacterial peptides, the underlying molecular mechanism is thought to involve incorporation of oxidized nucleotides into nascent DNA coupled with a temporary suppression of mismatch repair so that they are not removed [27, 28].

6. Cell cycle arrest and filamentation

Filamentation can occur following disruption or inhibition of peptidoglycan synthesis and inhibition of DNA synthesis is inhibited or DNA damage by a process known as the SOS response [29]. DNA damage can influence cell filamentation and inhibit cell division, because the DNA repair system prevents formation of septum until the DNA damage has been repaired [26]. Until the damaged DNA can be repaired, DNA repair system represses septum formation, the delay preventing the transmission of damaged DNA to daughter cells, [29]. In the absence of antibacterial agents or other stressors, bacterial cell filamentation occurs at a low frequency [29]. Bacteria postpone division by synthesizing protein SulA, an FtsZ inhibitor that halts Z-ring formation, thereby stopping penicillin-binding protein 3 activation and recruitment [29]. Cell filamentation could be observed, a marker of cell cycle arrest generally mediated under stress conditions by SulA [30, 31]. Oxidized DNA by ROS needs to be repaired by the bacterial DNA repair systems. Then, during the repair process, cell division is arrested by cell cycle checkpoint regulation to provide enough time for recovery before the serious phases in DNA replication [25]. In the bacterial cells, the cell cycle composes three periods; Phase I, the time from cell division to initiation of chromosome replication, is similar to phase G1 in the eukaryotic cells, while R phase was equivalent to S phase which is the phase to replicate the chromosome.

Once R phase was completed, the bacteria entered D phase directly without entering G2 phase, the time from

termination of replication to cell division [16]. While cell division is arrested, the bacterial chromosome remains condensed and the phenomenon of filamentation takes place, indicating replication arrest.

7. Characters of apoptotic-like death

Cell death occurs in an unordered and accidental manner, known as necrosis or in a programmed form, known as apoptosis [9]. Other signals have been described to provoke apoptosis and other mechanisms of death [9]. When the concentration of intracellular potassium is normal, the cell death process is repressed by the suppression of the caspase cascade and inhibition of the apoptotic nucleases activity. Thus, the efflux of intracellular potassium ion could prompt cell apoptosis [9]. In the same way, calcium ions and ROS work as pro-apoptotic second messengers [9]. Recently, some AMPs have been reported to exert antibacterial properties via similar phenotypes mechanism known as apoptosis.

The cells undergoing AMP-induced apoptosis experience particular phenomena including ROS production, caspase-like peptide activation, phosphatidylserine externalization, membrane depolarization, and hydroxy radical formation, on suffering severe DNA damage [32]. The FITC-Annexin V and PI double staining analysis revealed that flip-flop to the outer leaflet of the membrane occurred following coprisin treatment and the normal distribution of phosphatidylserine on the inner layer of the membrane bilayer was disrupted [32]. Phosphatidylserine is known to have an important role in the regulation of apoptosis in response to particular calcium-dependent stimuli [33]. The normal distribution of this lipid on the inner leaflet of the membrane bilayer is then disrupted because of stimulation of enzymes such as scramblase or flippases, which can move phosphatidylserine in both directions across the membrane, and inhibition of amino phospholipid translocases, which returns the lipid to the inner side of the membrane [34]. Some cells with AMPs indicate that phosphatidylserine was externalized on the outer surface of the plasma membrane [34]. (Fig. 2)

8. RecA protein and SOS response

RecA protein is found in all organism and essential for genetic recombination and recombinational DNA repair [22]. A prototype of RecA family of proteins, has multiple roles in the *Escherichia coli*. RecA catalyzes the DNA strand exchange mechanism by coupling with ATP hydrolysis, promoting the recombination process [35]. RecA protein binds to the single-stranded DNA with one RecA monomer for every three bases of DNA and forms nucleoprotein filament accompanied by ATP hydrolysis [22, 35]. This RecA filaments promote alignment with a homologous duplex DNA, strand exchange and branch migration. Beside nucleoprotein filament formation, RecA also has coprotease activity, which facilitates the autocatalytic cleavage of the LexA repressor [22]. RecA also directly facilitate replicative bypass of DNA lesions by associating with DNA polymerase during SOS response [22]. RecA monomers bind to single stranded DNA in an ATP-dependent manner forming an active nucleoprotein filament [35]. RecA facilitates the autocatalytic cleavage of LexA repressor, which is required for inducing the SOS response [35]. Caspase is a key mediator that regulates apoptosis and immune signaling during development or infection, for homeostasis [36]. Several AMPs stimulates activation of caspase-like protein and increase the expression of RecA [32, 37]. The expression of RecA, which has been reported to be a caspase-like protein involved in bacterial apoptosis-like responses [28]. (Fig. 2)

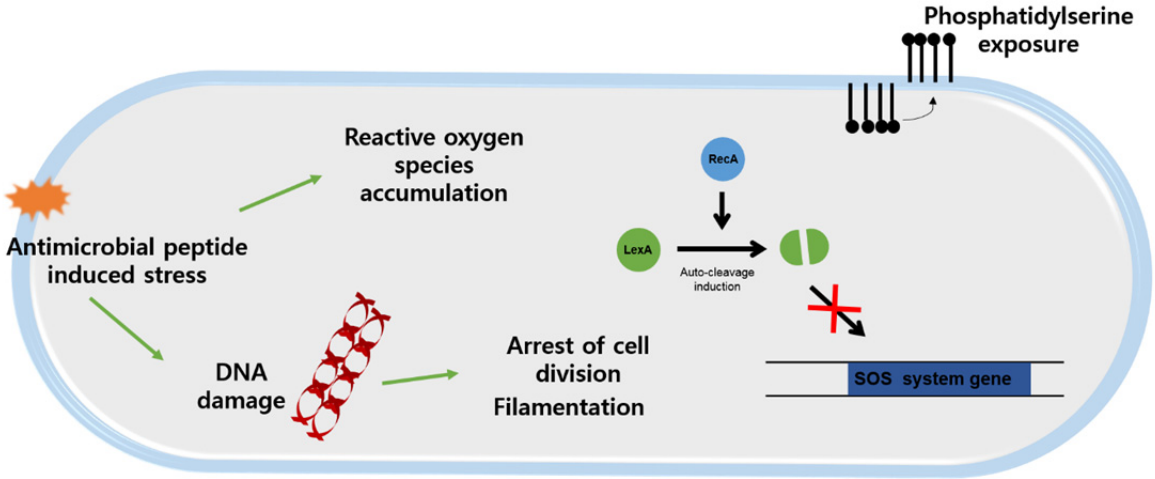

Fig. 2. Apoptosis like response in bacteria show that antimicrobial peptides can induce the pathway. DNA fragmentation and cell filamentation due to the halt of cell division progression. A relationship between RecA and caspase and assumed that RecA can act as a major regulator in both SOS repair system and apoptosis-like response in bacteria

9. Conclusions

AMPs that target these essential features of pathogenic bacteria are often considered to be bactericidal agents, because their action is so disruptive to the cell that viability is curtailed. Relationships between antimicrobial peptide and antimicrobial lethality may also be clinically significant. Also, antibacterial peptide can induce the apoptosis-like response in bacteria like as fungal apoptosis. RecA or other genes involved in the SOS response as potential novel drug targets to combat the ever-increasing problem of antibiotic resistance. Ongoing studies with a different approach, in terms of the understanding the diverse mechanism of AMPs, will contribute to the development of more potent bactericidal peptides without unexpected side effects.

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