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The Main Directions of Antimicrobial Peptides Use and Synthesis Overview

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Abstract

In this review, various classes of antimicrobial peptides (AMPs) were collected. Majority of these peptides are an integral part of the innate immunity of living systems; most of them are non-toxic to the human body, but have a detrimental effect on viruses, bacteria, parasites, tumors and fungi, which makes them indispensable in the fight against infections, while pathogenic strains have already acquired resistance to a wide range of antibiotics. Urgent need to develop new AMPs, many laboratories around the world are now developing AMPs. For this, first, a classification of AMPs has been drawn up; secondly, the main mechanisms of AMP action on the cells of the human body and their destructive action on pathogens are being studied. According to the new classification, AMPs are sorted into antibacterial, antifungal, antiparasitic, antineoplastic, etc. classes. The main factor ensuring the attachment of AMP to the membrane of a normal cell is its charge, and the hydrophobicity of AMP contributes to the formation of pores in the membrane of the microorganism; for this reason, the activity of AMP is related to its amphiphilicity. Thus, the placement of histidine with buffering properties into AMPs provides damage to microorganisms without hemolysis of host cells; the same purpose is served by obtaining hydrophobic sites in the newly synthesized AMP. The material presented in this work helps the synthesis of new effective AMPs capable of stopping the development of severe infectious processes, with Allah Support.

Keywords

AMP, Amphiphilicity, Hemolysis, Hydrophobicity

1. Introduction

Antimicrobial peptides produced in the body of almost all living beings are responsible for protection against foreign elements, mainly microorganisms. In the

human body, they are produced mainly in self defense cells, such as neutrophils, monocytes and macrophages; their synthesis increases sharply when tissues face damage by pathogens, namely gram-positive and gram-negative bacteria, viruses, fungi, and with tumor growth as well. They are also formed in plants and in the hemolymph of insects. Due to their low toxicity and selectivity of action, the attention of scientists around the world is focused on obtaining new synthetic AMPs that can withstand chemotherapy-resistant strains of microorganisms in order to treat diseases considered incurable.

2. AMP as an Alternative Medicinal Preparations

Recently, scientists have been attracted to short-chain antimicrobial peptides (AMPs) that are synthesized in all organisms and play a protective role against a wide range of pathogens in living organisms. Currently, scientists in leading laboratories around the world have focused on AMPs whose toxicity is low for the human body, and the antimicrobial activity rate is high [1]. The antimicrobial activity of AMPs is a result of their role in the body as the integral part of a widespread self-defense system in living organisms. The AMPs are also advantageous in that the detoxification work performed by AMP in a few minutes is carried out using antibiotic hours. The application of AMPs in medicine and the synthesis of their new types are of particular importance as there is resistance to antibiotics recently. Due to this, the possibility of co-administration of AMPs as an alternative to antibiotics and/or to reduce their dose and toxicity is currently being investigated. AMPs penetrate the membrane quickly and causes microbial cell death in a few minutes [2]. The rapid destruction of microorganisms by AMPs is not only due to their interaction with membranes: AMPs can also affect wound healing, chemotaxis, and cell migration by reacting with metabolic products and interacting with cellular signals in the cytoplasm [3], ergo the investigation & synthesis of the new generation of AMPs can be considered one of the most pressing problems of science.

AMP types include: epinesidine-1, BMAP-28, hCAP109-135, LL-37, MPI-1, polybia-MPI, SALF (shrimp antilipopolysaccharide factor), magainine-1, magainine-2, melittine, pardaxine, pleurocidines NRC -03 and NRC-07, protegrine 1, taxiplezine, cecropins A and B, lactoferricine B, brevinine-2R, hepsidine TH1-5, gomezine, dermaseptine B2, HNP-1, HNP-2 and HNP-3 defensins, NP-1 and NP-2-defensins.

3. AMP Discovery History

The first known AMP in science was lysozyme discovered in 1922 by Alexander Fleming in vegetables, as well as in animal tissues and secretions, showing bacteriolytic activity. Dubos then isolated an AMP called gramicidin from the peptide chain from the soil *Bacillus* and proved that the peptide could be active against pneumococcal infection and could be used in the local treatment of wounds and ulcers [4]. In 1956, leucine and phagocytin were the first AMPs isolated from

mammalian leukocytes; leucin and fagocitin of mammals have a disastrous effect. A few years later, it was shown that other bactericidal proteins also accumulate in the lysosomes of human leukocytes. In 1985, the Lehrer group identified three small peptides called "defenzins" from human neutrophils, also known as HNP-1, HNP-2, and HNP-3 ("human neutrophil peptides"); these peptides could be lethal to *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E.coli.* Numerous other natural and synthetic AMPs have been obtained in recent decades, and the amount of detected AMPs increased so much that the classification of AMP classes has arisen. AMPs may be classified according to their clinical signs. According to this new classification, AMPs are currently sort into 18 categories. These categories include antibacterial, antiviral, antifungal, antiparasitic, anti-HIV, anti-tumor peptides, and so on. The main part of AMPs (about 60%) exhibits antibacterial activity, followed by AMP with antifungal action (in about 26% of cases); and the remaining AMPs, mainly 2% - 5% in each class, have antiviral, antiparasitic, and antitumor, anti-HIV etc properties.

4. Antibacterial AMPs

Since listed below AMPs slow down the progressive development of the bacterial strains, they are referred to as antibacterial AMPs. Such skin and soft tissue infectious bacteria as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species are resistant to almost all common antibiotics [5]. Antibacterial peptides are used in clinical medicine for *Acinetobacter baumannii*, *S.aureus*, *Salmonella*, *Listeria monocytogenes*, *E. coli* and *Vibrio parahaemolyticusa* s. They inhibit the progressive growth of pathogenic bacteria, while some of them show cytotoxic effects. Defensins are antimicrobial peptides of innate immunity. Many natural and synthetic AMPs, such as nisin cecropins, and defensins, show significant inhibitory activity against gram-positive and gram-negative bacteria [6]; nisin is promising in the treatment of *Clostridioides difficile* [7]. Cecropin is a natural binding to ergosterol lytic peptide not lethal to mammalian cells, but with antifungal activity. Cecropin was first obtained from the bacteria-infected silkworms. Cecropin A is able to lyse gram-positive and gram-negative bacteria; it binds to the negatively charged bilipid layer of the membrane via the N-terminus, which is highly positively charged. Then the hydrophobic C-terminus acts as a blowing agent, making the membrane permeable, which leads to the death of bacteria. Synthetically derived from interferon-I P5 and P9 AMPs from *Aristicluthys nobilia* show a weak cytotoxic effects [8].

5. Antifungal AMPs

Antifungal AMPs show strong inhibitory activity against drug-resistant fungal infections [9]. AMPs of this group have a high antifungal effect in clinical medicine against the fungi *Aspergillus* and *Candida albicans*, as well as *the fungus Aspergillus flavus*, which are widespread in food and agriculture [10]. With the *exception of brevinin*, *ranaturin*, *and secropins*, many synthetic AMPs also have

significant antifungal activity [11]. For example, AurH1, obtained from aurein 1, 2 [12] by the semi-synthetic method, can significantly prevent *Candida albicans* infections that have a mortality rate of up to 40%, and can therefore be used as a treatment in these infections. Many AMPs can inhibit the development of aflatoxin, a carcinogen produced by *Aspergillus flavus* harmful to the human body. 37 AMP from *Lactobacillus plantarum* and their mixture can impair the development of *Aspergillus flavus* spores [13]. Two chemically obtained radishes AMP can have a significant inhibitory effect against yeasts such as *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii*. An ultrashort peptide made of the sequence amidated peptide H-Orn-Orn-Trp-Trp-NH2 also exhibits antifungal activity against food contaminants [14]. Fernández de Ullivarri *et al.* give the examples of semisynthetic and synthetic antifungal AMPs with their action on *C. albicans*, *A.fumigatus* and other fungi [15].

6. Antiviral AMPs

Antiviral AMPs have a strong destructive effect against viruses, mainly by preventing the virus binding to the host cell membrane, or by destroying the viral sheath, or by stopping viral replication. AMPs as therapeutic agents are especially promising against viral pathogens for which neither vaccines, nor treatment are available, for instance in case of dengue virus and Zika virus [16]. Recent studies have shown that Epi-1 AMP (Epinecidin-1, an Antimicrobial Peptide derived from Grouper (*Epinephelus coioides*), can inactivate viruses [17], as well as have significant inhibitory activity against viruses that cause infections of the oral cavity and feet. The synthesized peptide Epi-1 has many additional pharmacological properties, for example antimicrobial, immunomodulatory, anticancer and wound healing properties as well. However, SIAMP-type AMPs can significantly reduce mortality in chicks embryos infected with the infectious bronchitis virus [18], ergo this AMP can be used in this type of infection.

7. Anti-HİV (Human Immunodeficiency Virus) AMPs

Anti-HIV peptides that act against HIV, which causes acquired immunodeficiency in humans, also belong to the class of promising antiviral peptides. We may count gramicidin and bacteriocins obtained from bacteria, plant cyclotides, taken from insects melittins and cecropins, and piscidins from fish. Ascaphins, caerins, dermaseptins, as well as esculentins, and maximins from amphibians, cathelicidins and defensins from vertebrates are also recognized as anti-HIV agents [19]. Defensins are the most important subgroup of these peptides. Amig them are α- and β-defensins acting on different mechanisms, gramicidin D, serine 1, maximin 3, magaynine 2, dermaseptin-S1, dermaseptin-S4, siamycin-I, LL-37; some of them are already used in medicine as anti-HIV drugs [20].

8. Action and Sources of Anticoronaviral AMPs

In connection with the COVID-19 pandemic, scientists have also considered an-

tiviral peptides against coronavirus. Coronaviruses belong to the family *Coronaviridae*; they are helical symmetrical, positive single-stranded, coated viruses with an RNA genome ready for replication. The recent emergence of COVID-19, as well as coronaviruses SARS-CoV and MERS-CoV, which cause severe acute respiratory syndrome, pose a serious threat to human life, as these types of viruses can cause very serious respiratory diseases. The δ-strain of India has been replaced by the spreading rapidly ʎ-strain mainly distributed in North America. The coronavirus consists of a protruding glycoprotein and an adjacent shell, as well as a membrane and an RNA-encapsulated into the capsid, that facilitates infection. Antiviral AMPs can bind to the protruding glycoprotein, the spike protein, to prevent the virus binding to the host cell, thereby preventing the spread of infection. The SARS-CoV spike-protein domain contains the amino acid sequences HR1 and HR2; The HR2 peptide and its lipid-binding component are very similar to the membrane S-protein adjacent ferredoxin involved in the folding of the virus. It is even thought that this peptide may be completely identical to it. Such AMPs as defensins & temporins are effective against COVID, MERS-CoV & SARS-CoV [21]. It has been shown that AMP termed temporin has a therapeutic effect against MERS-CoV; two AMPs called K12 and K29 were obtained from nsp10, a non-structural protein of SARS-CoV [22], which can also inhibit SARS-CoV replication. In addition, the incidence of SARS-CoV fatalities is significantly reduced after treaing with rhesus thet a-defensin 1 [23]. Theta-defensin 1 (RTD-1) is a novel cyclic AMP also proposed as a prophylactic antiviral agent [24]. RTD-1 mediates its antifungal effects *in vivo* by host directed mechanisms rather than direct fungicidal activity. This new class of host-directed compounds may be used for treatment of disseminated candidiasis.

According to recent studies, LPD-12 is the best lipopeptide that after binding with spike protein which interrupt its affinity of binding with angiotensin-converting enzyme-2 significantly [25], it is the most potent "binding inhibitor" against COVID, preventing COVID-19 from binding to the host cell via the spike protein. Lipopeptides can be used against SARS-CoV-2 by interacting with viral fusion proteins. Small antimicrobial lipopeptides block the viral membrane fusion to the host cell by lipid membrane alternation. One of rhesus theta-defensin-1 action mechanisms is its immunomodulatory effect. It has also been found that anti-COVID AMP peptides prevent COVID-19 entry host cells or disrupt the interaction of COVID-19 with enzyme 2, which catalyzes the conversion of angiotensin. EK1C4, a lipopeptide of EK1 origin may inhibit SARS-CoV-2. Xia S. *et al.* developed a pan-coronavirus fusion inhibitor EK1 targeted the HR1 domain inhibiting SARS-CoV and MERS-CoV. They generated a series of lipopeptides derived from EK1, one of which, termed EK1C4, is the most potent fusion inhibitor against SARS-CoV-2 infection [26].

In general, anti-coronaviral AMPs can be derived from the followings: 1) peptides derived from HR1, HR2 or the receptor-binding domain of spike-protein; 2) peptides derived from non-structural proteins of the virus; 3) peptides derived

from other AMPs. It should be noted that anti-coronavirus AMP drugs are administered intranasally.

9. Antiparasitic AMPs

Primitive parasites can be transmitted from animal to human or human to animal in the living environment: through water, soil and food. Recently, the need for new antiparasitic drugs has increased due to increased parasites resistance. The activity of antiparasitic AMPs is studied on parasites that cause malaria and leishmaniasis [27]. Such AMPs as catelicidin and temporin-SHd has been shown to be highly active against parasites. Recent studies have shown that a synthetic AMP Epi-1 can destroy the membrane of *Trichomonas vaginalis* violating it [28]. It has been discoveredç that bee's milk is a valuable source rich in AMPsI bee's milk jellein peptide and comprised from 4 amino acids AMP can significantly weaken the *Leishmania parasite*. Peptides of C*yanobacteria* differ from the AMP of eukaryotes in that their antiparasitic effects are due to specific proteins. Thus, by examining the target proteins of antimicrobial peptides derived from C*yanobacteria*, it is also possible to differ the of parasite types, even if they belong to the same class.

10. Antineoplastic AMPs

Antineoplastic peptides inhibit the growth of tumor tissue in various ways. The mechanism of action some of them is based on the activation of immune cells with further destruction tumor cells; AMPs, on the other hand, destroy tumor cells by inducing necrosis or apoptosis in them. AMPs also inhibit angiogenesis, prevent the formation of a vascular network that nourishes tumor tissue, and prevents metastasis [29]. Another important mechanism of action of antineoplastic peptides is based on the disruption of vital functions by interfering with the gene transcription and translation in the tumor cell. Overexpression of phosphatidyl-serine on the surface of tumor cells is a sign of neoplasia. When AMP is added to a tumor cell culture, the cytotoxic effect of AMP is based on damage to the cytoplasmic membrane of the target cell, which results in tumor cell necrosis. Unlike normal eukaryotic cell surface, which is rich in phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, the nembrane surface of tumor cells contains 3 - 11 times more negatively charged phosphatidylserine, as in prokaryotes, which in fact promotes tumor recognition and targeting by AMPs. Such AMPs as LTX-315 damage tumor associated molecular agents by alterations in the cancer cells organelles indolysidine and puroindoline A [30] may have antineoplastic activity *in vivo*. AMPs may also induce apoptosis in tumor cells. The tumor membrane-lytic effect of AMP develops in a similar way to the mechanisms by which they affect the membranes of microorganisms. The antineoplastic effect may develop on internal or external mechanisms. The internal antineoplastic effect is triggered by the induction of the apoptosis as a result of mitochondrial damage. External antineoplastic action is a receptor-mediated

mechanism [31]. Apoptosis is triggered by recognition of the phosphatidylserine-rich tumor cell membrane. Over expression of phosphatidylserine on the outer surface of the cell membrane is one of the earliest indicators of apoptosis. Apoptosis is also accompanied by an increase in the expression of genes belonging to the caspase class [32], however, in necrotic cells primarily membrane damage is observed.

As can be seen, AMPs have a wide range of effects, and the study of their action mechanism stimulates investigators to the synthesis of AMPs with new properties and guides in the production of synthetic and semi-synthetic peptides with protective functions. It is very likely that in the future, AMPs will displace the bulk of the chemicals, albeit not completely in medicine. This is due to the high activity & low toxicity of AMPs to the human body.

11. Basic Principles of AMP Synthesis

For the synthesis of AMPs, chemical, enzymatic, biological pathways and enzymes of microorganisms are used. In the case of biological synthesis, recombinant microorganisms, such as *E. coli* are used as vectors [33]. The microorganism enzymes are used to break down certain proteins and form short peptides [34], after which the properties of these peptides are studied and the appropriate ones are selected and used for treatment. Actually, this method is another type of enzymatic pathway, because when peptide is obtained enzymatically, these enzyme can be obtained from any (usually plant and animal) source. For chemical synthesis, solid-phase synthesis and/or liquid-phase synthesis are used [35]. AMPs are also added to food products [36], and in this case their toxic effect, hemolytic activity and degree of proteolysis, broken down by enzymes of the organism, are taken into account; the cation charge, hydrophobicity, amphiphilicity of AMPs are also of great importance. The efficacy of AMP depends on their interaction with the negatively charged cell membrane. Alkaline arginine and lysine amino acid residues create initial interaction with the membrane, facilitating the binding and entry of AMP into the negatively charged membrane. In addition, these positively charged amino acid residues can also participate in the formation of covalent and non-covalent bonds with the residues of valine, tryptophan, leucine, alanine, tyrosine and phenylalanine located in the membrane [37]. Due to the fact that arginine has a higher positive charge than lysine, the placement of arginine in the peptide during the synthesis of synthetic AMPs leads to more pronounced antimicrobial properties. However, the disadvantage of placing arginine in the peptide is that it also causes a significant increase in hemolytic activity. Arginine side chain may also interact with microorganisms living in the hydrophobic layer of the membrane. Thus, Grassi *et al.*, (2017) found that the inclusion of arginine residues into temporin-Shf analogues increases their activity against the fungus *Saccharomyces cerevisiae* [38]. Thus, the placement of arginine in the newly synthesized AMP can be considered as a factor determining the degree of activity of this peptide. To limit the hemolytic ef-

fect of arginine, it is recommended to place it and lysine in the hydrophobic site of the α-helix. The placement of arginine in this site also affects the amphiphilicity of the peptide [39]. Arginine and lysine is better to be replaced by histidine, as the latter has a relatively low isoelectric point (close to neutral); in an acidic medium this amino acid can gain the additional positive charge required to bind to the membrane. The placement of this amino acid into the AMP is also beneficial from the point of view that arginine and lysine have a negative effect on the amphiphilicity of the peptide, because they are charged and limit the hydrophobic site of the AMP. From this point of view, the use of histidine in the synthesis of synthetic AMPs is more appropriate, than arginine and lysine [40]. In the synthesis of artifical AMPs hydrophobic amino acids are also required, since they promote AMP binding to the membranes of microorganisms. These include alanine, valine, glycine, etc. amino acids; they also reduce the overall charge of AMP. As the hydrophobicity of the peptide increases, their membrane-destructive and cytotoxic effects reduce [41]. Thus, although there is a need to increase its hydrophobicity during the synthesis of synthetic AMP, this can only be done in moderation. Thus, it is advisable to increase or decrease the hydrophobicity of AMP only in a certain range, because as soon as you go beyond this range, the antimicrobial activity of AMP decreases rapidly [42]. Note that AMPs with high hydrophobicity immediately enter the membranes of mammals and cause hemolysis there. This property of AMP was proved by Zhao *et al.* by showing a change in the hydrophobicity of *Helicobacter pylori* by replacing a single amino acid in the HPRP-A1 peptide [43]; HPRP-A1 is an amphiphilic α-chain of anti-cancer activity derived from the N-terminus of the ribosomal L1 (RpL1) protein of *Helicobacter pylori* [44]. The increase in hemolytic activity in AMP with increasing hydrophobicity is explained by the fact, that such peptides can easily interact with the lipid bilayer. Thus, in order to reduce such toxic effects on mammalian cells, it is very important to maintain the balance of hydrophobic and hydrophylic radicals in the synthesized AMPs. To obtain amphiphilicity, both hydrophobic and hydrophilic radicals must be present in the AMP. Hydrophilic positively charged amino acid groups allow AMP to enter the phospholipid bilayer of the membrane, while hydrophobic sites facilitate AMP penetration into the microbial membrane in the second stage of AMP exposure (Sato et Feix, 2006). Hydrophilic domains in the membrane are usually located in the surface, allowing it to interact with intracellular and extracellular fluids, and alpha helical structure AMPs are one of the most suitable peptide types with high efficacy. For example, alpha-purotionium derived from wheat and Ah-AMP1 derived from horse chestnut, the magainins, cecropins, LL-37, temporins, fowlicidins, melittin are in alpha-helix form, while defensins (type A), plectasin, lactoferricin, tachyplesin, thanatin are in β-sheet form [1] [29] [45].

Drin *et al.* (2009) hypothesized that the AMP helix is sensitive to the torsion of peptides located in the membrane [46]; since along with the increase in hy-

drophobic motifs, there is an increase in helical configuration of AMPs, they state that the inclusion of non-polar amino acids in the newly synthesized AMPs will also increase its membrane-damaging, destabilizing properties. Saint Jean *et al.* (2018) also showed that the replacement of polar amino acid groups with hydrophobic amino acid residues increases the affinity against erythrocytes in the newly formed AMP, and consequently hemolysis [47].

Hollman *et al.* found, that the replacement of two lysine residues in AMP with tyrosine and leucine increased its toxicity to membranes as a result of the high amphiphilicity of the newly formed hydrophobic domain [48]. Zhang *et al.* (2016) conducted the exact opposite experiment. That is, he made in his laboratory changes in AMP composition that dramatically reduced the amphiphilicity of cationic proteins [49]. As a result, the hemoitic effect of newly acquired AMPs decreased. However, it should be noted that simultaneously, the inhibitory effect on microorganisms of this artifically obtained peptide was also reduced by 2 times.

Since AMPs are an integral part of innate immunity [50], and antibiotic resistance is growing every year [51], these data can help in the search for new AMPs and save the lives of many thousands of patients.

12. Conclusion

By altering the amphiphilicity and hydrophobicity of AMPs during synthesis, many different active peptides with low hemolytic properties can be obtained, that may be used in medicine to treat various pathologies caused by microorganisms and parasites. With optimization of AMP stability, formulation and delivery ways, artificial AMPs may become very effective drugs.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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