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Mini review

Antimicrobial peptides: Current perspectives and future promises

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ABSTRACT

The large increase in antimicrobial resistance is demanding effective treatment against infection as the effectiveness of conventional antibiotics is decreasing due to the global emergence of multi-drug-resistant (MDR) bacterial pathogens. The development of novel and alternative therapeutic agents is required to control and reduce the effects of these pathogens. Antimicrobial peptides (AMPs) are potent agents with broad-spectrum activity against many Gram-positive and Gram-negative bacteria. This review mainly focuses on antimicrobial peptide diversity and the future scope for AMPs. The review also provides insight on approaches to overcome the current commercial limitations of AMPs.

Introduction

Antibiotics are being used as an anti-infective solution for patients in the treatment of life-threatening infections. The use of antibiotics has become more difficult due to pathogen resistance. The magnitude of bacterial resistance is likely to increase due to similarities in their activity spectrum and mode of action. This problem is expected to threaten global public health, and therefore, alternative therapeutic strategies are needed to overcome these challenges. Antimicrobial peptides (AMPs) have been discovered in insects, mammals, reptiles, and plants to protect against microbial infection [1-3]. They are found to be active as an antimicrobial and immunomodulatory agent [4]. The antimicrobial peptide was isolated from the skin of the African clawed frog, *Xenopus laevis*, named as "magazine" [5]. Researchers subsequently isolated the AMPs from roots, seeds, flowers, stems, and leaves from a wide variety of plant species [6]. Plants produce these AMPs in high numbers to defend against the infectious agents. AMPs are produced in abundance by human skin and prevent the colonisation of host tissues by pathogens. A substantial amount of progress has been made on the development of AMPs for the treatment of skin infections. The bacteria of the commensal flora, as well as probiotic bacteria, are also reported to induce AMPs [7, 8].

Diversity in sources of antimicrobial peptides:

The use of antibiotics as a magic bullet against various life-threatening infections has had a profound impact since the development of the first antibiotic, penicillin [9-11]. Since then, more attention has been paid to new antibiotic development and commercialization. Until the 1960s, natural AMPs were not considered an alternative to antibiotics. The rise of multidrug-resistant microbial pathogens sparked interest in AMPs. Antimicrobial peptides are produced by bacteria, plants, vertebrates, and invertebrates. Nisin was one of the first AMPs to be isolated and characterised from *Lactococcus lactis in 1947 [12*].

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It was found to be active against gram-positive bacteria and used as a food preservative [13]. In 1963, Zeya and Spitznagel described a group of basic proteins in leukocyte lysosomes endowed with antibacterial activity [14]. Higher plants also produce a broad range of AMPs, and they are generally rich in cysteine residues, which form multiple disulfides. They generally act as the first line of defence against various pathogenic bacteria, parasites, and fungi in plants. In addition to medical use, AMPs can also be employed for plant protection in agriculture and might help in the development of high-quality agricultural products [15]. An interesting anticancer activity was shown by some peptides from wild bee venom. And amphibian skin glands are also found to be a rich source of AMPs, with more than 500 AMPs reported [16,17].

In ancient times, insects were used in the treatment of a number of different ailments. The first insect AMP (cecropin) was extracted from the pupae of *H. cecropia* in 1980 [18, 19]. The AMPs derived from different insects exhibit different activities. The cecropin A derived from the silk moth (*H. cecropia*) showed only antibacterial activity, whereas the cecropin A from the mosquito *Anopheles gambiae* showed both antibacterial and antifungal activities. Probiotics are used to suppress infections due to antibiotic-resistant pathogens. Probiotics have been found to be safe and prevent pathogen colonization. They have the ability to produce antimicrobial agents. Probiotic microorganisms have been used in the production of fermented dairy products [20, 21]. The marine organisms and microorganisms were found to be rich sources of pharmacologically active molecules. The marine bioactive compounds with antimicrobial activity were first identified in a fish. Epinecidin, a 21-amino acid long peptide, was isolated from the fish species *Epinephelus coioides* and found active against gram-negative bacteria, including *P. aeruginosa*, *Vibrio vulnificus*, and *Riemerella anatipestifer* [22].

Antimicrobial peptides' future as modern therapeutics

Natural and synthetic peptides can act as a promising antibacterial agent, and there are several ways in which we can improve their credibility as a drug. The challenge of designing an agent with excellent pharmacologic properties that kills disease-causing microorganisms without harming the host is one that needs to be overcome. The AMPs produced by the human body can act as a template for designing broad-spectrum antimicrobial agents, as AMPs isolated from humans could be clinically safe. The bacteria of the commensal flora and probiotic bacteria are known for AMP production [23,24]. Identification of such AMPs and their overexpression in the skin could effectively protect the host from infectious agents without inducing inflammatory reactions.

The cationic nature of AMPs can prevent bacterial endotoxin shock [25, 26]. The endotoxin is released after bacteriolysis and triggers the release of pro-inflammatory cytokines at uncontrollable levels, which can result in endotoxic shock. The design of strong cationic peptides to neutralise the effect of endotoxins can expand the AMP horizon in the medical field. Another approach where bacterial colonisation can be targeted along with bacterial survival is with AMPs designed to selectively target regulatory pathways such as replication and protein synthesis that may be useful to block the proliferation of drug-resistant bacteria. Some enzymes involved in the synthesis of products that are not targeted by antibiotics in current use, such as isoprenoids [27] or those involved in ATP generation [28], may be future targets of AMPs. Another promising direction for antimicrobial peptides is to make their action more specific by grafting them onto a carrier molecule, such as an antibody, which may selectively target pathogenic organisms.

Strategic approaches to overcome the current commercial limitations of AMPs

The diversity in application of AMPs likely to increase their demand globally. The synthesis rates of AMPs that occur naturally are quite low and may not satisfy the demand in the future. An important limitation in natural production is that many interesting products are not expressed by the organism, and their genes remain in a "silent" state until a change in the environment promotes their transcription. To produce peptides and proteins in high quantity, a gene encoding the target peptide of interest is cloned into a specific expression vector. The choice of promoter has a profound impact on yield. The T7 promoter is mostly used and considered a strong promoter when *E. coli*, particularly strain BL2l (DE3), is used as a host. It is extremely popular for recombinant protein expression, as the target protein can represent 50%

of the total cell protein in successful cases [29]. Several strategies with innovative approaches have been developed during the last two decades for *E. coli*. It is characterised by rapid growth at low cost, with a cell doubling time of approximately 20 min [30], higher yields, established expression protocols, and cost-effectiveness.

Moreover, target AMPs is expressed as a fusion protein i.e. combination with a carrier protein and later cleaved from the same [31]. This is primarily to avoid the toxicity of the AMP to the host strain. These fusion partners are used to improve protein solubility and purification efficiency. Common fusion partners that have been used to express AMPs include thioredoxin, the Small Ubiquitin-like Modifier (SUMO), glutathione S-transferase (GST), and the Biotin Carboxyl Carrier Protein (BCCP). Thioredoxin (Trx) is well known for its high expression of peptides in soluble form and is the most preferred. For high-yield production of small peptides, coding sequences can be cloned as tandem repeats interspersed with single methionine residues. These peptides can then be cleaved with CNBr to release monomeric peptide units. Optimizing recombinant protein expression can also be carried out at the level of the composition of the culture media and related additives used.

Chemical synthesis provides custom-made peptides in small quantities, but production approaches based on transgenic organisms might be more cost-effective for large-scale peptide production. The transgenic expression of antimicrobial peptides in plants is emerging as one of the most promising platforms for the cost-effective production of therapeutics. The tobacco plant (*Nicotiana tabacum*) has been most popularly used as a transgenic expression system. An important advantage of using tobacco as a plant production system is the high volume of biomass that can be produced with only a few processing steps.

Conclusion:

Antimicrobial peptides offer several advantages as compared to current antibiotic drugs, as they represent a naturally occurring defence mechanism. Vast diversity in natural and synthetic AMPs provides an opportunity to develop improved antimicrobial therapies. Their potential role as immune-modulators and adjuvants to antimicrobial therapy suggests that they are promising agents in the field of antimicrobial chemotherapy; therefore, further research is needed to address the growing burden of drug-resistant infections.

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