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Recombinant Antimicrobial Peptides - Concepts and Applications

*Komal Ghauri[1], Muhammad Afzal Ghauri[2] and Amer Jamil[3]**

[1]Biotechnology of Prebiotics and Antimicrobials Lab, National Institute for Biotechnology and Genetic, Pakistan

[2]Industrial Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Pakistan

[3]Department of Biochemistry, University of Agriculture, Pakistan

Abstract

Antimicrobial Peptides (AMPs) are cationic, amphipathic, which are effective against a number of pathogens and hold a novel place in pharmaceutical field. Mode of action includes the attack on pathogen's membrane that increases permeability of the membrane resulting in uncontrolled ion-transport and finally the membrane is ruptured. The expression systems mainly used are of *E. coli* and *Pichia pastoris*. Codon preference is also considered while expressing protein. Some examples of AMPs include Hepcidin, Magainin, ORBK, Fowlicidin, Porcine protegrin-1, AMP-MP1102, Plectasin, NZ17074, Dybowskin-2 CAMA, Enterocin L50, SRCAM 602, OR-7 E-760 and L-1077. Among these Hepcidin, Magainin, Fowlicidin and Porcine protegrin-1 are commercially available. The increasing trend of drug resistant against bacterial pathogens has paved the path for the development of new antibiotic backbones. In the present scenario, PepSAVI-MS pipeline has been developed for bioactive peptides. This platform uses mass spectrometry and statistical approaches to identify bioactive peptides from complicated biological samples. The antimicrobial peptides have a great potential in drug discovery and commercial applications.

Keywords: AMPs (Antimicrobial Peptides); Mode of action; Applications; History

Introduction

The Antimicrobial Peptides (AMPs) are cationic, amphipathic peptides having less than 50 amino acids with many arginine and lysine residues exhibiting inhibitory concentrations as minimum as 0.25 to 41 g/mL [1]. AMPs are of great importance as they possess potential usage as novel antibiotics. In comparison with extraction from natural and chemical sources, recombinant AMPs provides cost-effective and suitable methodology for large scale production. Various systems are in vogue for heterologous production of proteins, *Escherichia coli* being used most commonly. The AMPs that are produced in *E. coli* are usually expressed as fused proteins to suppress harmful effects of the peptides in host. It also prevents host machinery from proteolytic degradation [2]. Many AMPs have been discovered so far, each having specific characteristics and differences from each other on different bases such as vector used and various functions in living organisms (Table 1). The AMPs are in fact host defense peptides, and are part of the innate immune response. They work against external pathogenic infections and are found among all varying classes of life [3]. Mode of action of AMPs is usually their attack on membrane of pathogens as shown in Figure 1.

Concept and History of AMPs

When World War I was going on, that time is regarded as the phase when latest drugs came into picture *via* foundation laid down by compounds like the opiate morphine and cyclic penicillin. These agents gave a new vision and a new set up against cure of various ailments prevailing among mankind ecosystem. It is a fact that protein-based agents i.e. peptides have successfully secured their position as the first ones among discoveries done in the field of therapeutics, minute sized species got more importance in the medicine-based sector, due to easy methods of their preparation, easy to take them (usually orally) and very good pharmacodynamics features. On the other hand, due to rapid decomposition of peptides by enzymes in living systems and more crucial ways of their ingestion led to more ignorance towards this class in the main drug production process. Presently, the medicine-based sector is going through immense changes. Highly secure rules, long procedures for drug making and extreme economic struggles show an apprehension that, in spite of enhancing assets of research, originality in medicinal area is on the way out. Particularly the

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Correspondence:
Amer Jamil, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan, E-mail: amerjamil@yahoo.com

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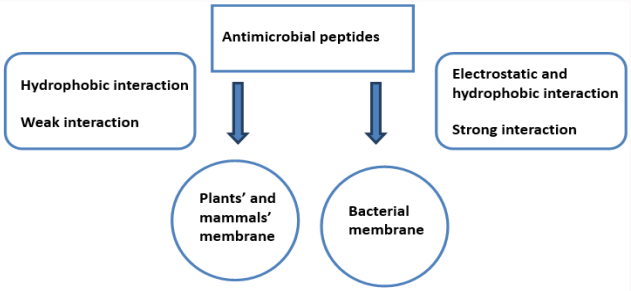

Figure 1: Events taking place in plasma membrane by action of AMPs.

last century has gone through a major exemplar swing in capacity of the medicinal industry, paying more focus on unexplored drugs and lessening fabrication costs, as to tolerate the high operating cost linked with medicine expansion. Vlieghe et al. stated that the zonal expectation is partly hidden in the peptides. About forty years ago, average frequency of the peptides making way into clinical trials was just one per year. There are about 60 approved peptidyl drugs that had generated approximately US$ 13 billion by the end of 2010. Although it represents only 1.5% of all drug product sales the numbers increased with annual growth rate of 7.5% and 10%. There are about 140 peptides in our clinical usage and about another 500 to 600 in pre-clinical development (By Rodney Lax).

Antimicrobial Peptides in Market

Drug manufacturing pipelines have been ruled by minute molecular agents across time span of first century of industrial sector. In order to reach the stage of gaining authenticity from market for commercial use the new drug-based products have to cope-up with many hurdles and difficulties like efficacy and admissibility. Due to these tight regulations for drug approval almost 90% of drug-based agents have to face failure during identification proceedings and before reaching market for commercial use [4].

Antimicrobial Peptides- Defense Providers

Antimicrobial peptides are the first line of defense, belonging to a class of immune-related peptides, used by many living organisms against many potent pathogens. As described earlier one of their mode of action is killing of bacteria by adhering to their respective membranes. They principally stop the synthesis of macromolecules which thus prevent large damage to the membrane. Although their exact mode of action is still not clear, it is possible to synthesize such compounds that can mimic the classical and stable AMPs. They have extensive broad-spectrum activities. From quantitative studies related to the permeability of membrane using electron microscopy it has been found that both AMPs and ampetoids are involved in the aggregation of biomacromolecules causing bacterial death. They are fast killers in a way that they aggregate bacterial ribosomes.

Antimicrobial Peptides in Lung Donors

Due to extensive use of alcohol the users are more prone to lung disorders. During lung transplantation allografts from alcohol user donors are more prone to develop graft dysfunction in the receivers. A study on effect of alcohol on the lungs of organ donors' immune response in the lungs of donors revealed that several antimicrobial peptides and cytokines were produced in the lungs as part of innate immune response. AMPs and cytokines were recorded in various groups including that of drinkers, non-drinkers and excessive alcohol users and they found in case of cytokines that only IL1-β ($p=0.046$) showed a 3.7 fold increase in donors who were recent drinkers and had an infection compared to donors who were recent drinkers with no infection, while in case of AMPs it was recorded that cathelicidin, hCAP18/LL-37, concentration was increased up to 2 fold in recent alcohol users with an infection in comparison to the non-drinkers with an infection ($p=0.0229$). Whereas when measures of β defensin-2 were recorded it was found that they showed a non-significant trend that decreased in excessive alcohol users both with and without infection.

Mechanism of Action

The AMPs show their activity by disruption and permeabilization of the target Plasma Membrane (PM), although exact mechanism of action is not yet fully known. Major events (Figure 1) include stoichiometric accumulation of AMPs on outer side of the PM by their binding to Phospholipids (PL), and once a gradient is produced, permeability of the plasma membrane is increased by formation of pores which are composed of mainly peptides or mixed peptide-phospholipid pores, resulting in destruction of membrane structure [5]. Amphipathic peptides usually adopt alignments in parallel direction to the bilayer surface in this way polar and non-polar side chains of helices face the environmental changes at the top surface.

Table 1: AMPs with respect to different vectors used, their identification methods and activity.

Name of AMP	Expression system	Vector used	Identification of peptide	Activity of peptide	Reference
Hepcidin	*Pichia pastoris*	pPIC9K-H	N-terminal protein sequencer	Treatment for iron homeostasis disorders	Rashid et al. [17]
Magainin	*E. coli*	pET-21a	Metal ion chromatography	Therapeutic applications	Ramos et al. [1]
ORBK	*E. coli*	pUC57-ORBK	His tag	Active against *E. coli* and *Staphylococcus aureus*	Li et al. [3]
Fowlicidin	*E. coli*	pET-21a	Tricine SDS PAGE	Antibacterial efficacy and lipopolysacchide neutralizing activity	Feng et al. [8]
Porcine protegrin-1	*Pichia pastoris*	Puc57-PG-1	6 his-tag	Anticancer activity	Niu et al. [9]
AMP-MP1102	*Pichia pastoris*	GAPMP1102	By MALDI-TOF MS	Active against *S.pneumoniaand S.aureus*	Mao et al. [18]
Plectasin from fungus	*E. coli*	pGEX-4T-1	BCA method	Active against *Enterococcus faecium*	Chen et al. [10]
NZ17074	*Pichia pastoris*	pPICZαA	SDS PAGE	High antimicrobial activity and low hemolytic activity against human red blood cells	Wang et al. [19]
Dybowskin-2 CAMA	*Pichia pastoris*	pPICZαA	Purity by SDS PAGE and molecular weight by MALDI TOF MS	Inhibit growth of a wide spectrum of bacterial agents while showing low level of hemolytic activity	Jin et al. [20]
Enterocin L50	*Pichia pastoris*	pPICZαA	Gel overlay assay	Target important pathogens of food spoilage bacteria	Basanta et al. [21]
SRCAM 602OR-7, E-760, L-1077	*Pichia pastoris*	Derivatives of plasmid pPICZαA	Mass spectrometry	Natural food preservatives	Arbula et al. [22]

This distribution has been found to show considerable disruptions in packing of lipid organization, transient openings at lower level of peptide concentration and when peptide to lipid ratio is at higher top membrane disintegration was observed.

Use of Fusion Partners in Synthesis of AMPs

In recent studies fusion strategy is used for expressing active peptides. A heat-stable and low molecular weight soluble protein like thioredoxin is expressed in the prokaryotic cytoplasm, shows chaperone-like activity. These fusion proteins reduce the lethal effects of antimicrobial peptides to the host cells and protect them from proteolytic destruction [6].

Applications of AMPs in Cecropin

Antimicrobial peptides may be applied in Cecropins which are a group of small basic polypeptides usually found in the hemolymph of insects, consisting of 31 to 39 amino acid residues and have a very broad spectrum, have high heat stability and possess bacteriostatic activity. Artificial synthesis of the cecropin gene is noted by a low efficiency and have high cost. For manufacturing large quantities of active peptides, expression of recombinant peptides can be used for manufacturing large quantities of active peptides *via* cost-effective way [6].

Cecropin XJ

It belongs to cecropin family, which was first isolated from the larvae of the Xinjiang silkworm (*Bombyx mori*) by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The whole amino acid sequence of the molecule was found out. It was successfully expressed in expression system of *Pichia pastoris*, however the levels were relatively low. *Cecropin XJ* shows the advantages of high expression rates, easy build-up and cost-effective growth medium. A vector obtained from the commercial pET32a (+) was developed. The thioredoxin gene was present in expression vector and T7 promoter contained a 6xHis-tag to ease the purification step and increase the expression levels of fusion proteins in *E. coli*. A high level of soluble recombinant *Cecropin XJ* was obtained and purified [6]. The recombinant *Cecropin XJ* displayed strong antimicrobial activity towards bacteria and fungi, as well as cytotoxicity to several types of human cancer cells.

Cecropin A

It is one of the natural antimicrobial peptides that show fast, potent and long-lasting lytic activity against a wide spectrum of pathogens. Transgenic rice plants with a codon-optimized synthetic cecropin A gene obtained from an endosperm-specific promoter. The transgenic rice plants showed stable transgene integration and inheritance [7].

Accumulation of Cecropin A

It accumulates in protein storage bodies in the endosperm of rice, particularly in type II protein bodies, supporting the fact that the glutelin N-terminal signal peptides play an important role in directing the cecropin A to organelle, independently of being tagged with the KDEL endoplasmic reticulum retention signal [7].

Production in Seeds

The production of cecropin A in transgenic rice seeds did not affect the seed viability or growth of seedling [7]. Furthermore, transgenic cecropin A seeds showed high resistant to infection by fungal and bacterial pathogens showing that the plant-produced cecropin A is biologically very active.

Expression Systems for AMPs

There are a variety of expression systems available for the mass production of AMPs. In contrast to synthesis from natural sources or chemical production, recombinant technology is cost effective method for mass production of AMPs [5]. Isolation of AMPs from the host tissues such as skin of frog and insect lymph fluid need cumbersome procedures like fractionation and purification [5]. It has been found that the recombinant antimicrobials peptides possess high antibacterial activity against the Gram-positive and Gram-negative bacteria and even drug-resistant strains [8]. The available expression systems for manufacturing of AMPs utilize *E. coli*, *Pichia pastoris* and *Saccharomyces cerevisae* as hosts [2].

Different Types of AMPs and their Unique Characteristics

Hepcidin

Hepcidin is a cysteine rich cationic peptide, which is synthesized in hepatocytes and is a key regulator of iron absorption in intestine. Human urine contains two dominant types of hepcidin comprising 20 and 25 amino acids respectively differing only by N-terminal truncation. Close hepcidin homologues have been identified in vertebrates ranging from fish to mammals, hepcidin is not related to any other previously known peptides family investigated the existence and cellular localization of hepcidin in the liver. Suggested that hepcidin may be medically applied in the treatment of various iron homeostasis disorders.

Magainin-2

Magainin-2 is a polycationic antimicrobial peptide, which is reported to be isolated from the skin of the African clawed frog *Xenopus laevis* [1]. It acts by permeabilization of membranes, either in a detergent-like manner or by making well-structured pathway channels. It causes the fast destruction of both hematopoietic and solid tumor cell lines at concentrations 5- to 10-fold. Expression vector for Magainin-2 was constructed with MAG2 at the N-terminal (MAG2-LK-CBM3) of the pET-21a vector [1].

ORBK

ORBK is a cyclic cationic peptide, which was cloned into pET28a vector with Maltose-Binding Protein (MBP) as a fusion partner and an N-terminal 6-HISAS an affinity tag for easy identification using *E. coli* BL21 (DE3) as expression host. It has higher absorption efficiency in comparison to other antimicrobial peptides [3].

Chicken AMP Fowlicidin

Fowlicidin is a member of Cathelicidin family, which was identified in chicken. It possesses high antibacterial activity and Lipopolysaccharide (LPS) neutralizing ability. It was successfully manufactured by *Escherichia coli* recombinant expression system. High antibacterial activity against the Gram's-positive and Gram's-negative bacteria, and even drug-resistant strains [8].

Porcine Protegrin-1

Porcine protegrin-1 is a β-hairpin antimicrobial peptide, which is amongst the shortest AMPs in sequence length and was isolated from

porcine leukocytes. It interacts with the membranes of pathogens and kills the pathogen by releasing its cellular contents thus causing destruction of pathogenic membrane [9].

Plectasin

Plectasin was isolated from *Pseudoplectania nigrella*. It binds to the pyrophosphate moiety of lipid II and thus prevent the formation of the bacterial cell wall, which alternatively inhibits the colonization of pathogens**.** NZ2114 is a novel variant of plectasin that has significantly more potent activities than its parental peptide plectasin [10].

Engineered Synthetic Cationic Antimicrobial Peptides

The increase incidence of antibiotic resistance over the last decade has emerged as one of the major global concern, this concern has paved the path for the discovery of novel synthetic antimicrobial peptides as an alternative. AMPs have very low chance of developing resistance in microbial agents. With the advancing era protein epitope mimetics have provided useful and very strong tools to study biomolecular recognition in many areas of chemical biology [11]. Using latest approach, a new mesocellular silica have been developed successfully on the surface of activated carbon and for enhancement of surface and porous area activated carbon was removed by process of calcination and the resulting products were made to be functional by polymixin B as an antimicrobial agent [12]. A study was carried out keeping in consideration the untreatable problem of infections caused by burns due to *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, the antimicrobial effects of CM11 peptide and 1% silver-doped bioactive glass against drug-resistant strains of the said microbes isolated from effected patients. It was found that the isolated bacterial species from patients were resistant to almost all commonly used antibiotics and silver treatment [13].

The 'PepSAVI-MS' Pipeline for Natural Product Bioactive Peptide Discovery

The new platform available today named PepSAVI-MS was developed to investigate bioactive peptides from plants using mass spectrometry and statistical approaches. The latest example of discovery of a bioactive peptide using this approach is from a botanical species *Viola odorata*. The cyclotides obtained through this botanical species has antimicrobial activity against *A. baumannii* [14].

Plants as Promising BioFactory Systems

Plants are excellent systems for producing AMPs as they are economical to grow (ref), easily scalable (ref) and generally considered as safe because of the low risk of contamination with human and animal pathogens (ref). Plants have successfully been used for the synthesis of different proteins for therapeutic and technological uses. Plants are one of the most promising bio-factory for commercial scale production of recombinant proteins due to low cost, scalability and safety. Influenza has been combated now with the help of recombinant vaccines based on two targets, the major surface hemagglutinin and the transmembrane protein M2 [15]. One of the most optimal platforms for massive production of oral vaccines is the system of plants. Tobacco based vaccines expressed either by nuclear or plastid system represents the best examples of potent synthetic polycistrons and viral sequences to modify plant cells as a potential source of biofactories to manufacture multicomponent vaccines in a single transformed line [16].

Future Perspectives

In near future, some very authentic peptide molecules are now in final stages of clinical trials and are very close to commercialization. Out of these almost half are prepared for cancer related studies as well as for heart related ailments. From safety point of view, the protein-based molecules-peptides have low levels of toxicity. From this it can be deduced that drug based industrial sector is becoming more stable and harmonize with advancing years as new agents are gaining the acceptance status more rapidly. Many drug-based sectors have attained the level of stability and proper maintenance in the market. The new protocols so far discovered have capacity to formulate new protein-based agents for use in numerous ways. From these highlights it can be predicted that a bright future for peptides is ahead.

The need of the hour is to develop more of such peptides which are highly cost-effective, easy to manufacture, highly stable, non-toxic, useful against a number of diseases and ease us in fighting against numerous diseases. Focus should be laid on more commercialized ventures of already designed AMPs. It is necessary that modification of existing AMPs is done for enhancement of their efficacy and effectiveness. Positive development should be made possible for designing of novel peptides for various applications in different phases. Use of economical resources should be made possible so that they pave the path for the manufacturing of more effective and more advantageous AMPs in an economical and commercial ways.

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