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Properties / Characteristics of Antimicrobial Peptides

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ABSTRACT

Antimicrobial peptides (AMPs) are natural antibiotics, synthesized by ribosomes, with an effect on the natural immunity of animal organisms. AMPs differ in composition [sequence and type of amino acids (AAs)] and structure, which contribute to rapid lysis and have a varied spectrum of antimicrobial activity. AMPs are: peptides (Pps) with alpha-helix structure (i.e., cecropins, magainins, mellitin, etc.); cyclic Pps and ring with several cysteine residues (i.e., defensins, protegrins, etc.); Pps rich in one or another AA (i.e., proline arginine-rich PR39, histidine, glycine, etc.). Most AMPs are characterized by hydrophobic and cationic properties, adopt an amphipathic structure (alpha-helix, spiral beta or alpha-helix / spiral beta), which is essential for antimicrobial activity. AMPs have the potential for therapeutic use in medicine. One of the major concerns of life sciences research is finding new ways to enhance the body's defense against pathogens. One way is to produce drugs based on AMPs. The AMPs are a class of small Pps that have the ability to destroy pathogens of microbial and viral origin. The mechanisms of action are known only partially and for a small number of AMPs, and the toxic action of AMPs is generally considered to be based on the induction by these molecules in the outer membrane of the pathogen of aqueous pores that facilitate nonspecific ion transfer, which ultimately leads to lysis of the target cell.

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Background

The human body produces many antimicrobial Pps that help the immune system eliminate infections. Scientists hoping to use these Pps as potential antibiotics have found that other Pps in the human body may have strong antimicrobial effects. AMPs, which are found in almost all living organisms, can kill many microbes, but are usually not strong enough to act as antibiotics on their own. The AMPs are an essential part of the immune system that act against microbial infections. They are found in both plants, insects and higher vertebrates [1].

These natural Pps are basic compounds, with a primary structure of 12-50 AAs, with over 800 such compounds in the existing databases to date. Examples of such AMPs along with their source would be: insect defensin A (fungi), cecropin A (insects), melittin (bee venom), magainin 2 (amphibians), tachyplesin 1 (crabs), α- defensin HNP-1 (humans) [2].

Their AA composition, amphipathicity, cationic electric charge, and their secondary structures allow these Pps to adorb and insert into lipid membranes to form transmembrane pores. Although there have been numerous attempts to elucidate their mechanism of action by various models such as classical transmembrane pores, toroidal pores or membrane destruction, there is still debate about how or how to act on microorganisms *in vivo*.

Numerous studies have described the influence of important structural parameters of Pps: net electrical charge, helicity, intrinsic hydrophobicity, hydrophobic moment, and the size of polar and hydrophobic domains on the membrane permeability effect, antimicrobial and hemolytic effect. Experiments show that these parameters can be a strong basis in optimizing the structure of Pps, correlated with their antimicrobial activity [3,4].

The predominance of a membrane permeabilization mechanism is determined by the cumulative effect of the structural characteristics of the Pp and their influence on the adsorption and permeabilization steps of the membranes [5]. The main characteristics that determine the activity and specificity of antimicrobial Pps are: primary sequence, secondary structure, helicity, hydrophobic moment, hydrophobicity, hydrophobicity angle and electrical charge of the Pp.

General properties / characteristics of antimicrobial peptides

Man has used nature since ancient times in his daily life to solve nutritional problems, or to combat or prevent a number of diseases. AMPs or defense host Pps are of natural origin, with antibiotic properties, usually 12 to 50 AAs, which are part of the innate immune response and are found in all kinds of life forms. These Pps function as broad-spectrum antibiotics, which have great potential for use as new therapeutic agents [6].

The AMPs kill Gram-negative and Gram-positive bacteria (including strains resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), enveloped viruses, fungi, and even transformed or cancerous cells.

Unlike most conventional antibiotics, it appears that AMPs may also have the ability to stimulate immunity to function as immunomodulators [7].

AMPs generally have a low molecular weight (max. 10 kDa), ie a relatively small number of AAs, being the simplest weapons of cellular defense, belonging to the non-specific immune system. Despite research, the biochemical mechanisms underlying the cytotoxic Pp membrane insertion process are incompletely known, but there is interest in finding new therapeutic agents for resistance to many of the antibiotics commonly used in anti-infective therapy [8]. In addition to the antibacterial activity and modulating the immune response of these Pps, studies have shown that some of these cationic Pps have cytotoxic activity on cancer cells and do not act on normal mammalian cells. Most of the available anticancer drugs allow control of tumor growth only at concentrations that also affect healthy cells, resulting in undesirable side effects. Thus, it is imperative to find new products with innovative mechanisms of action and one of the current directions of research is the use of cytototoxic AMPs [9].

The main features underlying the activity and specificity of MPAs are:
Primary sequence - most AMPs contain basic AAs in the primary sequence: lysine (Lys – K) and arginine (Arg – R), hydrophobic AAs: alanine (Ala – A), leucine (Leu – L), phenylalanine (Phe – F) or tryptophan (Trp – W) as well as other AAs such as isoleucine (Ile – I), tyrosine (Tyr – Y) and valine (Val – V); some AMPs may contain repetitive sequences of such AAs; the quantitative ratio between hydrophobic AAs and AAs having a net electric charge can vary from 1: 1 to 2: 1; The number of AAs in the structure of AMPs ranges from 6 AA residues for anionic Pps to 59 AA residues for cationic AMPs [10].

Net electrical charge - anionic AMPs have a negative net electrical charge at neutral pH, mainly due to the content of acidic AAs, aspartic acid (Asp – D) and glutamic acid (Glu – E) and cationic AMPs have an excess of positive net electric charge due to the content of basic AAs: arginine (Arg – R) and lysine (Lys – K); anionic Pps that are complexed with zinc, as well as strongly cationic Pps are often much more active than electrically neutral AMPs or those that have a lower net electrical charge [11].

Secondary structure - most AMPs do not have a well-defined secondary structure in aqueous solutions but at the level of cell membranes they can assume a variety of secondary structures such as α – helix structures (the most widespread in nature), β – sheet or structures cyclical; amphiphatic Pps with α – helix secondary structure are more active than AMPs that do not have a well-defined secondary structure; AMPs, which have a "core" in their secondary structure consisting of parallel β-sheet structures, such as defensin molecules, are often very active [12].

Hydrophobicity - represents the percentage of hydrophobic AA residues existing in the primary sequence of the Pp and in general for AMPs is ~ 50%; this feature is of particular importance in the process of insertion of AMPs into membrane lipid bilayers and the formation of transmembrane pores capable of lysing the cell membrane [13].

Amphiphaticity - is the property of AMPs to have a sequence of hydrophilic AAs and hydrophobic AAs arranged on either side of the α-helix structure; for α – helix Pps, amphiphaticity is often expressed by the hydrophobic moment which is the sum vector of the hydrophobic indices, normally oriented to the helix axis and indicates the orientation of the hydrophobic AAs relative to the α – helix axis; some AMPs rarely show a good delimitation of polar residues compared to hydrophobic ones, which makes hydrophobicity more difficult to quantify [14].

Polar angle – is a measure of the relative proportion of the polar part of a helix structure to the hydrophobic part; for example in a hypothetical situation where one α-helix structure of a Pp is arranged in such a way that one half of the helix in α its axis length is made up of polar AAs and the other half is made up of hydrophobic AAs, the polar angle is 180 degrees; an increase in the number of hydrophobic AAs will reduce the value of the polar angle; studies have shown that the polar angle is correlated with the stability and lifetime of transmembrane pores induced by AMPs; Pps with a low hydrophilic angle and a high average value of hydrophobicity tend to form transmembrane pores, while a Pp with an equal number of hydrophobic and hydrophilic AAs prefers to adopt a parallel orientation on the surface of biomembranes [15].

Because invertebrates do not have an acquired immune system, their defense is based solely on non-specific immune system resources. Considering that this group of organisms has had an evolutionary success, it becomes obvious that these resources of their non-specific immune system are extremely efficient. Studies have been performed on many species of insects, with *Drosophila melanogaster* becoming a true model for the study of innate immunity, leading to the discovery of immune response strategies (e.g., the discovery of pathogen recognition receptors, toll receptors), which are preserved by throughout evolution, to the human level [16].

Many of the Pps with an immunological role were initially discovered in insects, and later proved to be similar to Pps in mammals. In insects, these Pps are found in the hemolymph, in phagocytes, and in some epithelial cells.

They may be constitutively expressed (eg in shrimp, crabs) or may occur in response to an invasion of pathogens (e.g. *Drosophila antifungal* Pps). The most important achievements in this regard are related to plants (infusions, decoctions, tinctures, extracts). But in the last decades in the pharmacological science a new field is asserting more and more sharply, having as main object of study the insects, these constituting about 1.5 million species compared to the 300 000 of the plants. As biologists have found, insects are able to produce an immensity of biologically active substances that can serve as a model for imitation or, more importantly, a new source for obtaining medicinal preparations. We can mention that these intentions have already given results, resulting in the introduction in medical practice of food supplements or entomological drugs with various properties [17].

A wide variety of living things produce AMPs (PAMs) that are part of the body's first line of protection against pathogens. AMPs are low molecular weight, positively charged substances, isolated from single-celled organisms - insects, plants, birds, fish and vertebrates, including the human body. Data on about 1,200 (50% of insects) of such identified Pps prove their importance in the innate immune system. The expression of these Pps can be constitutive and inducible by infectious or inflammatory stimuli such as proinflammatory cytokines, bacteria or bacterial molecules (lipopolysaccharides – LPS), which induce innate immunity. PAMs are able to alter phagocytosis, release prostaglandins, neutralize the septic effects of LPS, contribute to the accumulation of various immune cells in the infectious site, initiate angiogenesis and regeneration, stimulate chemotaxis of monocytes and T cells.

Many of these Pps are prototypes. of medicinal remedies with antibacterial, antifungal, antiviral, immunomodulatory, antitumor, etc. action. [18].

The antimicrobial protection system can be achieved by fast-acting or late-reacting factors that persist permanently or are induced and that have an effector or regulatory action. AMPs are an important component of the immediate reaction protection system and are considered as an alternative to classical antibiotics (of microbial origin) [19].

These Pps are molecules containing about 12–100 AA residues, different in sequence, but which are synthesized in the form of predecessors that undergo changes (release of residues, glycosylation, halogenation) with the formation of active Pps.

The PAMs of different genesis and type have common characteristics:

- are amphipathic molecules that contain hydrophobic (reacts with lipids) and hydrophilic (interacts with water and negatively charged ions)
- are cationic molecules that have positive charges at physiological pH (+ 2 → + 7)
- are hydrophobic (hydrophobic AAs make up over 50%)
- are synthesized by genes that allow targeted action of PAMs (antibiotics of microbial origin are products of the metabolism of microorganisms)
- they have a low molecular weight as opposed to proteins
- they possess antimicrobial activity in low concentrations (μg / mL) [20].

It should be noted that horseshoe crab Pps are among the most potent antibacterial and antifungal substances, with a MIC of 2μg / mL. Polyphemusin also has antiviral activity, including against HIV. Insects make up 55% of the total biodiversity of living things and about 85% of the animal world. Insects are thought to have appeared in the Devonian era, 350 million years ago, and colonize all terrestrial and aquatic niches, all climates from poles to deserts. The healing properties of bees, ants, etc. have been known since ancient times.

In recent years there has been a growing interest in the study of biologically active substances and the immune system of insects. The immune system of insects, in contact with microorganisms, synthesizes and releases a series of molecules of the immune-Pp response with antimicrobial and immunomodulatory action. Deciphering their structure will allow their chemical and biological synthesis, and will later serve as a basis for obtaining new drugs of entomological origin. About 1,200 such Pps of natural origin are described, of which 50% are from insects. They were formed during the evolution and aim at the role of a new generation of antibacterial, antiviral and antifungal preparations [21].

The cellular mechanisms of insect protection are an object of study in immunology. Thus, cytotoxic chemokines (analogs of natural kilers) have been detected in insects, which appear before metamorphosis and destroy the larval tissues. In vitro studies have shown that these chemokines show antitumor action. The ubiquity of insects in all ecological systems stimulates scientific research in order to obtain new therapeutic agents. Among the biologically active Pps / polyPps in insects were characterized by Pps with antimicrobial action, which are fascinating scientific discoveries for use in therapy. In any case, relatively little data have been found on insect molecules with antiviral and antitumor activity. For these reasons, this field of research is an important source of antiviral and antitumor drugs [22].

Currently, biologists have found that insects are able to produce a lot of biologically active substances (Pps, hormones, pheromones, etc.) that can serve as a model of imitation or, more importantly, as a new source for obtaining medicinal preparations. These intentions have already given the first results resulting in the introduction in medical practice of food supplements or entomological drugs with antiviral, immunomodulatory, antibacterial, hepatoprotective, antitumor, etc. properties. In the case of vertebrates, although there is a more advanced immune system with an adaptive component, a Pp component is still maintained as the first line of defense. Due to their direct or indirect antimicrobial defense roles, in vertebrates the immunologically active Pps are found especially in places where contact with pathogens is more frequent: mucosal surfaces, skin, granules of cells of the immune system. The glands in the skin of amphibians have proven to be the richest source of such Pps, so far isolating no less than 500. Among the prototypes, we mention magainins with α-helical structure, which have a strong pore-forming activity on the membranes of gram-positive and gram-negative bacteria, fungi, yeasts and viruses [23].

The relationship between structure and function and the mechanisms of action of magainins have been extensively studied, based on which the first antibacterial therapeutic means based on Pps were developed (but were not clinically successful).

Dermaserpines, Pps isolated from the skin of a South American frog, have a broad spectrum of action and have been studied for other drugs. In addition to the skin, these Pps were also isolated from the stomach lining, indicating a possible role in protection against ingested pathogens. The best characterized Pps in this class are buforin and buforin II, isolated from an Asian frog. These buffers are formed by cleavage of histone 2A [24].

Another group of AMPs found in vertebrates is the cathelicidin group.
They are structurally characterized by a well-preserved N-terminal domain (cathelinic domain) which is proteolytically cleaved to release the C-terminal domain, which is in fact the mature, active Pp. Most cathelicidins are found in circulating cells in an inactive form. The predominant source of cathelicidine is the secretory granules of neutrophils, but can also be found on the surface of the oral mucosa, genitourinary tract, lung and keratinocytes on skin surfaces with inflammatory conditions. Beyond the N-terminal end, the structure of cathelicidins is complex, with α-helix, β-hairpin or proline or arginine-rich regions. The structural diversity of cathelicidins is an indicator of their functional diversity, these Pps having a wide range of antimicrobial and immunomodulatory activities. Cathelicidins have been isolated from many species of mammals, mice, rabbits, horses, sheep, cattle, humans. One of the best characterized cathelicidines is the bovine antimicrobial Pp BMAP-28, an α-helical Pp that rapidly permeabilizes the membranes of a broad spectrum of microorganisms at relatively low concentrations *in vitro* [25].

Bovine Pp rich in proline Bac 5 proves selectivity, preferring to attack only gram-negative bacteria. Only one cathelicidine – LL – 37 (hCAP18) was expressed in humans. Evidence to support its defensive role is to increase the expression of its gene in response to skin infections, as well as the fact that LL-37 deficiency leads to chronic periodontitis. LL-37 also plays immunomodulatory roles through its chemoattractant effect and

modulation of the inflammatory response. A second important group of immunologically active Pps in mammals is defensins. These are cyclic Pps, classified according to the number of disulfide bridges made between the six cysteine residues preserved during evolution (α– and β-defensin and the macrocyclic structure θ-defensin). As with cathelicidins, defensins are also synthesized as inactive precursors, requiring proteolytic cleavage for activation [26].

While α and β-defensins are widespread in the mammalian world, θ-defensins have so far been identified only in neutrophils and monocytes in monkey species. Depending on the species, α and β-defensins are found in the granules of neutrophils, macrophages, NK cells, Paneth cells, some cells on the mucosal surface (genitourinary and respiratory tract).

The expression of defensins may be constitutive, for example hBD – 1 in most tissues, or may be induced, for example hBD – 2, whose expression in monocytes is increased by monocyte exposure to bacterial lipopolysaccharides. *In vitro* studies have shown that defensins generally have poor antimicrobial activity, depending on salt concentration (they are inhibited at 100mM monovalent salt or 2mM divalent salt). However, the concentrations of α-defensin found in the intestinal crypts or in neutrophil granules are sufficient to have a bactericidal effect even in the salinity conditions mentioned above. θ– defensins and hBD – 3 maintain their bactericidal activity in conditions of physiological salinity, in addition they have antiviral activity (anti-HIV) [27].

Experiments were performed with transgenic mice and knockout mice for proteases required to cleave defensin precursors. Thus, in the case of MMP – 7 knockout mice (they lack MMP – 7 protease), they showed a decrease in clearance for E. coli and mortality on contact with *Salmonella enterica* increased substantially. HHD – 5 knockout mice (Paneth cells) showed immunity to *Salmonella enterica* serovar *Typhimurium infestation*. It is important to note that in each experiment a correlation was observed between the antibacterial activity expressed by the Pps in vitro and the activity manifested in vivo. Another important observation is that during infections, the level of defensins in human plasma increases from a normal level of 40 ng / mL to 1μg / mL [38].

Concluding Remarks and Future Perspectives

In recent years, interest in the etiology of neurodegenerative diseases has increased considerably, and one of the hypotheses proposed by studies conducted so far in this direction indicates the disorder of metal homeostasis in the brain as the main cause. To induce an abnormal conformation of the Pps and proteins involved in the body's biological processes. In most cases, improper packaging of Pps and proteins leads to oligomerization, aggregation, and ultimately to the formation of insoluble wounds. A wide range of neurodegenerative diseases are affected by the processes of aggregation of Pps and proteins, although it is not yet known whether the formation of fibrils is the cause of the disease or just a consequence.

Most PAMs have hydrophobic and cationic properties, adopt an amphipathic structure (alpha-helix, spiral beta or alpha-helix / spiral beta), which is essential for antimicrobial activity. For these reasons, WFP has a great potential for therapeutic use in medicine.

High-resolution investigations of the structure of proteins inserted in lipid membranes and recent studies of the role of individual AAs in mediating protein-lipid interactions have shown that polar-aromatic residues, tryptophan (Trp) and tyrosine (Tyr), have a specific affinity for the lipid region, at the membrane interface - aqueous medium.

The membrane-aqueous interface represents a relatively large part of the total thickness of the lipid bilayer and has a complex chemical environment, which offers many possibilities for non-covalent interactions with the side chains of Pps and proteins. This interface has a polarity gradient, from very apolar near the region of the hydrocarbon tails that form the hydrophobic core of the membrane, to very polar near the aqueous phase. Lipid carboxyl groups, phosphatcholine groups, and water molecules around the polar ends of phospholipids offer the possibility of dipole-dipole, cation-π interactions, the formation of hydrogen bonds with the AA side chains of Pp and protein structures.

Scientists believe that by modifying these Pps to enhance antimicrobial activity, they can develop synthetic Pps that could be used as antibiotics against drug-resistant bacteria.

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