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Biologically active natural peptides

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Abstract
This paper presents a bibliographical synthesis of the main research and valorisation directions in the field of some natural peptides of major biological importance. The natural distribution, classification, structure, conformational and functional competences together with the most accessed block schemes of isolation are also mentioned.

Key words: antimicrobial peptides, defence peptides, peptide antibiotics, antifungal peptides, endogenous peptide antibiotics.

1. Introducing the problem

Peptides, organic combinations with polyamidic structure, are formed generically through condensation of a limited, generally defined number of amino acids (AA). Progresses achieved in the last century in the chemistry and biochemistry of biologically active polypeptides have endorsed both their structure and synthesis together with the formulation ***(postulation) of a biological correlation structure –activity.*** The basis for classifying these natural representatives consists of the ***chemical (structural) criterion*** and ***biological activity*** (antibiotics, hormones, enzymes, toxins, etc). The object of the present overview is exclusively and selectively constituted of peptidic antibiotics. Although there are over ***200 polypeptidic antibiotics***, the term *defined structure* refers only to ***75*** and *exhaustive synthesis* only to ***15***, respectively.

If penicillin contains only two fragments of AA, ***gramicidin A*** has ***15*** structural units ***AA***, and saramycetin reaches ***14.000***. A general characteristic of polypeptidic antibiotics is its type of action (***inhibiting microorganisms development***) with high degree of similitude (***Figure 1***).

Action specificity is advanced and any structural modification affects decisively biological activity. Some natural polypeptidic structures contain ***lipidic*** fragments [6-methyl-heptanoic and octanoic acids (polymyxin)] and ***"non-specific" AA*** to plants/animals [β-lysine (capreomycin)] respectively, the former responsible for unicellular architecture liposolubility on the whole, while the latter are responsible for the biological activity.

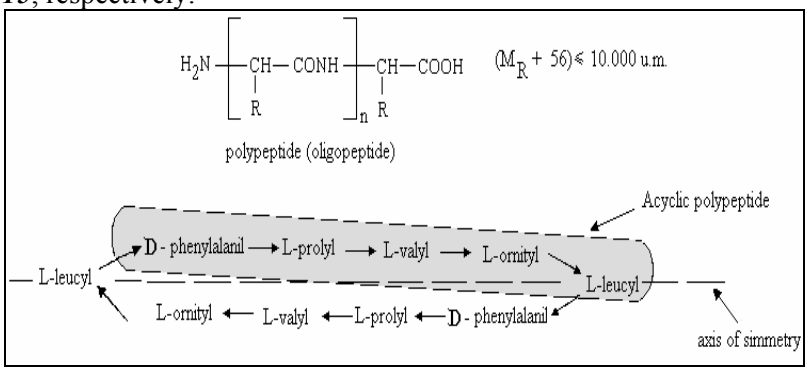

Figure 1. Gramicidin S (cyclopeptide antibiotic) (cyclic decapeptide) [1]

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Physiologically active natural polypeptides (antibiotics) are important ***food preservatives*** [***nisin (E - 234)*** (***34 AA***) (*Streptococcus lactis subsp*.), ***natamycin*** (***E - 235***) (*Streptomyces naturalensis* and *lactis*), ***lysozyme (E - 1105***) (***129 AA***)].

2. State of knowledge.

Chemistry and biological applications of polypeptidic chains (oligopeptidic) as such and/or derivatised lead to macromolecular biocompatible structures intensely studied and popularised in the literature of the last nine decades. The widespread antibiotics after ***1940*** induced the quick development and adaptation of the pathogens resistant to the action of traditional biostructures. Favourable acknowledgement after ***1990*** of the first "***protein conjugates***" by the ***USA Food and Drug Administration*** in the prophylaxis of acute limphoblastic leukaemia consecrated these structures and technologies of isolation, purification, and characterisation. New protein polypeptidic architectures pointed to in literature confirm the increaseing interest in the field.

Nanobiomaterials (which also include successfully biologically active natural peptides) as ***incapsulation and sequestration matrices*** recently ***"rediscovered"*** are bibliographical landmarks that bring forth already known biocompetences not enough exploited. Of major interest are ***antimicrobial peptides (AMPs)*** noted initially in non-specific native mechanisms fighting infections in humans and animals.

Although the first antimicrobial peptide (***nisin***) was discovered in ***1928***, most information concerning their diversity and action mechanism come from studies carried out the last four decades [2].

AMPs can be anionic/cationic structures capable of adopting a conformation in which cation hydrophobic ***AA*** are spatially organized in a ***"winged"*** or ***"amphipatic"*** design influencing both water solubility and capacity of interacting energetically with biological membranes. Relatively small peptidic chains (***12-100 AA***), positively charged (with a net charge between ***+2*** and ***+9***) (***Tabel 1***), amphiphile ***AMPs,*** were isolated from unicellular microorganisms, insects (invertebrates), plants, amphibians, birds, fish, and mammals(including humans)[3,4].

The presence of ***AMPs*** represents a universal characteristic of the defence systems of all living organisms being represented from the level of bacteria and plants to the level of much evolved mammals because they belong to the ***"archaic"*** area of the ***native, non-specific immune system*** – the main defence instrument of the body.

Table 1. Abbreviations, electrical properties and hydropathy of amino acids [5,6]

Amino Acid	*3 letter*	*1 letter*	*Side chain charge (pH 7)*	*Hydropathy index**
Alanine	Ala	A	neutral	1,8
Arginine	Arg	R	positive	-4,5
Asparagine	Asn	N	neutral	-3,5
Aspartic acid	Asp	D	negativ	-3,5
Cysteine	Cys	C	neutral	2,5
Glutamic acid	Glu	E	negative	-3,5
Glutamine	Gln	Q	neutral	-3,5
Glycine	Gly	G	neutral	-0,4
Histidine	His	H	positive	-3,2
Isoleucine	Ile	I	neutral	4,5
Leucine	Leu	L	neutral	3,8
Lysine	Lys	K	pozitiv	-3,9
Methionine	Met	M	neutral	1,9
Phenylalanine	Phe	F	neutral	2,8
Proline	Pro	P	neutral	-1,6
Serine	Ser	S	neutral	-0,8
Threonine	Thr	T	neutral	-0,7
Tryptophan	Trp	W	neutral	-0,9
Tyrosine	Tyr	Y	neutral	-1,3
Valine	Val	V	neutral	4,2

*The hydropathy index of an ***AA*** characterized hydrophobic or hydrophilic properties of its side-chain. The larger the number is, the more hydrophobic the amino acid.

In most cases, their role consists of annihilating invading pathogens but recently they have also confirmed other biological competences immunomodulators of the non-specific defence response and the transfer role to specific immune response to evolved organisms [8-11].

Table 2. Reprezentative antimicrobial peptides [7]

Group	*Peptide*	Amino acid sequence(*1*)	*Origin*
I α-helix	Temporin L	FVQWFSKFLGRIL	*Rana temporaria* (2)
	Temporin B	LLPIVGNLLKSLL-***Am***	*Rana temporaria* (2)
	Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS	*Xenopus laevis* (2)
	SMAP29	RGLRRLGRKIAHGVKKYGPTVLRIIRIAG	sheep myeloid
	LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	humans, leukocytes, epithelia
	Cecropin A	KWKLFKKIEKVGQNIRDGIKAGPAVAVVGQATQIAK-***Am***	*Hyalophora cecropia*
II β-sheet	Protegrin-1	RGGRLC$_1$YC$_2$RRRFC$_1$VC$_2$VGR-***Am***	porcine leukocytes
	Tachyplesin-1	KWC$_1$FRVC$_2$YRGIC$_2$YRRC$_1$R-***Am***	*Tachypleus gigas*
	Polyphemusin-1	RRWC$_1$FRVC$_2$YRGFC$_2$YRKC$_1$R-***Am***	*Limulus polyphemus*
	Androctonin	RSVC$_1$RQIKIC$_2$RRRGGC$_2$YYKC$_1$TNRPY	*Androctonus australis*(3)
	Human β-defensin-1	DHYNC$_1$VSSGGQC$_2$LYSAC$_3$PIFTKIQGTC$_2$YRGKAKC$_1$C$_3$K	several human tissues
III extended structure	Indolicidin	ILPWKWPWWPWRR-***Am***	bovine neutrophils
	Histatin-5	DSHAKRHHGYKRKFHEKHHSHRGY	human saliva
	Bactenecin-5	RERPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLRFP	bovine neutrophils
	PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFP-***Am***	pig intestine
IV loop	Bactenecin-1	RLC$_1$RIVVIRVC$_1$R	bovine neutrophils
	Ranalexin	FLGLIKIVPAMIC$_1$AVTKKC$_1$	*Rana catesbeiana* (2)
	Thanatin	GSKKPVPIIYC$_1$NRRTGKC$_1$QRM	insect hemocytes
	Brevinin 1E	FLPLLAGLAANFLPKIFC$_1$KITRKC$_1$	*Rana esculenta* (2)
	Lactoferricin	FKC$_1$RRWQWRMKKLGAPSITC$_1$VRRAF	cow and human milk

Note: ***Am*** – C-terminal amidate; (*1*) sequence of amino acids (one-letter code) [5] ; index numbers represent the frequency of chain disulfide bridges; (2) - skin; (3) - hemolymph.

3. Classification and structure.

The number of known ***AMPs*** representatives is high. A reasonable classification can be done depending on the secondary structure (***Table 2***) [12,13].

****α-Helical peptides*** (***Figure 2, E***) adopt disordered structures in aqueous solution while fold into an ***α-***helical conformation upon interfering with hydrophobic solvents or lipid surfaces. This peptides are often found to be amphipathic and can either adsorb onto the membrane surface or insert into the membrane. The majority of the helical peptides are cationic, with selective toxicity to microbes. One of the most studied of the cationic, antimicrobial, amphipathic helical peptides is ***magainin*** (***$C_{114}H_{180}N_{30}O_{29}S$***) [7]. There are also hydrophobic, slightly anionic ***α-***helical peptides, witch exhibit less selectivity towards microbes compared with mammalian cells. An example of a hydrophobic, negatively charged cytotoxic peptide is ***alamethicin*** (***$C_{92}H_{150}N_{22}O_{25}$***)[7].

****β-Helical peptides associated with intramolecular disulfide bridges (Figure 2, A)*** are cyclic peptides constrained either by disulfide bridges: ***β-defensin-2*** (human) [14], ***tachyplesins***[15], ***protegrins*** (***Figure 3, A_1, A_2***) [16], or by cyclization of the polipeptide backbone, as in the case of ***gramicidin S*** (***Figure 1; 3, C***)[17], ***polymyxin B*** [19] and ***tyrocidines*** [19].

These peptides present ***β-sheet*** conformation in aqueous solution that may be further stabilized upon interactions with lipid surfaces [7]. Defensins are among the most studied and characterized ***β-sheet*** - forming ***AMPs*** (***Figure 4***).

****Linear peptides with an extended structure***, characterized by overrepresentation of one or more ***AA*** (***Figure 2,F***).

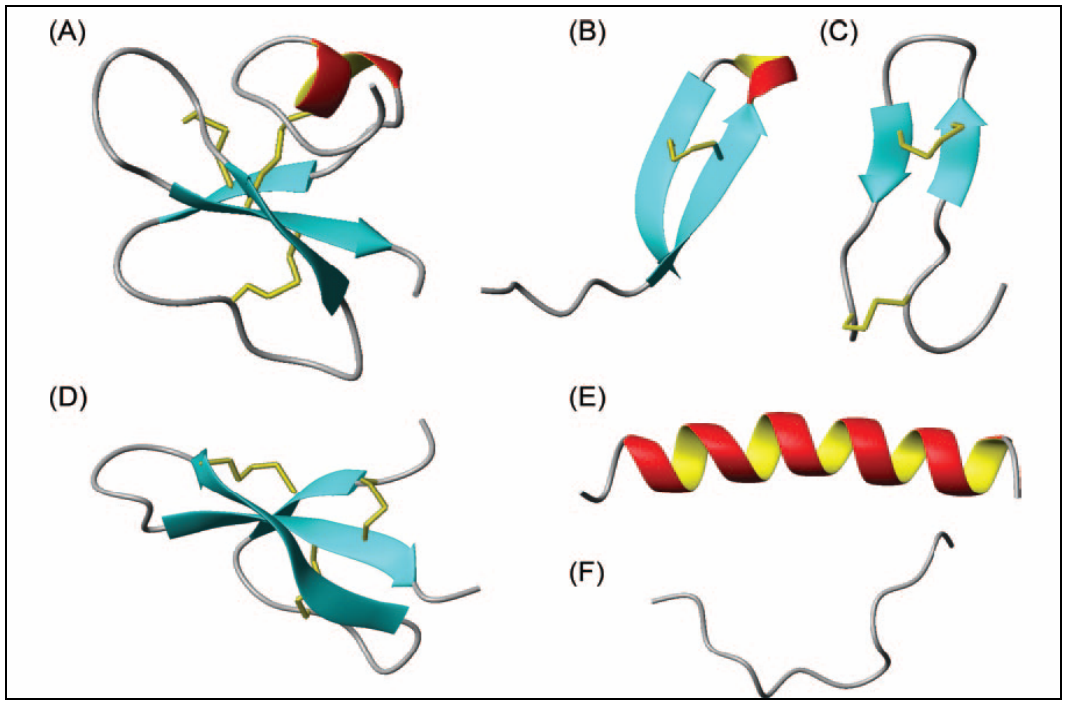

Figure 2. Scheme of the structure of some natural biologically active polypeptidic chains **(A)** β-defensin-2 (human) [20]; **(B)** thanatin [21]; **(C)** β-sheeted polyphemusin [22]; **(D) d**efensin-1 (animal) [23]; **(E)** α-helical magainin-2 [24]; **(F)** extended indolicidin [25] (yellow – the disulfide bridges).

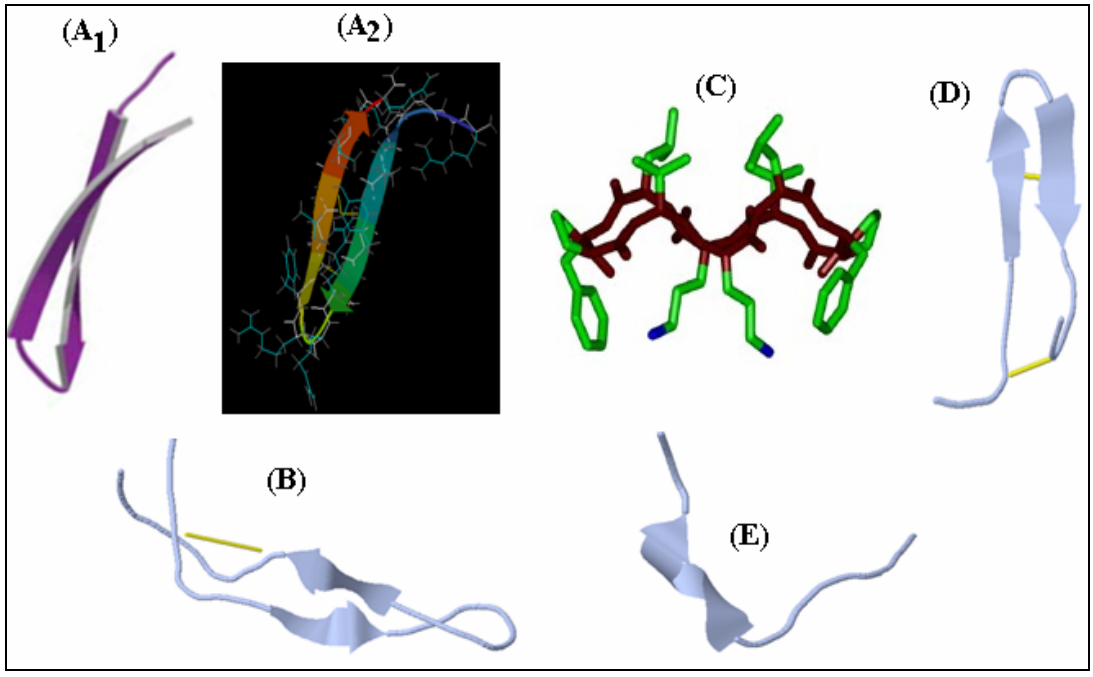

Figure 3. Scheme of the structure of some natural biologically active polypeptidic chains (**A**$_1$) protegrin 1 [26**]**, (**A**$_2$) protegrin 1 [27]; (**B**) lactoferricin [28]*;* (**C**) gramicidin S [29]; (**D**) tachyplesin 1 [30]; (**E**) tritrpticin [31].

Histatin (***$C_{133}H_{195}N_{51}O_{33}$***), a peptide present in saliva, is highly rich in histidine fragments (***Table 2***) [5,32-34]. The peptides produced by ***porcine neutrophils*** are very rich in proline and arginine (***PR-39***) or proline and phenylalanine (***prophenin***) [7, 35,36].

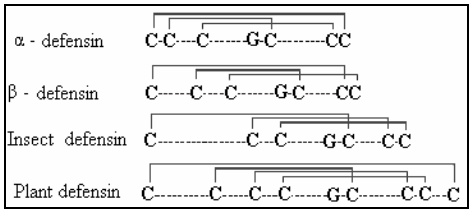

Figure 4. Disulfide bridges of defensins [37]

**** Peptides containing a looped structure*** (***Figure 2,B***). In contrast to other ***AMPs***, proline-arginine-rich peptides cannot form amphipathic structures due to the incompatibility of high concentration of proline residues in such structures and have been proposed to adopt a polyproline helical type-***II*** structure [38,39].

Another ***AMPs*** classification was proposed based on the charge, the amino acid composition and conformation [13,26, 40-42] (***Table 3***).

The first group is represented by ***anionic antimicrobial peptides***. They are small (***721.6–823.8 Da***) peptides present in bronchoalveolar lavage fluid and airway epithelial cells [26,43-45]. In combination with zinc (***cofactor***) this peptides are active against gram-positive and gram-negative bacteria [26].

The second group contains approx. ***290 cationic peptides***, which are short (less than ***40 AA*** residues) (***cecropins***, ***andropin***, ***moricin***, ***magainin***, ***CAP18*** and ***LL-37***).

The next group contains approx. ***44 cationic peptides*** enhanced in certain ***AA*** [41, 46]: ***PR-39*** (***33-49%*** proline and ***13-33%*** arginine residues), ***prophenin*** (***57%*** proline and ***19%*** phenylalanine residues), ***indolicidin*** (tryptophan residues) [26].

The fourth group of ***anionic and cationic peptides*** have approx. ***380*** mem bers, contain cysteine residues and form disulphide bridges.

This group includes: ***protegrin*** from porcine leukocytes (***16 AA*** residues, including four cysteines linked by two intramolecular disulphide bridges), ***55 α-defensins***, which include ***human neutrophil peptides*** and ***cryptdins*** (***29–35 AA*** residues, including six cysteines linked by three intramolecular disulphide bridges) [47], about ***90 β-defensins*** [from humans (***HBD***) and animals] (***36–42 AA*** residues including six cysteines linked by three intramolecular disulphide bridges) [48-50], approx. ***54 insect defensins***, about ***58 plant defensins*** and a ***rhesus θ-defensin (RTD-1)***,an ***18***-residue ciclyc peptide with three disulphide bridges [26,51,52]. The last group is represented by ***anionic and cationic peptides*** similar to the ***AMPs*** described above. Their role in native immunity is not clearly defined.

Table 3. ***AMPs*** classification based on the amino acid sequence and origin [26]

Anionic peptides:
- Maximin ***H5*** (*amphibians*);
- Small anionic peptides rich in glutamic and aspartic acids from (*sheep, cattle, humans*);
- Dermicidin (*human*);
Linear cationic α-helical peptides:
- Cecropins (***A***), andropin, moricin, melitin and ceratotoxin (*insects*);
- Cecropin ***P1*** (*Ascaris nematodes*);
- Magainin (2), dermaseptin, bombinin, brevinin-1, buforin II (*amphibians*);
- Seminalplasmin, BMAP, SMAP (SMAP29, ovipirin), PMAP (*catle, sheep, pigs*);
- LL37 (*humans*); CAP (*rabbits*);
Cationic peptides enriched in:
- Proline: abaecin (*honeybees*);
- Proline and arginine: apidaecins (*honey-bees*); drosocin (*Drosophila*); pyrrhocoricin; bactenecins (*cattle, sheep, goats*); PR-39 (*pigs*);
- Proline and phenylalanine: prophenin (*pigs*);
- Glycine: hymenoptaecin (*honeybees*);
- Glycine and proline: holotricin (*beetles*);
- Tryptophan: indolicidin (*cattle*);
- Histidine: histatins (*man);*
Anionic and cationic peptides that contain cysteine , with disulphide bridges [-(S-S)-]$_n$:
- ***n = 1***: brevinins;
- ***n = 2:*** protegrin (*pigs*) and tachyplesins (*horseshoe crabs*);
- ***n = 3:*** α-defensins (humans), *rabbits; rats*); β-defensins (*humans, cattle, mice, rats, pigs, goats*); rhesus θ-defensin; (RTD-1) (*rhesus* monkey);
- Defensins: defensin A (*insect*);
- ***n > 3*** disulphide bridges: drosomycin (*fruit flies*), plant antifungal defensins;
Anionic and cationic peptide fragments of larger proteins :
- Lactoferricin (*lactoferrin*);
- Casocidin I (human *casein*).

4. Natural AMPs distribution and applications.

AMPs produced by ***bacteria*** were among the first to be isolated and characterized [53]. While they do not protect against infection in the classical sense, they contribute to survival of individual

bacterial cells by killing other bacteria that might compete for nutrients in the same environment. ***Bacteriocins*** (***bacterial AMPs***), (***1.9 – 5,8 kDa***) are produced by gram-positive bacteria [54] and are more efficient than most peptides produced by other evolved organisms. The bacteriocins constitute a structurally diverse group of peptides, and it was recently proposed that they be classified into two broad categories: ***lantibiotics*** (containing lanthionine) and ***non-lantibiotics*** [55].

Knowing bacteriocins covers their processing, purification, and characterisation (***Figure 5***).

Due to their physical and chemical properties, ***bacteriocins*** can be used as ***natural preservatives in the food industry***. Most of them are thermally stable, resistant to pasteurisation while keeping their activity, active within a wide range of ***pH***, and resistant to organic and inorganic solvents [57-65]. The most extensively studied lantibiotic is ***nisin***, (*Lactococcus lactis*) which has been commonly used for nearly 50 years as a ***natural food preservative*** without significant development of resistance.

In ***plants***, ***AMPs*** play an important and fundamental role in defense against infection by bacteria and fungi. Observations to support this role include the presence and expression of genes encoding ***AMPs*** in a wide variety of plant species investigated thus far, demonstrations of their bactericidal and fungicidal activity in vitro, and correlations between expression levels of peptides and susceptibility to a particular pathogen. In plants have been identified ***thionins*** and ***defensins*** (***Figure 6***) [66].

Thionins are bioactive against bacteria and fungi. Studies utilizing ***transgenic plants*** have confirmed that heterologous expression of thionins can confer protection against bacterial challenge [68, 69].

Defensins isolated from leaves, flowers, seeds, and tubers, confer protection in vitro against bacteria and fungi[70]. They are recommendable for different crops to diminish losses caused by pathogens, thus supplying major advantages: natural products, quick biodegradation. An important disadvantage of defensins would be their lower efficiency compared to synthesis fungicides [71].

At present, defensins (insects and mammals) are tested clinically to allow recommendation in bacterial and fungal infections. ***AMPs*** (plants) interact with specific structures from the fungal membrane (phosphoinositol sphingolipid or glycozil-ceramides, etc.) suggesting advanced selectivity in the prophylaxy of fungal infections [71].

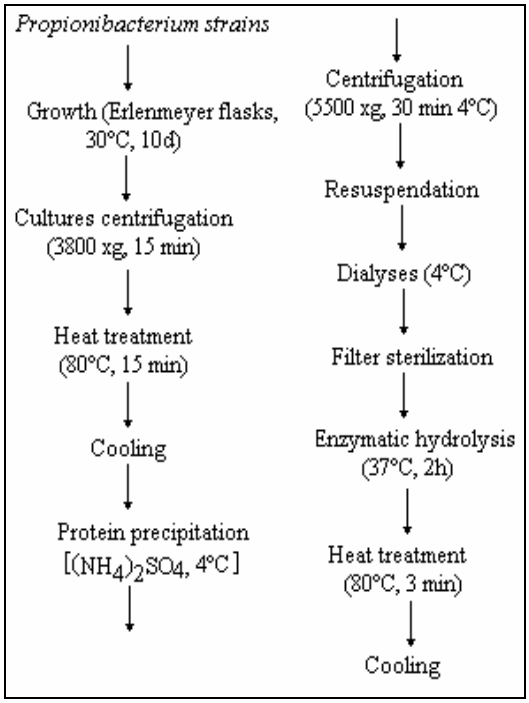

Figure 5. Partial purification scheme for bacteriocins [56]

Since ***invertebrates*** do not have a native immune system, their defence is based exclusively on the reservoir of non-specific immune competences. If we admit the fact that these organisms have known a remarkable progress, these competences of their non-specific immune system are extremely efficient.

Many of the ***AMPs*** with immunologic role initially identified in insects were also confirmed in mammals. In invertebrates, they can be found in hemolymph, in phagocytic cells and epithelial cells of shrimps, crabs [72,73] as a response to pathogen invasion (antifungal peptides of *Drosophila*) [74].

Among the many peptides with ***antimicrobial functions*** in invertebrates, we retain exclusively ***prototypical*** ones: ***cecropins*** (***α-***helical peptides from fly hemolymph), ***α-helical melitin*** (bee venom), and the ***β-hairpin***-like peptides ***tachyplesin and polyphemusin*** isolated from horseshoe crab [75].

The most abundant group of ***AMPs*** in invertebrates are the defensins (acyclic peptide

structure, with three or four disulfide bridges) [76].

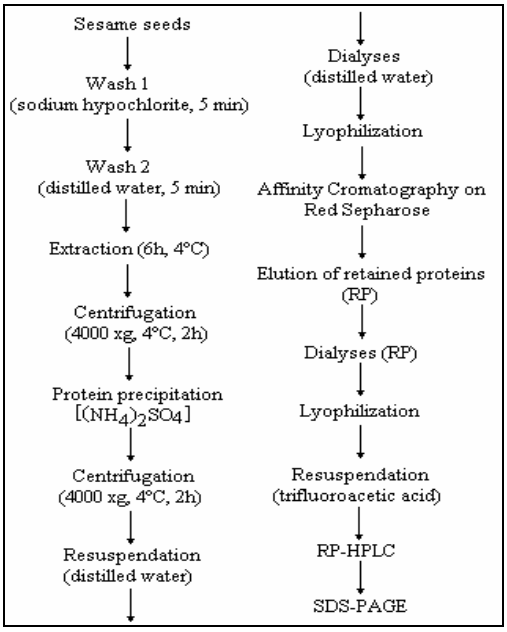

Figure 6. Purification scheme for AMPs from sesame seeds [67]

AMPs have been isolated from a wide range of ***vertebrate*** species, including fish, amphibians, and mammals, indicating that, even in the presence of an adaptive immune response, these peptides have an important role in „***host***" defense. Direct microbicidal activity is associated with vertebrate ***AMPs,*** under certain physiological conditions, representing the "***first line of defense***", especially where they are found in very high concentrations (granules of phagocytic cells or in the small intestine) [8-10,77,78]. In the case of vertebrates, though there is an ***evolved immune system*** with adaptive competence, the ***peptidic component*** is accepted as ***"first line of defence".***

In addition to direct microbicidal activity, cationic peptides perform critical immunomodulatory functions and may be involved in the control of inflammation [9, 10,78, 79].

Due to the mission of direct or indirect antimicrobial defence in vertebrates, ***AMPs*** locate particularly in areas where pathogen contact is more frequent (mucous surfaces, skin, and granules of some immune system cells) [9,10,78,79].

In addition to their presence in the skin, ***AMPs*** have been isolated from the mucosa of the stomach, indicating a role in protection from ingested pathogens. The most systematically characterised peptides in this class, (***buforin I*** and ***buforin II)*** (Asian frog) are generated by cleavage of histone ***2A*** [76,80, 81].

Cathelicidines are an important group of ***AMPs*** in vertebrates, structurally characterized by a conserved ***N-***terminal segment (the cathelin domain) that is proteolytically cleaved to generate the ***mature, active peptide*** contained within the ***C-***terminal segment. Most structures in this group are found in circulating cells in an inactive form, the predominant source being secretory granules of neutrophils, the surface of the buccal mucous, of the genital and urinary tract, of the lungs, and in the keratinocytes on the dermatological surfaces of inflammatory conditions [82] (***Figure 7***).

Another group of immunologically active mammalian ***AMPs*** is the ***defensins*** [83, 84], cyclic peptides which are categorized on the basis of the disulfide bridges between their six conserved cysteine residues (***α-*** and ***β-***defensins) their macrocyclic nature (***θ-***defensins).

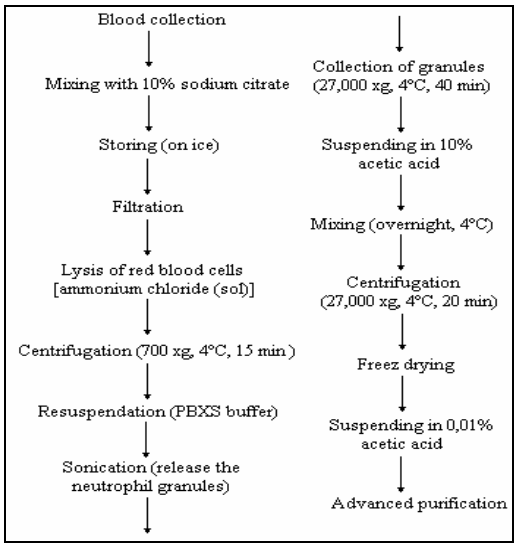

Figure 7. Purification scheme for cathelicidin peptides from ovine neutrophils [85]

While ***α-*** and ***β-***defensins are widespread in vertebrates species, ***θ-***defensins have been identified so far only in the neutrophils and monocytes of some species of monkeys [51]. Depending on the species, ***α-*** and ***β-***defensins are found in the granules of neutronphils, macrophages, ***NK cells***, intestinal ***Paneth cells*** and some cells on the mucosal surfaces (respiratory passage and urinogenital tract).

5. Biologically active activity.

Antibacterial, antifungal, sometimes antiviral, antiparasitic, or antitumor activity is manifest since these biomacromolecules ***act as "warning systems" for immune cells involved in chemotaxis or activation.***

AMPs are often efficient against pathogens resistant to conventional antibiotics. It has also been confirmed that some structures have an ***antiendotoxin effect***.

Representatives from all structural classes of peptides, confirmed ***significant capacity to inhibit viral infections***. The antiviral activity of ***AMPs*** was frequently associated to the viral adsorption and entry process [86] or the result of a direct effect on the "***viral envelope***" [87,88]. The spectra of viruses that are affected comprise ***enveloped RNA and DNA viruses***, with the exception of ***nonenveloped adenovirus*** [89, 90], ***feline calicivirus*** [91] and ***echovirus 6*** [92].

α-Helical peptides (***cecropins***, ***clavanins*** and the cathelicidin ***LL-37***) have confirmed minimal or no activity against herpes simplex virus (***HSV***) [93-95], while ***α-helical magainins***, ***dermaseptin*** and ***melittin*** have shown quite potent anti-***HSV*** activity [88,87, 95,96,111].

Conversely, ***β-sheet peptides*** (***defensins***, ***tachyplesin*** , ***protegrins***) and ***β-turn*** peptide ***lactoferricin*** showed activity potentiated towards ***HSV*** [95,97-102]. Within the different peptide subclasses, activity may evolve considerably. Protegrin analogues lacking one or both disulfide bridges can be highly active or inactive against ***HSV*** infections [95].

For more natural ***AMPs***, were obtained synthetic analogues in an attempt to ***correlate major structural competences*** with ***antiviral activity***. Different strategies for design of such peptides have been pursued.Several researchers have drawn their attention to the importance of electric charge of aromatic ***AA,*** since antiviral peptides are frequently strongly cationic and amphiphilic [95,98,103,104]. The hydrophobic character of the peptides has been investigated for a hybrid peptide of cecropin ***A*** and magainin-***2*** [105], while the substitution of ***D-*** or ***L-*** forms of ***AA*** has been studied on a set of ***θ-***defensins [102]. The creation of a series of lactoferricin analogues and study of their activity towards ***HSV*** revealed a ***relationship between the peptide charge and its antiviral activity*** [99, 106]. However, the spatial conformation of the charged ***AA*** has a decisive role for antiviral activity than the actual net charge [99]. For lactoferricin the nature of the aromatic ***AA*** appeared to be of minor importance for the antiviral activity, although its contribution to the secondary structure can be crucial [106].

Detailed studies concerning the impact of secondary structure on anti-***HSV*** activity confirmed that the spatial form "***α-helix***" does not explain its antiviral activity [107], which justifies the claim that the electric charge of the ***AA*** fragments (***Table 3***) are of great importance for anti-***HSV*** activity [103], according to results reported in a study carried out on peptides derived from bovine lactoferrin and chicken ovotransferrin [108]. It was concluded that the presence of the hydrophobic character and of positive charge of the ***AA*** fragments is important but not decisive for antiviral activity [108].

For ***θ-***defensins have been structured a series of analogues focused on the substitutions ***Ile→Tyr*** and ***Arg→Tyr***, and have demonstrated the importance of electric charge and spatial conformation of these peptides [102]. ***Lactoferricin*** and ***polyphemusin*** have ***β*** structures stabilized by one and two internal disulfide bridges, respectively. These disulfide bridges have been shown to be crucial for the antiviral activity of the peptides [99,109,110].

Despite their diverse structures, many peptides have similar antiviral modes of action [99, 103]. A possible explanation is based on the observation that antimicrobial host defense peptides are known to ***adopt amphipathic conformations*** that are intrinsic to antibacterial activity. Although the ***"viral target"*** of these peptides appears to vary, the demonstrated antiviral effects are similar [111].

The most elaborated category of ***cationic antimicrobial peptides (CAMPs)*** is represented by ***antibacterial activity*** ones [112]. It is known that, no matter their action target, they need to interfere with the bacterial cytoplasmic membrane [113]. The first step in this interacttion is the attraction between the peptide and the "***target" cell***, due to electrostatic bonding between cationic peptides and negatively charged components present in the outer bacterial membrane (phosphate groups within the lipopolysaccharides of gram-negative bacteria or lipoteichoic acids present on the surfaces of gram-positive bacteria). Bioprocesses taking place on the membrane

surface have been the subject of several studies. They've been proposed:

***the „aggregate" model** (**Figure 8, A**) (in this model peptides reorient to span the membrane as an aggregate with micelle-like complexes of peptides and lipids, but without adopting a particular orientation);

the "toroidal pore" model** (**Figure 8, B**) (the peptides insert themselves perpendicular to the plane of the bilayer, with the ***hydrophilic regions oriented to the phosphorlipid groups and the ***hydrophobic regions*** to the lipid zone;

*** the "barrel-stave" model** (**Figure 8, C**) (the peptides insert in a perpendicular orientation to the plane of the bilayer, forming the "***staves***" in a "barrel"-shaped cluster, with the ***hydrophilic regions*** of the peptides facing the lumen of the pore and the ***hydrophobic regions*** interacting with the lipid bilayer);

*** the „carpet model"** (**Figure 8, D**)

(the aggregates of peptide align parallel to the lipid bilayer, coating local areas in a carpet-like fashion. At a given threshold concentration, this is causing formation of micelles and membrane pores[111].

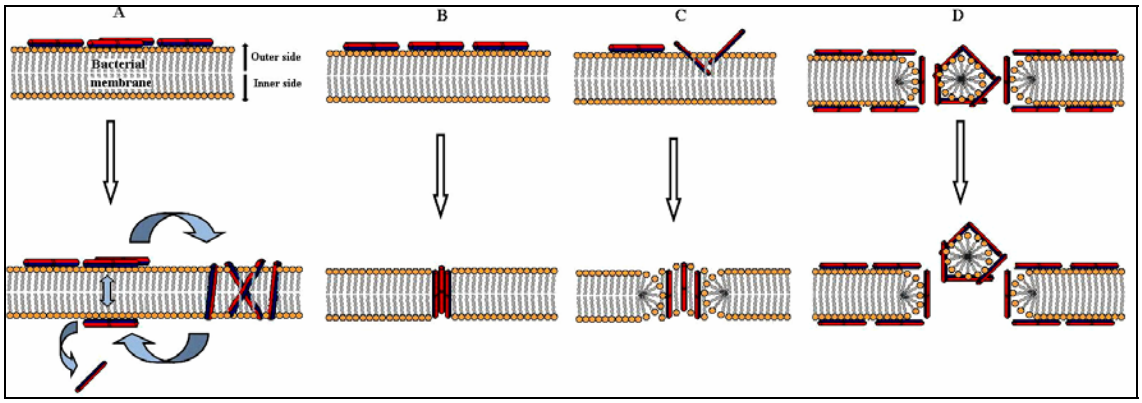

Figure 8. Mechanisms of action of antibacterial peptides (the peptides are shown as cylinders rods, where the hydrophilic regions are marked with red color and the hydrophobic regions with blue) (adapted after Jenssen, H., [111])

Though all peptides should interact with the cytoplasmatic membrane, it was demonstrated that several ***AMPs*** do not cause the membrane permeabilization, yet producing bacterial destruction.

A large number of ***AMPs*** can translocate across the membrane and accumulate intracellularly, where they target a variety of essential cellular processes to mediate cell killing. This new confirmed models include ***inhibition nucleic acid biosynthesis, protein biosynthesis, cell wall biosynthesis and enzymatic activity*** [111].

Physical forces behind antibacterial activity were evaluated in detail [114-116] and they include ***net positive charge*** (enhancing interaction with anionic lipids and other ***"bacterial targets"***), ***hydrophobicity*** (necessary to insert in the membrane architecture), and ***flexibility*** (that allows the peptide to transition from its natural and native conformation to patial architecture proper for interaction).

Each of these parameters can vary substantially, but are essential for the functioning of peptides as antimicrobial agents and for their interaction with bacterial membranes [***critical control point (PCC) to exercise antimicrobial competences***] [111]. Although ***CAMPs*** are divided into four structural classes: ***α***-helical, ***β***- sheet, loop and expanded [40, 42], there are structures that do not fit in this simplified classification system. Many peptides produced by bacteria are represented in two different conformational domains (***α-helical*** and ***β-sheet***, respectively)[117]. For some peptides, these secondary structures are observed only in interaction with membranes (native bovine neutrophil indolicidin is unstructured in aqueous medium, but adopts a different conformation when interacting with membranes) [118].

Conformational interconversion flexibility of the secondary structure of indolicin has been suggested to permit different interferences with distinct molecules, including ***DNA*** and ***membranes*** [119].

A way to increase the antibacterial activity of ***CAMPs*** is to change their flexible secondary structures. By modifying the membrane-associated shape of indolicidin (making the terminal areas ***N*** and ***C*** vicinal), the activity against gram-negative bacteria increases. This shape could also be stabilised by grafting a fragment of cysteine to each area and by creating a disulphide bridge [25]. In exchange, introducing a covalent cross-link between ***Trp6*** and ***Trp9*** allowed the development of a synthesis indolicidin analogue [120]. Both changes induced a decrease of protease sensitivity, but did not inhibit antimicrobial activity. Similar attempts to stabilize specific structural elements have been made with a ***cecropin-melittin hybrid complex***, in which the ***α-***helical structure in solution was stabilized by the introduction of a covalent lactam bond between two residues of four ***AA*** apart [111, 121]. Stabilization of the helical structure in ***cecropin A*** has demonstrated the importance of this structural domain in antibacterial activity against *E. coli* [122]. The introduction of a disulfide bridge within the C-terminal ***α-***helix of ***sakacin P*** to increase the amount of ***α-***helical structure led to the expansion of the spectrum of activity [111, 117].

CAMPs activity can also be monitored through ***hydrophobicity change*** and ***peptide net electric charge***. Several studies have demonstrated that high levels of hydrophobicity can decrease selectivity between the desired bacterial targets and host cells [123,124].

The incorporation of charged residues above a certain maximum (varying with each peptide) does not lead to an increase in antibacterial activity [114]. The balance between hydrophobicity and electric charge initially assessed as empirical manner is experimentally verified and adaptable to each studied ***CAMP***.

The inclusion of a particular peptidic architecture in a certain structural class does not provide certainty about the mode of action and spectrum of activity. In fact, some ***CAMPs*** with similar secondary structures have opposite characteristics with respect to antibacterial activity [111]. ***The α-helical melittin*** (bees) that penetrate the membranes of eukaryotic and prokaryotic organisms, falls within the ***α-***helical structural class. Conversely, ***buforin*** (true toad) is transferred into cells and acts on macromolecular biosynthesis.

Several studies confirmed that ***CAMPs*** with similar secondary structures and minimal differences in the primary sequence can posess different antibacterial activities [125]. Some ***CAMPs*** can change membrane permeability at their minimal efficient concentration or at higher or lower concentrations. Nowadays it is admitted that antibacterial peptides are capable to effect antimicrobial activity due to their amphipathic (amphiphile) feature and to the presence in the structure of structural areas with high concentrations of positively charged fragments [22].

The mode of action of ***antifungal peptides (AFPs)*** involves either the fungal cells lysis or interference with fungal cell wall biosynthesis [126]. However, as the numbers of known ***AFPs*** increase, new modes of action are being identified. It is interesting that peptides with primarily fungicidal activity (peptides isolated from plants) tend to be rich in polar and neutral ***AA***, suggesting a unique structure-activity relationship [127].

Two new peptides derived from human lactoferrin with different anti-*Candida* activities but with a very similar sequence were identified [128]. One of these peptides has a similar sequence to brevenin-1Sa [129]. However, other studies have shown that ***AFPs*** vary substantially in sequence and structure, and peptides as structurally diverse as ***eucommia*** (with five disulfide bridges) [130], ***the α-helical P18*** [131] and ***indolicidin*** (extended structure) [132], plant ***defensins*** and a ***β-***sheet peptide isolated from *Acrocinus longimanus* [133], have all shown antifungal activity [111]. Thus, as well as for antibacterial peptides, there is no obvious structural field that ensures antifungal activity [111].

Modification of ineffective ***AMPs*** has revealed that even the slightest structural change can induce antifungal activity. Grafting undecanoic acid or palmitic acid to magainin leads to peptidic analogues that have activity against both yeast and fungal infections [134].

The directed fusions of parts of ***magainin 2*** and ***cecropin A*** to form the hybrid peptide ***P18*** resulted in a peptide with potent fungicidal activity against pathogenic *Candida albicans*, *Trichosporon beigelii*, *Aspergillus flavus*, and *Fusarium oxyspovrum* [131]. Studies with protegrin (***18***-residue pig peptide), which has both antibacterial and antifungal activities,

demonstrated that the antibacterial activity could be retained in a ***12***-residue deletion peptide but only two residues could be deleted for the potent antifungal properties of this peptide to be retained [111, 135].

Although no conserved sequences are evident for the ***AFPs***, several have been demonstrated to possess specific biochemical characteristics (chitin [130,136] or heparin [137,138] binding abilities). Structure-activity relationship studies on three synthetic bovine lactoferricin (derived peptides with ***17 – 30 AA***) showed a significant positive correlation between the values of isoelectric point of peptides (***pH***) and their activity agains *Candida* [139]. Another study also confirmed a direct correlation between the ability of peptides to form macromolecular aggregates of lipids and their antifungal activity [140].

Magainin-2 was one of the first ***AMP*** demonstrated to display ***antiprotozoan activity*** (***antiparasitic activity***), leading to swelling and eventual bursting of *Paramecium caudatum* [141]. More recently a synthetic ***AMP***, ***Oct-CA(1-7)-M(2-9)***, has been shown to be effecttive for prophylaxis of canine leishmaniasis [142], which is caused by the parasite ***Leishmania***, (a major cause of morbidity and mortality in humans)[143].

The antinematodal effect of the porcine cathelicidin ***PMAP-23*** has been confirmed against the eggs and worms of *Caenorhabditis elegans*, effect exerted through disruption of the cell membrane via pore formation or via direct interaction with the lipid bilayers [144], resembling the antifungal mode of action for ***PMAP-23*** [111, 145].

Several ***AMPs*** possess an antiprotozoan mode of action that indicates parallels with their antibacterial, antiviral, or antifungal modes of action. Analogues of mussel defensins have been demonstrated to efficiently kill *Leishmania major* and *Trypanosoma brucei* in a temperature-, time- and dose-dependent mode of action.

These peptides were found to interact with the external epithelium of *T. brucei*. However, structure-activity relationship studies indicated that the antiprotozoan and antiviral activities were mediated by different mechanisms [146].

Conclusions

Natural ***AMPs*** are, due to their diversity, biologically active competences, fields of inter- and pluri-disciplinary application, of real interest for both the science of products and of food processing in the near future.

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