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Antimicrobial peptides – natural antibiotics

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Abstract

The antimicrobial peptides are small, cationic, amphiphilic peptides, characterized by microbicidal activity against bacteria, fungi, viruses and other pathogens. The knowledge of their quick and strong antimicrobial action, as well as the non-specific membrane-mediated mechanism of antimicrobial peptide-induced cell death has led researchers to study substitution of conventional antibiotics by antimicrobial peptides. Understanding the correlation between structure and function of antimicrobial peptides is the key element for the development of nontoxic therapeutics in many infectious diseases. This review presents some recent progresses in the study of antimicrobial peptides, recognized as the most important elements of non-specific host defense systems and the innate immunity in fungi, plants, invertebrates and vertebrates.

Key words: antimicrobial peptides, defensins, therapeutic potential, biomarkers

Introduction

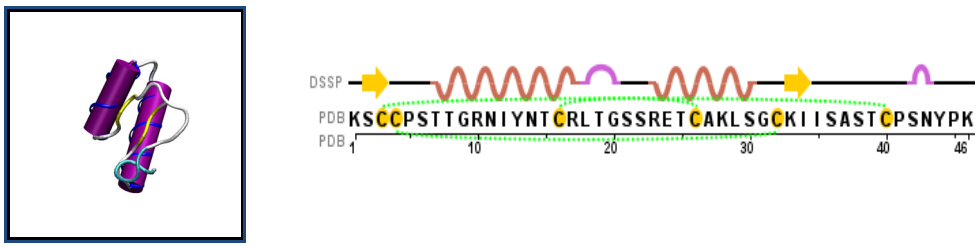
This review presents some recent progresses in the study of antimicrobial peptides. The antimicrobial peptides are secreted by fungi, plants, invertebrates and vertebrates and protect them from invasion of bacteria, fungi, viruses and other pathogens [1, 2]. The results of studies from the last decade led to the idea that all multicellular organisms possess these forms of non-specific host defense systems and the innate immunity. Antimicrobial peptides have diverse structures and functions and interact with cell membranes of invader cells by disturbing the membrane integrity. This action leads to cell lysis and, later, to their death [3 - 5]. Microbes are the cause of many infectious diseases. Increasing microbial resistance to common antibiotics has become a grave threat in maintaining public health. Due to their features, antimicrobial peptides have become attractive and safe targets for researchers that are looking for new antibiotics [6 - 9]. Another research direction is studying antimicrobial peptides as potential biomarkers of such diseases as: various forms of cancer, psoriasis, HIV / AIDS [10 -13]

The investigation of the properties of the antimicrobial peptides is a field of remarkable interest to discover its mechanism of action and develop strategies for new biotechnological applications in medicine and plant protection. The most widely used methods of investigation of the antimicrobial peptide structure are nuclear magnetic resonance (NMR) spectroscopy and X-ray diffraction. The termodynamic parameters of interactions, the structure and the stability of antimicrobial peptides have not been studied in detail. The computer simulations have become a tool increasingly widely used in the last 20 years to study the structure of various biomolecules. The important progresses in the computing resources and improvements in accuracy of force fields used to describe biomolecules have allowed the provision of the new data in molecular dynamics simulations. These progresses

have given the possibility of comparison of simulation data with experimental data, of refinement of experimental data and replacing laboratory experiments with simulation predictions, all at once saving time and financial resources [14 - 17].

Plant antimicrobial peptides

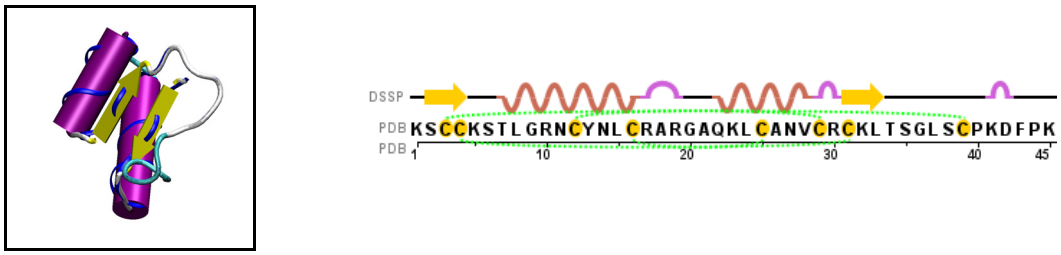
Plants are constantly exposed to attack from a large range of pathogens. Under attack conditions plants synthesized antimicrobial peptides as innate defence. Thionins were the first antimicrobial peptides to be isolated from plants, and normally consists of 45-48 amino acids. Thionins are a family with low molecular weight (approximately 5 kDA), rich in arginine, lysine and cysteine residues and include three or four conserved disulfide linkages. There are positively charged at neutral pH, due to the presence of several basic amino acid residues, and are mainly found in seeds. Thionins have toxic effects against bacteria, fungi, yeast, plant, and various mammalian cell types [18 - 21]. This lytic activity of thionins has been shown for fungi cell membranes and, also, for mammalian cells, where a selectivity to certain cell types can be discerned [22 - 24]. Viscotoxins belong to plant thionins and are toxic against a various number of cell types. They are produced from the leaves and stems of the European mistletoe (*Viscum album*). The fluorescence spectroscopy studies showed that both viscotoxin A3 and viscotoxin B present a high conformational stability and a similar conformation in solution and when bound to membranes. Viscotoxins induced the appearance of imperfections on the surface of membranes that lead to the destabilization and disruption of the membrane bilayer. [25]. By NMR spectroscopy has been determined the three-dimensional structure of the plant viscotoxin C1, from the Asiatic *Viscum album*. The viscotoxin C1-fold is very similar to that found for other related thionins. The viscotoxin has a high toxicity against tumoral cells. By sequence and structural alignment analysis have been identified the residues responsible for the modulation of viscotoxin cytotoxicity [26]. The three-dimensional structures of viscotoxins A1 (fig. 1) and B2 has been solved by X-RAY crystallography method and both form dimmers in the crystal. The viscotoxin B2 is coordinated by sulfate or phosphate anions [27].



a) b)

Fig. 1. Viscotoxin A1, plant antimicrobial peptide - 3C8P a) structure image, b) detailed SEQRES sequence.

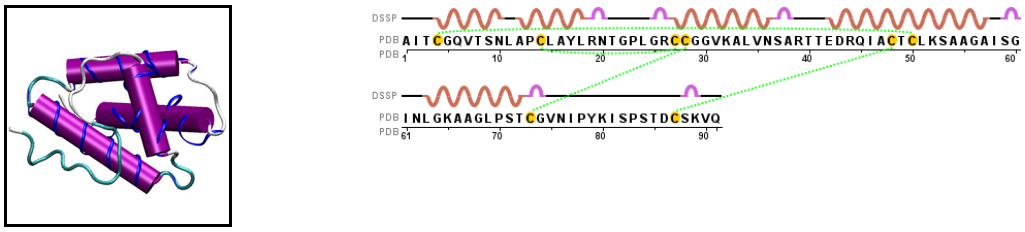
Whereas the mechanism of cation inhibition of membrane disrupting activity of thionins is not known, the mechanism of action of the beta-purothionin (fig. 2), a natural antimicrobial peptide from the endosperm of wheat seeds, assume inserts into the hydrophobic core of the lipid bilayer. These are results of studying the interaction of beta-purothionin with multilamellar vesicles of dimyristoylphosphatidylglycerol (DMPG) by P solid-state NMR and infrared spectroscopy [28]. The structural properties and mechanisms of inhibition of wheat beta-purothionin by metal ions were investigated by unconstrained molecular dynamics simulations in explicit water [29]. Alpha(1)-purothionin is a wheat-germ protein and a basic lytic toxin. The three-dimensional structure of alpha(1)-purothionin was solved by molecular-replacement methods and revised by X-ray difraction method and refined to an R-factor of 15.5% [30].



a) b)

Fig. 2. Beta-purothionin, 1BHP, a) structure image, b) detalied SEQRES sequence

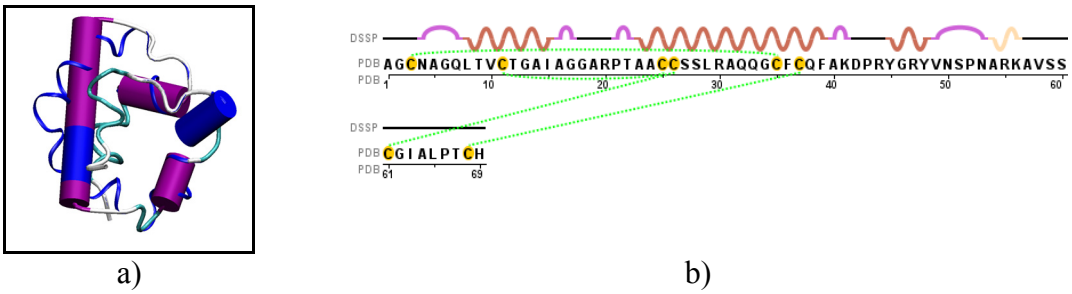
The plant Lipid Transfer Proteins (LTPs) are present in high amounts in various plant species and different plant tissues. LTPs are divided into two subfamilies with relative molecular masses of 9 kDa (LTP1s) and 7 kDa (LTP2s). LTPs bind a large range of lipid molecules to their hydrophobic cavity. LTPs were thought to participate in membrane biogenesis and regulation of the intracellular fatty acid pools. Because they are able to transfer various types of polar lipids, plant LTPs are also named "non-specific lipid transfer proteins". LTPs are involved in cutin formation, embryogenesis and defense reactions against phytopathogens, symbiosis, and the adaptation of plants to various environmental conditions [20, 31]. The structural and functional characteristics of a lipid transfer protein (LTP1_1) expressed in young aerial organs of *Nicotiana tabacum* was reported (fig. 3.). By nuclear magnetic resonance spectroscopy and molecular modeling techniques the three-dimensional structure of LTP1_1 was determined. The results suggest that LTP1_1 is able to bind only one LysoMyristoylPhosphatidylCholine molecule and LTP1 lipid binding properties could be modulated by subtle changes in a conserved global structure [32].



a) b)

Fig. 3. Lipid transfer protein, LTP1_1, 1T12, a) structure image, b) detailed SEQRES sequence

Similar binding cavities and hydrophobic interactions as in rice nsLTP1 were revealed in mung bean nsLTP1. Also, lipid transfer properties of mung bean nsLTP1 are comparable with those of rice nsLTP1 [33]. The three-dimensional structure of rice nonspecific lipid transfer protein (nsLTP2) has been solved by NMR solution method and is presented in fig. 4. The C terminus of the nsLTP2 is very flexible and forms a cover over the hydrophobic cavity that could shelter big molecules with rigid structures [34].



a) b)

Fig. 4. Nonspecific lipid transfer protein, nsLTP2, 1L6H, a) structure image, b) detailed SEQRES sequence.

Snakins are antimicrobial peptides with 63 amino acid residues (6,9 kDa) and have been isolated from potato tubers. The peptides snakin-1 (SN1) and snakin-2 (StSN2) have antimicrobial activity against bacterial and fungal pathogens of potato and other plant species. Snakin-1 and snakin-2 induce aggregation of both Gram-positive and Gram-negative bacteria [35, 36]. For the first time, snakin-1 (SN1) was expressed and purified using a prokaryotic expression system for generation and in vitro characterization of cysteine-rich plant peptides with potential antimicrobial activities against a wide range of phytopathogenic microorganisms, in order to select the most effective agents for future in vivo studies [37].

Plant defensins are a superfamily of antimicrobial peptides, with representatives in vertebrates, invertebrates and plants. First found in literature are known as gamma-thionins. A new sulfur-rich peptide, named as gamma-hordothionin, has been isolated from barley endosperm. Gamma-hordothionin consists of a chain of 47 amino acids with a molecular mass of 5250 Da, and contains four disulfide bridges. Gamma-hordothionin inhibits translation in cell-free systems derived from mammalian (rabbit reticulocyte, mouse liver) as well as non-mammalian (*Artemia* embryo) cells, at several levels [38] The NMR spectra of the gamma 1-purothionin (fig. 5. a), from Triticum turgidum and the gamma 1-hordothionin (fig. 5. b), from Hordeum vulgare has been performed by two-dimensional sequence-specific methods. The results show that both proteins have identical secondary and tertiary structure, the three-dimensional structures of the gamma-thionins differ remarkably from plant alpha- and beta-thionins and crambin and show a higher structural analogy with scorpion toxins and insect defensins [39].

According to their antifungal activity, the plant defensins can be divided in two groups: 1) plant defensins that inhibit fungal growth through morphological distortions of the fungal hyphae and 2) plant defensins that inhibit fungal growth without morphological distortion [18]. The *Vigna radiata* plant defensin 1, VrD1, (fig. 5. c) and 2, VrD2, (fig. 5. d) was isolated from the seeds of the mung bean, *Vigna radiata*. It was reported that although both peptides show a similar global fold, only VrD1 exhibit insecticidal activity and alpha-amylase inhibitory activity [40, 41].

Petunia hybrida defensin 1 (PhD1) is a subclass of plant defensins with five disulfide bonds. PhD1 has 47 residues and antifungal activity. The structure of PhD1 has been determined by NMR spectroscopy and suggests that the additional disulfide bond from PhD1 do not change its tertiary structure with respect to other plant defensins [42].

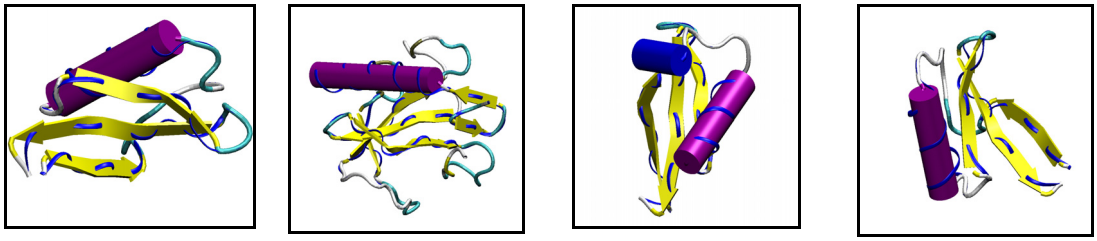


Fig. 5. Structure image of a) the gamma 1-purothionin, 1GPT, b) the gamma 1-hordothionin, 1GPS, c) the *Vigna radiata* plant defensin 1, VrD1, 1TI5, d) the *Vigna radiata* plant defensin 2, VrD2, 2GL1.

Bacterial antimicrobial peptides

Many bacteria, both Gram-positive and Gram-negative, produce and secrete both cationic and neutral antimicrobial peptides. The bacterial antimicrobial peptides are also referred as peptide bacteriocins [43]. Bacteriocins are lethal to bacteria other than the producing strain and are classified largely based on differences in their molecular weight. The main mechanism of action of antimicrobial peptides with bacterial origin is by permeabilizing

of the target cell membranes [44, 45]. Some peptide bacteriocins have specific mechanisms which inhibit bacterial functions. Thus, peptide microcin C7 inhibits protein synthesis and peptide mersacidin inhibits peptidoglycan biosynthesis. The peptide nisin is produced by *Lactococcus lactis* and has antibacterial activity against a Gram-positive bacteria. It was demonstrated that nisin resistance protein-mediated proteolytic cleavage represents a novel mechanism for nisin resistance in non-nisin-producing *L. lactis* [46]. The antibiotic epilancin K7 and 15X antibacterial peptides from *Staphylococcus epidermidis* has a potential application as food-preserving agents and as antibiotics. Both have closely similar primary and tertiary structure, the distribution of positive charges and a common mode of action [47, 48].

Carnocyclin A (CclA) is an antimicrobial peptide produced from *Carnobacterium maltaromaticum* UAL307. CclA shows activity against a broad spectrum Gram-positive organisms. CclA is a circular bacteriocin with 60 amino acid residues and has been structurally characterized by NMR studies [49]. There were studied the structure, function and/or mode of action for other bacteriocine peptides too, such as plantaricin (fig. 6.), lactococcin (fig. 7.), curvacin (fig. 8. a.), piscicolin (fig. 8. b.), pediocin (fig. 8. c.), carnobacteriocin (fig. 8. d.) and many others [50- 55].

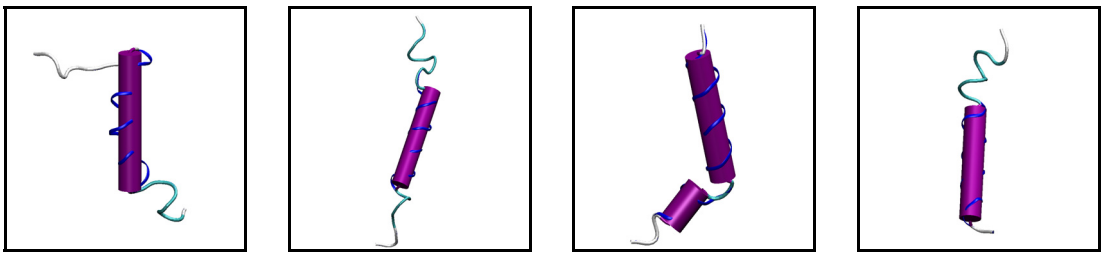


Fig. 6. Structure image of a) the plantaricin K in DPC-micelles, 2KEG, b) the plantaricin K in TFE, 2KEH, c) the plantaricin J in DPC-micelles, 2KHF, d) the plantaricin J in TFE, 2KHG.

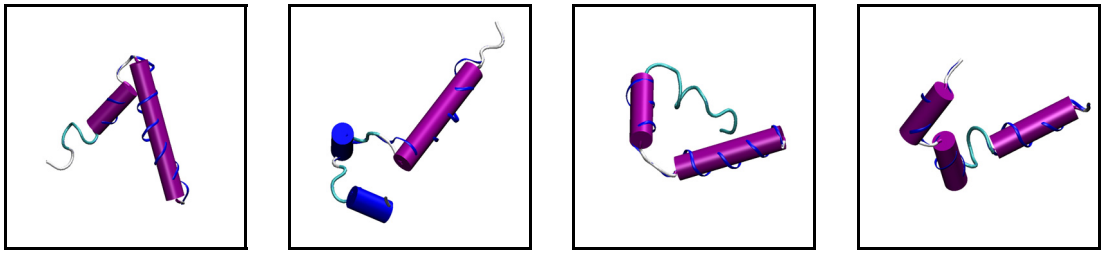


Fig. 7. Structure image of a) the lactococcin G-a in DPC, 2JPJ , b) the lactococcin G-b in DPC, 2JPK, c) the lactococcin G-a in TFE, 2JPL, d) the lactococcin G-b in TFE, 2JPM.

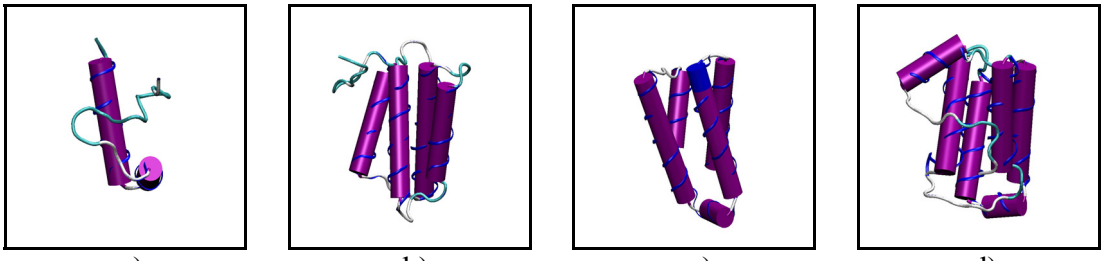


Fig. 8. Structure image a) the curvacin A, 2A2B , b) the piscicolin, 2K19, c) the pediocin, 2BL7, d) the carnobacteriocin 1TDP

Viral antimicrobial peptides

Lentivirus lytic peptides (LLPs), encoded by discrete C-terminal sequences of the human immunodeficiency virus type 1 (HIV-1) transmembrane protein, demonstrate potent antimicrobial and cytolytic activity. It was noted that the LLPs have a high proportion of arginines and no lysine residues. Between the peptides has been observed a difference in selectivity [56]. The mechanisms of action of bis-LLP1, a dimerized and amidated LLP1 derivative, against *S. marcescens* was studied [57].

Insect antimicrobial peptides

Insect antimicrobial peptides have been isolated both from inside of the insect and from outside the body. Although both classes are antimicrobial, the venoms tend to have cytotoxic activities. Insects can express different peptides depending on the type of pathogens [19]. When insects have complete metamorphosis, antimicrobial peptides are produced by the fat body and by various epithelia and when insects have incomplete metamorphosis antimicrobial peptides are produced by hemocytes in the healthy animal and secreted into the hemolymph upon infection. In the insects (primitive organisms), antimicrobial peptides replace the immune response [19]. The insect secrets antimicrobial peptides such as sarcotoxins, hyphancin, enbocin, spodopsin, ponericins, melittin, stomoxyn, spinigerin, ceratotoxins. The apidaecins represent a group of antimicrobial peptides and was isolated from lymph fluid of the honeybee (*Apis mellifera*). These peptides have antimicrobial activity against a wide range of plant-associated bacteria and some human pathogens [58].

The *Anopheles gambiae* defensin is active against *Staphylococcus aureus* at low concentration. This peptide and five hybrids designed by combining conserved its sequence regions and variable regions and were studied by NMR and molecular modelling. Toxicity was tested for evaluating their activity against *Staphylococcus aureus* strains sensitive and resistant to conventional antibiotics. The results led to obtaining one chimeric defensin with increased antimicrobial activity [59].

Stomoxyn (fig. 9. a) and spinigerin (fig. 9. b, c) are linear cysteine-free insect peptides class with antimicrobial activity against a large spectrum of microorganisms, parasites, and some viruses. Both peptides do not have lytic activity against mammalian erythrocytes. While stomoxyn has structural similarities with cecropin A, spinigerin has structural similarities with magainin 2, which leads to the idea of a similar mode of action [60].

Thanatin has been found to have a broad range of activity against bacteria and fungi. Thanatin (fig. 9. d), an insect defense peptide with 21 amino acids, has more similarities with the structures of different peptides, such as brevinins, protegrins and tachyplesins. These peptides have a two-stranded beta-sheet stabilized by one or two disulfide bridges [61].

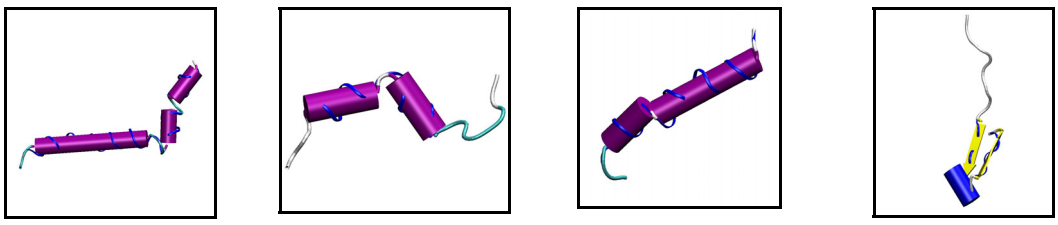


Fig. 9. Structure image of a) the stomoxyn, 1ZRX, b) the spinigerin in TFE 10%, 1ZRW, c) the spinigerin in TFE 50%, 1ZRV, d) the thanatin, 8TFV.

Mammalian antimicrobial peptides

According to their structural features, the antimicrobial peptides may be divided into four distinct groups: 1) cysteine-free α-helices, 2) extended cysteine-free α-helices with a predominance of one or two amino acids, 3) loop structures with one intramolecular disulfide bond, and 4) β-sheet structures which are stabilized by two or three intramolecular disulfide bonds [62].

Mammalian antimicrobial peptides can be found within the granules of neutrophils, in Paneth cells, in epithelial cells, or as the degradation products of proteins [63]. Neutrophils, polymorphonuclear leukocytes (PMNs), are the most abundant leukocytes. They are the first line of defense against infection. The main antimicrobial peptides contained by neutrophils are defensins, cathelicidins, bactenecins and indolicidins [64 - 68]. These antimicrobial peptides kill micro-organisms by non-oxidative mechanisms, such as by disturbing the microbial cell membrane integrity. Defensins are the most studied mammalian antimicrobial peptides and have six invariant Cys residues which form three structurally indispensable intramolecular disulfide bridges [69 - 70]. Defensins have been categorized into three main groups according to their structural differences: α-, β and θ-defensins, that differ in the location and connectivity of three disulfide bonds. θ-defensins, the last discovered, are found only in non-human primates [70]. Two main features of mammalian defensins are a positive net charge and a turn-linked β-strand structure [66]. The cryptdin-4 (Crp4) (fig. 10. a) is an alpha-defensin secreted by mouse Paneth cell. The 3D structure of this peptide and of their mutant (E15D)-Crp4 peptide, (fig. 10. b), in which a conserved Glu(15) residue was replaced by Asp, was determined by solution NMR method. The results led to conclude that bactericidal activity and proteolytic stability of the mature peptide are not influenced by the conserved salt bridge in Crp4 [71].

The defensin peptides found in humans are α-defensins consisting of 29-33 amino acids residues and β-defensins consisting of 35-72 amino acids. The human α-defensins have disulfide connections between Cys 1-6, 2-4 and 3-5, while the β-defensins have disulfide connections between Cys 1-5, 2-4 and 3-6 [72]. Defensins contribute to the regulation of host adaptive immunity against microbial invasion by using chemokine receptors on dendritic cells and T cells. Linkage of β-defensins or selected chemokines to an idiotypic lymphoma antigen yield potent antitumor vaccines [73]. The molecular basis of the Gly-Xaa-Cys motif conserved in all mammalian defensins was studied by replacement of the invariant Gly17 residue in human neutrophil alpha-defensin 2 (HNP2) by L-Ala or one of the D-amino acids Ala, Glu, Phe, Arg, Thr, Val, or Tyr. These studies identify an essential conformational prerequisite in the beta-bulge of defensins for correct folding and native structure [74].

The cathelicidins are a large family of antimicrobial peptides found in all mammalian species. They have been identified in fish, bird, cow, pig, rabbit, sheep, mouse, monkey, horse and human [75, 76]. The cathelicidins are synthesized by epithelial cells, neutrophils and macrophages. The best known human cathelicidin is LL-37 (fig. 10. c). The LL-37 sequences possess biological activity and are mature, processed, antibacterial form [77]. LL-37 protein has been found in epithelial cells, skin, gastrointestinal and respiratory tract, NK cells, T cells and B cells [78].

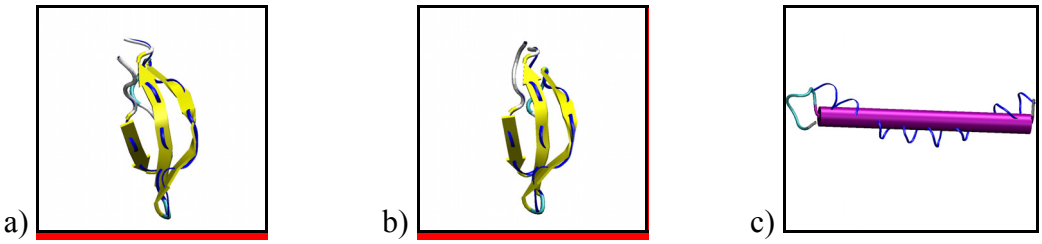


Fig. 10. Structure image of a) the cryptdin-4, Crp4, 2GW9, b) the mutant (E15D)-Crp4, 2GWP, c) the human cathelicidin, LL37, 2K6O.

There are 6 different human α-defensins: human neutrophil peptide (HNP) 1-4 first isolated from neutrophils and human defensin (HD) 5-6 identified in Paneth cells of the intestine. Human neutrophil peptide are found in monocytes, NK cells, macrophages, B cells and T cells, natural killer cells, immature dendritic cells and in neutrophils [79-81]. HNPs have microbicidal activity against both Gram-negative and Gram-positive bacteria, fungi, viruses, protozoa and Chlamydia. HNP 1-3 are the most abundant forms of human α–defensins and suppresses in vivo the activity and the replication of the HIV-1 virus. HNP 1-3 may serve as blood markers for colon cancer because it was shown that the expression of HNP 1-3 is up-regulated in the tumor tissue [82]. It was indicated the therapeutic potential of HNP-1 against experimental tuberculosis [83]. The three-dimensional structures of HNPs have been elucidated by NMR7 and X-ray experiments.

Human β-defensins (HBD) 1-4 have been isolated from both leukocytes and epithelial cells. Beta-defensins play an important role in the innate immune system. Recent research has demonstrated that beta-defensins have other important biological functions like inhibition of viral infection and interaction with Toll-like receptors. [84]. HBD 1-3 has microbicidal activity towards the Gram-negative bacteria and the yeasts *Candida albicans* and *Malassezia furfur*. In addition, HBD-3 has microbicidal activity against Gram-positive bacteria and HBD-4 bactericidal activity against *Pseudomonas aeruginosa* is stronger than that of the other known β-defensins [62]. Defensin research have indicated a major role for these in host defense against infection and revealed their potential in links between the innate and acquired immune system. Recent studies have suggested that defensins increase epithelial cell proliferation, α-defensins display anti-inflammatory activities and have a role in the pathogenesis of chronic obstructive pulmonary disease, cystic fibrosis and inflammatory cardiovascular diseases [85].

Acknowledgments

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