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Antimicrobial Peptides – A Milestone for Developing Antibiotics Against Drug Resistant Infectious Pathogens

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Abstract
Antimicrobial peptides appear to be ever-present and multipotent components of the innate immune defense mechanism used by both prokaryotic and eukaryotic organisms. These antimicrobial peptides differ in amino acid composition, range of antimicrobial specificities, hemolysis, cytotoxicity and mechanisms of action. AMPs have been isolated from a wide variety of animals, both includes vertebrates and invertebrates, and plants as well as from bacteria and fungi. Antimicrobial peptides interact with microbes initially it emphasize the binding of lipids. These peptides exhibit broad-spectrum activity against a wide range of microorganisms includes Gram-positive and negative bacteria, protozoa, yeast, fungi and viruses. A few no of peptides have also been found to be cytotoxic to sperm and tumour cells. In this current review we discuss about the antimicrobial peptides isolated from marine invertebrates, and these peptides may provide the impetus for the development of novel strategies for the prevention of bacterial infections in animals.

Keywords :
Antimicrobial peptides, Antimicrobial susceptibility, Marine invertebrates.

INTRODUCTION

The ubiquitous amount of antimicrobial peptides which are present in marine environment attests to their overall importance in building the defence mechanism in most of the organisms. They are measured part of the humoral natural defence of invertebrates against infections and hence they are also been termed as “natural antibiotics”. Antimicrobial peptides (AMPs) are oligopeptides with a unreliable number (from five to over a hundred) of amino acids. AMPs have a broad spectrum of target organisms rang from viruses to parasites. Previously AMPs have also been referred to as cationic host defense peptides [1], anionic antimicrobial peptides/proteins [2], cationic amphipathic peptides [3], cationic AMPs [4], host defense peptides [5], and α-helical antimicrobial peptides [6]. Over the past two decades there has been a intensive effort to isolate and characterize antimicrobial peptides (AMPs) as an alternative to antibiotics [7], as well as for the foundation of disease-resistant strains of fish through transgenesis [8,9,10]. These peptides are a major factor of innate immune systems, and are found in many tissues and cell types in numerous species, including mammals, insects, fish, and amphibians. Their mechanisms of action and structures are varied, but they all kill microorganisms rapidly [11].

AMPs can be classified into four groups based on their structures: α-helical peptides, extended peptides, β-sheet peptides and loop peptides [12]. The α-helical AMPs, including cecropin, magainin, and pexiganan, constitute a representative class of AMPs and this group of peptides is usually unstructured in aqueous solution and forms amphipathic helices in membranes or membrane-mimicking environments. α-helical AMPs disrupt bacterial membranes by forming carpet-like clusters of peptides. The β-sheet AMPs, such as α-helix, β-sheet defensins, and protegrin, are stabilized by disulfide bridges, and form relatively stiff structures. Many of β-sheet AMPs exert their antimicrobial activities by disrupting bacterial membranes. The extended AMPs, which are principally rich in specific amino acids such as proline, tryptophan, arginine and histidine. The extended AMPs are not active against the membranes of pathogens, but they can achieve their antimicrobial activities by penetrating across the membranes and interacting with bacterial proteins inside. The loop AMPs, including bactenecin, adopt a loop formation with one disulfide bridge. [12]

AMPs provide alternative ways of eliminating invading pathogens. The majority of AMPs share two features that enable them to interrelate with microbes: i) they have a net positive charge, which enable them to interact through electrostatic forces with bacterial membranes which in turn are predominately anionic, and ii) they can form amphiphatic structures in hydrophobic environments and thus break through into the bacterial phospholipid bilayer [13].Natural antimicrobial peptides exhibit broad spectrum activities against bacteria, fungi, viruses and play an important part in innate immunity [14,15]. Because many AMPs kill bacteria by disruption of membrane integrity.

The interest in AMP reflects both their relevance to intrinsic host defence, and their potential development as therapeutics. Marine invertebrate are expected to be an essential source for antimicrobial molecules. The field of marine invertebrate AMP was not broadly studied yet and in this review it gives an idea about the antimicrobial peptides in marine invertebrates.

ANTIMICROBIAL PEPTIDES IN MARINE INVERTEBRATES

Phylum Echinoderms

Two novel families of AMPs were isolated and characterized from the coelomocytes of 66 individuals of the green sea urchin, *Strongylocentrotus droebachiensis* [16]. The centrocins, purified from the sea urchin *S. droebachiensis*, are hetero-dimeric AMPs consisting of a heavy chain and a light chain connected with one Disulphide Bridge [16]. Here, the heavy chain alone exhibits the same antimicrobial activity as the native molecule. Hemolymphs of the three echinoderm species *H. tubulosa, Cucumaria* sp. and *P. lividus* contain no cationic peptide host defence, which is in agreement with previous attempts to isolate this kind of molecules from other echinoderm species [17].

Phylum Mollucsa

Animals classified under the phylum Mollusca are extremely diverse in form. Mollusks such as squid, chitons, clams, snails, slugs, octopus, and oysters have been [18] identified as Antimicrobial proline-rich peptides from the hemolymph of marine snail *Rapana venosa*. In the hemolymph of mollusks they have explored the isolation, identification and characterisation of 11 novel antimicrobial peptides. The isolated peptides from the hemolymph have molecular weights between 3000 and 9500 Da and this was determined by mass spectrometric analysis such as ultrafiltration and reverse-phase high-performance liquid chromatography (RP-HPLC). The N-terminal sequences of the peptides identified by Edman degradation method matched no peptides in the MASCOT search database, but it was identified as a novel proline-rich peptides. UV spectra confirm that these substances have the characteristics of protein peptides with acidic isoelectric points. Four of the Pro-rich peptides also showed strong antimicrobial activities against tested microorganisms including Gram-positive and Gram-negative bacteria. Three different concentrations in the solutions of the peptides (2, 10 and 50 l), isolated from the hemolymph of *Rapana* were analyzed for antibacterial activity against Gram-positive (*S. aureus*) and one Gram-negative (*K. pneumoniae*) bacteria. Seven to eleven peptides exhibit antimicrobial activity against these two bacterial strains.

The first AMP discovered in bivalve molluscs was in the 1990s through reverse genomics, i.e. from biochemical purification of active peptides, to cDNA and gene sequencing [19, 20] identified a putative antimicrobial sequences from the histone-H2A of backwater oyster *Crassostrea madrasensis*, rock oyster *Saccostrea cucullata*, grey clam *Meretrix casta*, shell *Ficus gracilis*, and ribbon bullia *Bullia vittata*. The 25 amino acid peptide exhibited high similarity to previously reported histone-H2A-derived AMPs from invertebrates indicating the presence of an antimicrobial sequence. Physicochemical properties of the peptides has the characteristic features of antimicrobial peptides, it indicates their potential role in innate immunity of molluscs. Hemocytes are predominantly responsible for innate immune defense and release AMPs [21] defensins were identified in hemocytes of the mussel *Mytilus galloprovincialis* (MGD-1 and -2) [22]. A novel Arg- and Cys-rich AMP named myticin B (40 amino acids) isolated from hemocytes of *M. galloprovincialis* showed antifungal activity against *F. oxysporum* with MIC 5-10 μM. Myticin A possessing a similar amino acid sequence to that of myticin B was not antifungal at 20 μM. Mytilin B, a 34-residue AMP containing 4 intramolecular disulfide bonds, purified from hemocytes of the same species exhibited antifungal activity against *F. oxysporum* with MIC 0.7-1.4 μM [23]. Mytimycin, a novel antifungal Cys-rich polypeptide of 6.2 kDa that hindered the growth of fungi, was isolated and partially characterized from *M. edulis* and were active against *M. luteus* [24]. Dolabellin B2, a 33-residue antimicrobial peptide isolated from the body wall of the sea hare *D. auricularia* and it shows fungicidal activity against *S. cerevisiae* (IC_{50}~25 μg/ml), and it shows fungistatic agaist *C. albicans* [25]. In the mollusk *M.galloprovincialis*, the different AMPs act complementarily, with mytimycin being a purely anti-fungal agent, defensins and myticins are essentially active against Gram positive bacteria, and the mytilins display a broader activity spectrum depending on the isoform [23]. A narrow antimicrobial spectrum of AMPs shows that cationicity or amphipathicity alone are not sufficient for microbial killing/inhibition. This is further supported by the relatively few, but still highly active, anionic AMPs which have been characterized in later years [26,27] have isolated antibacterial and antifungal peptides from the blood of immune-challenged untreated mussels (*Mytilus edulis*). They have characterized the two isoforms of a novel 34-residue, cysteine-rich, peptide with potent bactericidal activity and partially characterized a novel antifungal peptide of 6.2-kDa containing 12 cysteines.

Phylum Annelida

Aurelin, a novel antimicrobial peptide which are isolated from jellyfish *Aurelia aurita*. This peptide which consist of novel 40-residue antimicrobial peptide, aurelin, which exhibit activity against Gram-positive and in the mesoglea of a scyphoid jellyfish *Aurelia aurita* gram negative bacteria was purified by preparative gel electrophoresis and RP-HPLC method. Molecular mass of 4296.95 Da and complete aminoacid sequencing of aurelin was obtained. Two novel antimicrobial peptides such as theromacin and theromyzin were isolated and characterized from the coelomic liquid of the leech *Theromyzon tessulatum* [28]. Theromacin has 75-amino acid cationic peptide which contains 10 cysteine residues. It is arranged in a disulfide array showing no similarities with other known antimicrobial peptides. Another peptide Theromyzin has 86-amino acid linear peptide and it constitutes the first anionic antimicrobial peptide observed in invertebrates. Both these peptides exhibit activity against Gram-positive bacteria [29].

Phylum Porifera

Two AMPs produced by Porifera are Stylisin and Discodermin A [30], sponges contain a large variety of bioactive compounds, including cytotoxic and antimicrobial [31], many of which are considered as microbial symbiont origin. Discodermin A, the first

bioactive sponge peptide isolated from *Discodermia kiiensis*, contains a large number of D-amino acids and such unusual amino acids as *tert*-leucine (*t*-Leu), cysteinoic acid (Cya), and sarcosine (Sar) [32,16].Marine sponges of the genus *Theonella* are prolific in bioactive metabolites possessing unusual structures [33]. Theonellamide F is a bicyclic peptide isolated from a Japanese *Theonella sp.* containing several unusual amino acids, e.g. histidinoalanine, 3-methyl-*p*-bromophenylalanine, (2*S*,4*R*)-2-amino-4-hydroxyadipic acid (L-Ahad), and (3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8- (*p*-bromophenyl)-5,7-octadienoic acid (Aboa). It inhibited the growth of *C. albicans* with an MIC 6.3 µg/ml [32].

Phylum Chordata

Plicatamide [34] and the halocyamines [35], having 8 and 4 aa residues, respectively. These AMPs from tunicates show antimicrobial activity. Antimicrobial activities were detected in the two tunicates like *Microcosmus sabatieri* and *Halocynthia papillosa*. The characterization and separation of two novel peptides from *H. papillosa* hemocytes. These molecules exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. Complete peptide characterization was obtained by a combination of Edman degradation and mass spectrometry. The mature molecules, named halocyntin and papillosin, comprise 26 and 34 amino acid residues, correspondingly. Their primary structure exhibits no significant similarities with previously described AMP [36]. Mass spectrometry and analysis of the wide-scan spectrum expose the deduced molecular masses of 2731.55 and 3318.44 Da for the two molecules isolated from one of the fraction. Halocyamine A and B were the first, isolated from the solitary ascidian *Halocynthia roretzi* [35]. These two small tetrapeptides are reported to have antimicrobial activity against both Gram-negative and Gram-positive bacteria, but also to have cytotoxic activities against different eukaryote cell strains. Short AMP was characterized as an octapeptide named plicatamide and it was isolated from *Styela plicata* [34].

Phylum Arthropoda

A defensin- like peptide also takes part in the immune defense of the nematode *Ascaris suum* [37]. Sequence comparisons between all insect defensins reveal that similarities are ranging from 58 to 95% and 75% similarities are observed between the defensin isolated from the dragon y *Aeschna cyanea* which belong to the ancient order of the Odonata and the scorpion and mussel defensins. a defensin with an N-terminal extension of six residues has been isolated from the gut of the dipteran Stomoxys calcitrans [38]. Crustin (MrCrs) was isolated and sequenced from a freshwater prawn *Macrobrachium rosenbergii*. The MrCrs protein contains a signal peptide region at N-terminus between 1 and 22 and a long whey acidic protein domain (WAP domain) at C-terminus between 57 and 110 along with a WAP-type 'four-disulfide core' motif. The recombinant MrCrs protein agglutinated with the bacteria measured for analysis at a concentration of 25 mg/ml, except *Lactococcus lactis.* The bactericidal results exhibit that the recombinant MrCrs protein destroyed all the bacteria after incubation, even less than 6 h. These results imply that MrCrs is a potential antimicrobial peptide, which is involved in the defense system of *M. rosenbergii* against viral and bacterial infections [39].Horseshoe crab contains tachyplesin precursors it consist of 77 amino acids with 23 residues in a presegment, and that there are two types of mRNAs equivalent to the isopeptides tachyplesins I and II. Tissues of the horseshoe crab exposed that the tachyplesin precursors are uttered mainly in hemocytes and cardiac and brain tissues. The tachyplesin precursor consists of a single peptide of 23 amino acid residues, a mature peptide followed by an amidation signal "Gly-Lys-Arg" and an supplementary carboxyl-terminal sequence of 34 residues, including an acidic amino acid cluster[40].Two novel molecules isolated from hemolymph of evident scorpions of the species *Androctonus australis* are (i) androctonin, a 25-residue peptide with two disulfide bridges, active against both Gram-positive and Gram-negative bacteria and fungi and showed marked sequence homology to tachyplesins and polyphemusins from horseshoe crabs; and (ii) buthinin, a 34-residue both antibacterial peptide with three disulfide bridges. The third peptide contains 37 residues and three disulfide bridges and visibly belongs to the family of anti-Gram-positive insect defensins [41]. Penaeidins are 5.5- to 6.6-kDa antimicrobial peptides newly isolated from the plasma and haemocytes of the tropical shrimp *Penaeus vannamei*. These molecules diverge from the other classes of antimicrobial peptides in that they are composed of a proline-rich N-terminus and of a C-terminus containing six cysteine residues unavailable in three disulfide bridges. Two penaeidins (Pen-2 and Pen-3a) were uttered in *Saccharomyces cerevisiae*. The recombinant Pen-2 and -3a were characterized in terms of primary structure by Edman degradation, mass spectrometry and gas chromatography. Pen-2 and -3a activity express that penaeidins have a broad spectrum of antifungal properties associated with a fungicidal activity, and that their antibacterial activities are basically directed against Gram-positive bacteria, with a strain-specific inhibition mechanism [42]. The biological properties and primary structure of a small granular component S2, named tachycitin. This component was purified from the acid extract of hemocyte waste by two steps of chromatography. The purified tachycitin was a single chain protein with an evident Mr=8,500 by Tricine SDS-polyacrylamide gel electrophoresis. Ultracentrifugation analysis exposed tachycitin to be present in monomer form in solution. Tachycitin inhibited the growth of both Gram-negative and -positive bacteria, and fungi, with a bacterial agglutinating property. Additionally, tachycitin and big defensin acted synergistically in antimicrobial activities. The amino acid sequence and intrachain disulfide bonds of tachycitin were determined by amino acid and sequence analysis of peptides produced by enzymatic cleavages. The mature tachycitin consist of 73 amino acid residues which contain five disulfide bonds with no N-linked sugar. Tachycitin may correspond to a new class of chitin-binding protein family in animals.

CONCLUSION

Antimicrobial peptides (AMPs) are small, naturally occurring peptides that display strong antibacterial properties generally believed to be a result of selective bacterial membrane disruption. As a result there is an augment in consideration has been focused on AMPs due to their promising effect on novel therapeutics. Marine invertebrates, representing an enormous genetic and biological diversity, have proven to be a rich source for discovering potent AMPs with novel and unique structural motifs. Tachyplesins has 17,18 amino acid long peptides . This peptide was isolated from the haemocytes of the Japanese horseshoe crab, *Tachypleus tridentatus* [43]. It consist of β-sheet forming peptides which are synthesized as a large 77-residue precursor that contains an acidic amino acid cluster in its C-terminal portion [44]. Polyphemusin is an isoform of Tachyplesins peptide which has been purified from the haemocytes of *Limulus polyphemus*, a horse shoe crab [45]. crustins and penaeidins was more active against Gram positive bacteria [46]. In the mollusk *M. galloprovincialis*, defensins and myticins are essentially active against Gram positive bacteria, and the mytilins display a broader activity spectrum depending on the isoform [23]. This differentiated antimicrobial spectrum may be explained by differential lipid composition in the membrane of the microorganisms, or an inability to penetrate the outer membrane of certain Gram negative microorganisms. Nevertheless, a narrower antimicrobial spectrum of AMPs shows that cationicity or amphipathicity alone are not sufficient for microbial killing/inhibition. This is further supported by the relatively few, but still highly active, anionic AMPs which have been characterized in later years [26].Finally it is hoped that this review herein offers a comprehensive view of antimicrobial peptides in marine invertebrates.

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