Antimicrobial Peptides from the Marine Fishes

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Abstract: Fishes are one of the organisms that have managed to survive in a milieu of pathogenic organisms. The primary interference of fish with their environment happens through a mucous layer that covers its entire body. Marine fishes possess antimicrobial peptides as a part of their defense system, which are mainly present in the mucous layer indicating that they eliminate the pathogenic bacteria before they enter the skin barrier. A number of α-helical Antimicrobial Peptides (AMP) such as Pardaxins, Misgurin, Pleurocidins, Parasin, Oncorhyncin II and III, Chrysopholin and HFIAP (Hagfish Intestinal Antimicrobial Peptide) have been isolated from different species of fishes. However, studies on the role of antimicrobial peptides in fishes are very limited. Various mechanisms developed by multicellular organisms in nonspecific immunity raises questions on the role of antibiotic peptide as a deterrent against infection. The present study provides a general introduction to the subject with special emphasis on the role of bioactive peptides in marine fishes.

Key words: Marine fishes, antimicrobial peptide, mucous, immunity, bacteria

INTRODUCTION

Antimicrobial Peptides (AMPs) are regarded as an important component of the first-line defence in various animal species and are even more important in fish when compared with mammals as fish rely more on their innate immune system (Hancock, 1997; Hancock and Scott, 2000). In addition to the highly specific cell-mediated immune system, vertebrates and other organisms have a defense system made up of distinct groups of broad-spectrum antibacterial peptides (Boman, 1991, 1994, 1995; Zasloff, 1992). Rameshkumar et al. (2009a) proved that marine crabs Charybdis lucifera possess an antimicrobial peptide in their hemolymph. The main advantage of the antibacterial peptides as factors of innate immunity is that they can function without either high specificity or memory. An antibacterial peptide which is isolated from Thalamita crenata shows immense activity towards human bacterial pathogens (Rameshkumar et al., 2009b). Antibacterial peptides are promptly synthesized at low metabolic cost, easily stored in large amounts and readily available shortly after an infection. The haemolymph proteins of marine invertebrates are unique in composition, as they do not contain immunoglobulin or albumin like proteins and the protein (Rameshkumar et al., 2009c).

Several AMPs have been isolated from fish, such as Pleurocidins from winter flounder, Pleuronectes americanus (Walbaum), American plaice, Hippoglossoides platessoides

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Immune Mechanisms of Fish

Fish which form minor and major links in food webs of the aquatic ecosystems harbor a wide array and a time large numbers of parasites. Fish represent not only the earliest, but also the largest class of vertebrates. This triumph of fish has been accomplished despite the fact that they possess both slower and less developed adaptive immune systems than those of higher vertebrates. Due to their aquatic environment, fish have unique anatomical and physical characteristics. Fish live in intimate contact with an environment containing both saprophytic and pathogenic microbes capable of digesting and degrading fish tissues (Ellis, 2001; Plouffe et al., 2005). The slow adaptive immune response of fish makes innate immunity, which is fast acting and temperature independent (Ellis, 2001) the predominant system of fish host defense. This innate immune response is essential for the survival of this whole class of animals. The defense includes many elements such as antimicrobial peptides (Cole et al., 1997) and polypeptides (Fernandes and Smith, 2002) non-classical complement activation, release of cytokines, inflammation and phagocytosis (Ellis, 2001; Magnadottir, 2006) Concisely, fish have evolved a number of innate immune responses to defend themselves against infection.

Source of AMPs

So far, more than 750 different AMPs have been identified in various organisms ranging from insects to plants to animals including humans (Mendez et al., 1990; Liu and Hansen, 1990; Breukink and Kruijff, 1999; Schnapp et al., 1998). Besides these, bacteria themselves produce AMPs and about 50 of them have been isolated from various Gram-positive bacteria especially lactic acid-producing organisms (Luders et al., 2003) Most of these peptides are synthesized as a propeptide consisting of an N-terminal signal sequence (which aids in targeting of endoplasmic reticulum), a pro segment and a C-terminal cationic peptide that demonstrates antimicrobial activity after it is cleaved from the rest of the protein. These peptides have been grouped based on their primary structure, amino acid composition and their size.
Classification of AMPs

Nuclear Magnetic Resonance (NMR) has emerged as a useful technique for studying details of structures of most of the known antimicrobial peptides. Analysis of the three dimensional structure of these peptides has led to the better understanding of their function. Since a majority of these peptides are small in length, their three dimensional structures can be obtained using conventional two dimensional NMR methods. Based on the NMR structures of known peptides along with sequence analysis AMPs are broadly classified into five groups.

Helical AMPs

Much of the structural and biochemical work has been focused on Cecropins, which were the first to be identified and characterized (Steiner et al., 1981). All cecropins have helix-forming tendencies in certain organic co-solvents like Trifluoroethanol (Cammers-Goodwin et al., 1996). Initial studies with NMR showed that cecropin-A from H. cecropia exhibited a helical pattern in 15% Hexafluoroisopropyl alcohol (Holak et al., 1988). The results suggested a highly amphipathic helix with hydrophobic and cationic charged surfaces, a motif observed in many other AMPs. Magainins are another group of well characterized peptides composed of 23 residues isolated from the skin of the African clawed frog, Xenopus laevis (Matuzusaki, 1999). NMR studies showed that like cecropins, magainins also form amphipathic -helical structures in 25% Trifluoroethanol (Marion et al., 1988).

Cysteine Rich AMPs

The human neutrophil peptides HNP-1, -2 and -3 were first of the cysteine-rich peptides isolated from the human granules (Ganz et al., 1985). These defensins are 30 amino acid peptides rich in cysteine residues and are present in a wide variety of organisms. Most of these defensin molecules harbour a consensus motif of six cysteine residues forming three in trimolecular disulfide bonds. The positions of the disulfide bridges are mostly between C1-C4, C2-C5 and C3-C6. X-ray crystallography studies with HNP-3, in combination with sedimentation equilibrium centrifugation, suggest that the peptide exists as a dimer. The NMR structure of defensin shows the presence of three-stranded antiparallel-sheets. Dorosomycin, isolated from drosophila contain four disulfide bonds and are made up of three antiparallel strands with a helix in between the first two strands (Landon et al., 1997).

β-Sheet AMPs

A few of the known AMPs form a single hairpin structure and are approximately 20 residues long containing one or two disulfide linkages. Horseshoe crab peptides, tachyplesins and polyphemusin II, both share a hairpin motif stabilized by two disulfide bonds (Kawano et al., 1990; Tamamura et al., 1993). NMR studies along with 3D structures indicate that tachyplesin shows strong resemblance to protegrins, peptides isolated from porcine leukocytes. Both these molecules forms antiparallel -sheet connected to a turn and is composed of two disulfide bridges (Tamamura et al., 1993). NMR studies with thanatin isolated from the hemipteran insect P. maculiventris showed results similar to that of tachyplesin, including an antiparallel sheet maintained by a single disulfide bridge. Lactoferricin B, a 25 amino acid proteolytic derivative of lactoferrin in solution adopts a sheet structure stabilized by a single disulfide bond, as shown by NMR studies (Hwang et al., 1998). Schneider et al. (1998) have demonstrated that one can put together a novel combination of peptide synthesis modules and arrive at a novel structure.
AMPs Rich in Regular Amino Acids

Some AMPs are composed of high numbers of regular amino acids. The structural conformation of such peptides are different from the regular α-helical or β-sheet peptides.

Histatin, a peptide isolated from human saliva is rich in histidine residues and is active against *C. albicans* (Xu et al., 2005) while cathelicidins are proline rich peptides and have irregular structures, indolicidins (Selsted et al., 1993) and tritripticin (Lawyer et al., 1996), are rich in tryptophan. Bacteriocins Bac-5 and Bac-7, like cathelicidins, are proline-rich (Lawyer et al., 1996) while the peptide PR-39, is rich in arginine residues (Agerberth et al., 1991).

AMPs with Rare Modified Amino Acids

Few peptides are unusual as they are composed of rare modified amino acids. Best examples of such peptides are those produced by the bacteria themselves. Nisin, a lantibiotic, is one such peptide produced by *Lactococcus lactis* and is composed of rare amino acids like lanthionine, 3-methylanthionine, dehydroalanine and dehydrobutyrine (Devos et al., 1993). The peptide is active against Gram-positive bacteria and shows no defined structural conformation in water, while it reveals several turn structures when bound to dodecylphosphocholine. Another peptide leucocin A, a 37-residue AMP isolated from *Leuconostoc gelidum*, is shown to form an amphiphilic conformation well suited for interacting with membranes (Fregeau et al., 1997). Such peptides undergo post-translational modification that result in conformations not seen in other classes of antimicrobial peptides. The gramicidins are composed of several DH-amino acids that allow them to form an unusual cyclic hairpin (Gibbs et al., 1998).

Mechanism of Action for Antimicrobial Peptides

Considering that AMPs are natural barriers to bacterial infections, pathogens ought to have developed a variety of strategies that render them resistant to antimicrobial host defenses. The only currently available structural model explaining the mechanism of action of AMPs Matzusaki (1999) explained the action of these peptides is from the outside and over the pathogen's membrane either by increasing their permeability or by destabilizing membranes by changing the net charge of the composed system. Since biological membranes are indeed dynamic fluids, the generation of resistance appears to be less likely to occur. Nonetheless, pathogens have evolved countermeasures not to resist, but at least to limit AMPs' effectiveness, such as chemical modifications and/or alternation of energy-dependent pumps at the membrane level. The same is true for intracellular bacterial pathogens, in which resistance-limitation is less effective against mostly cationic peptide-driven antimicrobial activity existing in the phagosomes of circulating monocytes, neutrophils and some mucosal epithelial cells. Additionally, the fact that the common features for most peptides are a net positive charge and an amphipathic nature, allows them to persist at water-lipid interfaces and then to disturb microbial membrane components (Ruissen et al., 2001).

Most of the peptides without disulfide bridges have random structures in water and it is only when they bind to a membrane or other hydrophobic environment, or self-aggregate, that these peptides form a structure. For example, cecropins and melittin fold into amphipathic alpha-helices in membranous environments. It is known that the dual cationic and hydrophobic nature of the peptides is important for the initial interaction between the peptide and bacterial membrane. Cationicity promotes interaction with bacterial outer and cytoplasmic membranes. Also, hydrophobicity is important and e.g., increasing the hydrophobic moment of magainin analogues causes increased binding of the peptide to the
membrane due to increased hydrophobic interactions between lipid acyl chains and the hydrophobic helix core (Wieprecht et al., 1997). An overview of the proposed interaction of peptides with the cell envelope membranes of gram-negative bacteria is given in Fig. 1.

The mechanism by which antimicrobial peptides act has become a complex issue. It is important to understand how the peptides act to fully exploit the use of peptides as antimicrobial agents. Small sequence changes can lead to major changes in activity. Not only is antimicrobial activity difficult to predict, but so are cytotoxic activities. Other peptides are selective for tumor over normal host cells. It is also very difficult to predict which peptides will be active in vivo based on in vitro MICs. However, many peptides do have reasonable activities in animal models without obvious toxicity (Hancock, 1997) and thus have been considered for potential use in the clinic route for passage of ions. To try to resolve such a dilemma arising from model membrane studies, have devised an assay based on measurement of the effects of peptides on the trans cytoplasmic membrane potential gradient. This assay showed that only certain peptides completely depolarize the cytoplasmic membrane of Escherichia coli at their MICs. However, they cause partial collapse of membrane potential at concentrations well below their MICs (an observation that contradicts the carpet model, which suggests that when peptides achieve a threshold concentration, the membrane is destroyed a phenomenon that is also not visible in electron micrographs). Still other peptides (e.g., indolicidin and bactenecin) do not permeabilize the cytoplasmic membrane to any great extent at their MICs and a separate mechanism of action is suggested. For different cationic peptides this has been proposed to be an action on the nucleic acids of bacteria or a triggering of autolysis. The bactericidal effects of these peptides tend to be extremely and therefore, it is difficult to monitor the stages of bacterial killing. Human lactoferrin peptides have a relatively slow action and for these peptides, it has been shown that membrane potential collapses, followed by membrane integrity, resulting in cell lysis. It has been also observed that the structures of human lactoferrin peptides alter with time once the peptides are bound to bacterial cell wall constituents and that the peptide does not form pores.
Antimicrobial Peptides Isolated from Fishes

The low infection rate of fish is remarkable and has inspired further studies of their innate defense system. Few antimicrobial peptides have been identified in fish. However, the number of microbial peptides being isolated and identified from the epidermal cells or secretion of the skin, gills and intestines of bony fish (teleosts) is constantly increasing. Some of these antimicrobial peptides have high sequence homology to known proteins with other function, suggesting a derivation from cleavage products of larger proteins, such as Histones (Park et al., 1998; Patrzykat et al., 2001; Birkemo et al., 2003; Fernandes et al., 2003, 2004) and ribosomal proteins (Fernandes and Smith, 2002). Peptides considered to be dedicated to the innate immunity have been isolated or cloned from fish and their expression has been analysed.

Fish have been largely ignored as a potential source of antimicrobial peptides. Of approximately 600 peptide antibodies that have been isolated from various animals, relatively few have been identified from fish (Table 1).

A number of antimicrobial polypeptides and other defense components have been identified in many fish species. They includes natural antibiotics (Mendez et al., 1990) Apolipoproteins (Concha et al., 2003, 2004) a number of different isotypes of Muramidase (lysozyme) (Fernandes et al., 2004) permeability-increasing protein (Xu et al., 2005), Squalamine (Moore et al., 1993) and other unidentified antimicrobial factors (Ourch and Chung, 2004). However, sequences have been reported from only a minimal number of fish species (Douglas et al., 2003a, b), the largest vertebrate group containing over 23,000 species (Table 2).

Future Role of Antimicrobial Peptides as Therapeutic Agents

Microbial pathogens occupy and exploit a diverse variety of tissues and niches where they must confront antimicrobial peptide-mediated host defenses to survive. Thus, it is unrealistic to expect that no microbial pathogens are able to resist antimicrobial peptides. Rather, it is essential to understand whether a pathogen resists a given peptide and if so, through constitutive or inducible mechanisms. The main advantage of antimicrobial peptides

Table 1: Peptide antibiotics isolated from fish

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Fish</th>
<th>Approximate MW and No. of amino acids</th>
<th>Location</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFIAP</td>
<td>Hagfish intestinal atlantic hagfish antimicrobial peptide</td>
<td>3.5-4.6 kDa (30-37 AAs)</td>
<td>Intestine</td>
<td>Shinme et al. (1996)</td>
</tr>
<tr>
<td>Pardoxins</td>
<td>Red sea moose sole</td>
<td>3.3 kDa (33AA)</td>
<td>Skin (mucus gland)</td>
<td>Oren and Shai (1996)</td>
</tr>
<tr>
<td>Pleurocidins</td>
<td>Winter flounder</td>
<td>2.7 kDa (25 Aas)</td>
<td>Skin intestine</td>
<td>Cole et al. (1997)</td>
</tr>
<tr>
<td>Piscidins</td>
<td>Hybrid striped bass</td>
<td>2.5 kDa (22 Aas)</td>
<td>Skin, gill</td>
<td>Douglas et al. (2001)</td>
</tr>
<tr>
<td>Misgurins</td>
<td>Loach</td>
<td>2.5 kDa (21 Aas)</td>
<td>Whole fish</td>
<td>Park et al. (1997)</td>
</tr>
<tr>
<td>Hepcidins</td>
<td>White bass in other tissues</td>
<td>2.3 kDa (21 Aas)</td>
<td>Liver, low expression</td>
<td>Shi et al. (2002)</td>
</tr>
<tr>
<td>LCRP</td>
<td>Sea lamprey</td>
<td>2.2 kDa (19 Aas)</td>
<td>Skin</td>
<td>Conlon and Sower (1996)</td>
</tr>
<tr>
<td>Parxin-1</td>
<td>Asian catfish</td>
<td>2.0 kDa (19 Aas)</td>
<td>Skin</td>
<td>Park et al. (1998)</td>
</tr>
<tr>
<td>HSDF</td>
<td>Coho salmon</td>
<td>NR (26 AAs)</td>
<td>Mucus, blood</td>
<td>Patrzykat et al. (2001)</td>
</tr>
</tbody>
</table>

NR: Not reported, AA: Amino acids

Table 2: Antimicrobial polypeptides cloned from fish tissue

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Fish</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathelicidins</td>
<td>Atlantic hagfish</td>
<td>Uzzell et al. (2003)</td>
</tr>
<tr>
<td>Cathelicidins</td>
<td>Rainbow trout</td>
<td>Chang et al. (2005)</td>
</tr>
<tr>
<td>Hepcidins</td>
<td>Red sea bream</td>
<td>Chen et al. (2005)</td>
</tr>
</tbody>
</table>
for innate defense is that they are small molecules and can be synthesized in a matter of hours, unlike components of adaptive immune response, which take days and can eliminate two or more intruders at the same time without requiring specific recognition for each foreign invader (Boman, 1995). As was mentioned earlier fish too possess antimicrobial peptides. In nature, where there are fewer stress factors present, their native antimicrobial peptides may be sufficient to protect fish against infections. However, in aquaculture facilities fish not only have to live in a plethora of microbes, but also encounter stress and physical injuries caused by other fish or the environment itself. These conditions set up the optimal circumstances for pathogens to prey on the susceptible host (Thune et al., 1993; Pickering, 1974).

Besides revealing the elevated level of host protection against pathogens, in vivo studies proved that insect borne diseases such as malaria can be prevented using insects carrying symbiotic bacteria transformed with an antimicrobial peptide gene and transgenic mice can be used to produce tracheal antimicrobial peptides, potential antibiotics for the treatment of cystic fibrosis. Currently, antimicrobial peptides are in trials for their use in clinics for the treatment of skin infections associated with burns, diabetic wounds and eye infections (Boman, 1994, 1995). Evidently, the genes encoding these potent antimicrobial peptides represent good candidates for the genetic improvement of fish stocks to react to bacterial diseases.

CONCLUSIONS

Constitutive and inducible mechanisms of resistance to antimicrobial peptides are becoming clearer with the advent of genetically modified pathogens and the availability of reagent quantities of native or synthetic antimicrobial peptides. As with constitutive responses, it is not surprising that many of the mechanisms responsible for inducible resistance involve modifications of the pathogen envelope and/or extracellular facet of the cytoplasmic membrane directly offsetting mechanisms of peptide action.

In recent time has seen important progress in identification of new endogenous antimicrobial peptides and in the ascertaining of signals that can regulate their expression. The demonstration that a functional deficiency of endogenous antimicrobial peptides may contribute the persistent airway infections seen in patients with cystic fibrosis has increased attention and directed efforts to antimicrobial defenses of epithelial cells and mucus membranes. Expansion of work in this area should further clarify the effector mechanisms of innate immunity.

Recently marine peptides have opened a new perspective for pharmaceutical developments. The present review clearly shows antimicrobial peptides isolated from fishes would be a good source of antimicrobial agents and would replace the existing in inadequate and cost effective antibiotics.

ACKNOWLEDGMENT

We are grateful to thank our Director, Centre of Advanced Study in Marine Biology and Ministry of Earth science, Govt of India, for rendering encouragement and support.

REFERENCES


