ISJ 7: 157-164, 2010 ISSN 1824-307X

MINIREVIEW

# Defensins and cystein rich peptides: two types of antimicrobial peptides in marine molluscs

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*Accepted June 3, 2010*

## Abstract

This review focuses on defensins and cystein rich peptides, which are the most abundant natural antimicrobial peptides (AMPs) described in molluscs. These are compact peptides, 3-5 kDa in molecular mass, cationic and amphipatic; the presence of at least six cysteine residues forming three or four disulfide bridges is their prime structural characteristic. A 3-D structural characterization of these molecules has been included in recent investigations, using currently-available techniques. AMPs have been purified from hemocytes, epithelial tissue and plasma as well as cloned and chemically synthesized. Their antibacterial activity against Gram-positive and Gram-negative bacteria and fungi has been shown; only a synthetic mytilin fragment has displayed activity against viruses.

**Key Words**: defensins; cystein rich peptides; antimicrobial peptides; innate immunity; marine molluscs

### Introduction

Marine molluscs are exposed to microbial pathogens in their environment, which can number up to $10^6$ bacteria/ml and $10^9$ virus/ml of seawater (Ammerman *et al.,* 1984). In order to defend themselves against such condition, molluscs have developed very effective mechanisms that are part of their innate immunity (Tincu and Taylor, 2004). Antimicrobial peptides (AMPs) are the major component of the innate immune system in marine invertebrates (Destoumieux *et al.,* 1997, Mercado *et al.,* 2005; Arenas *et al.,* 2009; De Zoysa *et al.,* 2009). The first research on AMPs in bivalve molluscs through reverse genomics was done at the end of the 90s (Hubert *et al.*, 1996). AMPs are distinguished by their net positive charge and amino acidic residue amphipathic distribution; these key features explain their mode of action with the membrane of target microorganisms (Marshall and Arenas, 2003). In order for the synthetic peptides to maintain the antimicrobial activity, they must be able to form an amphipathic structure, i.e., they must be organized in hydrophobic and hydrophilic amino acid zones (Zasloff, 2002; Arenas *et al.*, 2009).

A general mechanism of action has been proposed describing the sequence of associated events occurring once the peptides are initially attracted to the target membrane of microorganisms by electrostatic attraction. Then, hydrophobic interactions with the membrane ensue, followed by accumulation of the peptide until a threshold concentration is achieved. The peptide then adopts a new dynamic conformation that causes a deformation of the membrane, followed by a transient peptide conformation which enables it to insert into the membrane. In the next step the peptides multimerize forming complexes such as barrel-staves or toroid pores. In the final stages the peptide is translocated to the cytoplasmic face of the membrane to exert its action on membranous cytosolic components. Different types of AMPs follow some or all of the steps described above. (Cudic and Otvos, 2002; Zasloff, 2002; Yeaman and Yount, 2003).

The purification procedure is summarized as follows: The homogenized samples are suspended in cold acetic acid 11 % (1:10) in order to solubilize cationic molecules and sonicated for 3x30 sec at 11 RMS in ice. The crude extract is centrifuged at 11,000x*g*, 35 min at 4 °C and the pellet is discarded. The supernatant is called acid extract (AE) and is further shaked at 37 °C for 1 h to favor sugar hydrolysis. The acid extract is loaded on a Sulfoethyl (SE) Sephadex C-50 cation-exchange chromatography column (Biorad), eluted with 1 M NaCl - 1 % acetic acid ( pH 3.0), in order to enrich cationic peptides. The eluate is applied onto a Sep-pak C18 Vac cartridge (Waters associates)

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equilibrated in acidified water (0.05 % trifluoroacetic acid in UPW (Ultra Pure Water). After a wash with acidified water, the peptides are eluted with 5 %, 20 %, 40 %, 60 % and 80 % acetonitrile (ACN), to obtain several hydrophobic fractions. The samples obtained are lyophilized and reconstituted in MilliQ water, total protein content determined by the bicinchoninic acid (BCA) microplate assay (Pierce) and tested for antibacterial activity. Only those with antimicrobial fraction activity are subjected to reversed phase HPLC. All purification steps are performed on a RP-HPLC model LaChrom D-7000 with a LaChrom model L-7455 photodiode array detector. Column effluents are monitored by UV absorption at 225 nm. Eluates are selected for further purification and loaded on a Sephasil C-18 (250x4.1 mm) column (LiChroCART). Elution is performed with a linear gradient of 5-60 % ACN in acidified water over 90 min at a flow rate of 0.6 ml $min^{-1}$. The resulting fractions are collected, lyophilized, reconstituted in ultra-pure water (UPW ) and frozen at -20 °C until antimicrobial activity testing (Bulet *et al.,* 1991; Charlet *et al*., 1996; Mercado *et al.*, 2005).

Among the different natural AMPs, those containing pairs of cysteine residues forming intramolecular disulfide bridges are particularly common (Dimarcq *et al.*, 1998; Bulet *et al.*, 2004; Reddy *et al.*, 2004; Yount *et al.*, 2006). This highly complex 3 - 5 kDa group has been extensively studied in mussels, *Mytilus edulis* and *Mytilus galloprovincialis,* where they were classified into four groups: defensins, mytilins, myticins and mytimycin (Charlet *et al.*, 1996; Mitta *et al.*, 1999a; Pallavicini *et al.*, 2008; Costa *et al.*, 2009; Parisi *et al.*, 2009). Defensins have been also recently described in oysters *Crassostrea virginica* and *Crassostrea gigas* (Seo *et al.*, 2005; Gueguen *et al.*, 2006; González *et al.*, 2007) and abalone *Haliotis discus discus* (De Zoyza *et al.*, 2010); mytilins and myticins, on the other hand, have been also described in clams *Ruditapes decussates* (Gestal *et al.*, 2007).

Defensins and cystein rich peptides from marine molluscs express a stronger activity against Gram-positive and Gram-negative bacteria and fungi (Charlet *et al.*, 1996; Mitta *et al.*, 1999a, b; Seo *et al.*, 2005; Gueguen *et al.*, 2006; Gestal *et al.*, 2007) and one synthetic mytilin fragment displayed activity against the white spot syndrome virus (Dupuy *et al.*, 2004; Roch *et al.*, 2008).

**Defensins and cystein rich peptides from mussels**

For the summary of the AMPs described in mussels and the relative alignments see Table 1 and Fig. 1.

Defensins A (4314.3 Da) and B (4392.4 Da) were purified from the hemolymph of *M. edulis* using chromatographic methods. Both exhibited six cysteines, forming three intramolecular disulfide bridges positioned in a highly conserved array, thus allowing a complex three-dimensional structure The cysteine consensus motif is identical to that found in the large family of arthropod defensins, *Phormia* defensin, described in detail for the *Phormia terranovae* (Charlet *et al.*, 1996). The latter corresponds to a central amphipathic α-helix with an extended NH2-terminal loop and a COOH-terminal antiparallel β-sheet. The helix is stabilized through two disulfide bridges to the β-sheet and the $NH_2$-terminal loop is linked to one of the strands of this sheet via the third disulfide bridge (Cornet *et al.*, 1995). Using the liquid growth inhibition method (Bulet *et al.*, 1993) it was determined the *Mytilus* defensins A and B were consistently more active against the Gram-positive strain *M. luteus* (MIC: 0.6 - 1.2 μM) than to the Gram-negative strain *E. coli* (MIC: 2.5 - 10 μM). The Minimal Inhibitory Concentration (MIC) values are expressed as an interval (a - b), where (a) represents the highest peptide concentration tested at which bacteria are still growing and (b) the lowest concentration that causes 100 % growth inhibition (Charlet *et al.*, 1996).

The defensin isoforms MGD-1 and MGD-2 (4 kDa), containing eight cysteines, were purified from the plasma and hemocytes of mussels, *M. galloprovincialis,* using conventional chromatographic methods. Two extra cysteines and one modified amino acid suggested that these molecules are new members of the arthropod defensins family (Mitta *et al.*, 1999b). The 3-D structure of MGD1 was established using NMR analysis, which mainly consists on the classical CSαβ structural motif (Cys4 - Cys25, Cys10 - Cys33 and Cys14 - Cys35 disulfide bonds). The two extra cysteines (Cys21 - Cys38) form an original fourth disulfide bond. Synthetic MGD1, correctly folded to form the four disulfide bonds, retains the antibacterial activity of the native molecule and presents a similar effect than the insect defensin A, thus proving the fourth disulfide bond of MGD1 was not significant for the biological activity (Yang *et al.*, 2000). A series of synthetic peptides, conforming the main known secondary structures of MGD1, allowed the location of the nonapeptide CGGWHRLRC corresponding to the residues between Cys25 and Cys33. The bacteriostatic activity of such sequence was strictly dependent on the bridging of Cys25 and Cys33. The antibacterial activity of this synthetic nonapeptide clearly evidenced an effect on Gram-positive when tested against the Gram-positive bacteria *Micrococcus lysodeikticus, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium* (MIC 0.6 - 0.8 μM) and the Gram-negative bacteria *Vibrio alginolyticus, Vibrio metschnikowii, Escherichia coli* 363, *Salmonella Newport* (MIC>75 μM) and *Fusarium oxysporum* (MIC 5 μM) (Romestand *et al.*, 2003).

Modelling studies evidenced that positively charged and hydrophobic residues of MGD1 were organized in two discrete domains; this feature would support the hypothesis that positive charges allow the initial attraction of the peptide with the membrane, as well as the hydrophobic domain insertion into the bacterial lipid bilayer (Romestand *et al.*, 2003).

The tissue location analysis through optical and ultrastructural levels showed MGDs were mainly located in the granular structures of the hemocytes and enterocytes, where they were synthesized as an 81 amino acid precursor and processed as active

**Table 1** shows a summary of the AMPs described in marine bivalves

| Peptide | Origin | Acces Number | MW Da | Sequence | Reference |
|---|---|---|---|---|---|
| Defensin A | *M. edulis* | sp \| P81610 | 4314.3 | GFGCPNDYPCHRHCKSIPGRXGGYCGG XHRLRCTCYR | Charlet *et al.*, 1996 |
| Defensin B | *M. edulis* | sp \| P81611 | 4392.4 | GFGCPNDYPCHRHCKSIPGRYGGYCGG XHRLRCTC | Charlet *et al.*, 1996 |
| MGD-1 | *M. galloprovincialis* | sp \| P80571 | 4000 | GFGCPNNYQCHRHCKSIPGRCGGYCG GWHRLRCTC | Mitta *et al.*, 1999b |
| MGD-2 | *M. galloprovincialis* | gb \| AAD52660 | 4000 | GFGCPNNYACHQHCKSIRGYCGGYCAS WFRLRCTC | Mitta *et al.*, 1999b |
| AOD | *C. virginica* | sp \| P85008 | 4265.0 | GFGCPWNRYQCHSHCRSIGRLGGYCA GSLRLTCTCYRS | Seo *et al.*, 2005 |
| Cg-Def | *C. gigas* | gb \| AJ565499 (EST) emb \| CAJ19280 | n.a. | GFGCPGNQLKCN NHCKSISCRAGYCDAATLWLRCTC | Gueguen *et al.*, 2006 |
| Cg-defh1 | *C. gigas* | gb \|DQ400101 | n.a. | GFGCPRDQYKCNSHCQSIGCRAGYCDA VTLWLRCTC | Gonzalez *et al.*, 2007 |
| Cg-defh2 | *C. gigas* | gb \|DQ400102 | n.a. | GFGCPGDQYECNRHCRSIGCRAGYCDA VTLWLRCTC | Gonzalez *et al.*, 2007 |
| Abalone defensin | *H. discus discus* | gb \|FJ864724 | 4900 | KRVTCDLLSLQIMGNSFGDSACAAHCIG LHHSGGHCSGGVCVCR | De Zoyza *et al.*, 2010 |
| Mytilin A | *M. edulis* | sp \| P81612 | 3773.7 | GCASRCKAKCAGRRCKGWASASFRGR CYCKCFRC | Charlet *et al.*, 1996 |
| Mytilin B | *M. edulis* | sp \| P81613 | 3974.3 | SCASRCKGHCRARRCGYYVSVLYRGRC YCKCLRC | Charlet *et. al.*, 1996 |
| Mytilin B | *M. galloprovincialis* | gb \|AAD52661 | n.a. | SPSDMMPQMNENENTEFGQDMPTGET EQGETGI | Mitta *et al.*, 2000 |
| Mytilin C | *M. galloprovincialis* | gb \|AAD45013* | n.a. | SCASRCKSRCRARRCRYYVSVRYGGFC YCRC | Mitta *et al.*, 2000 |
| Mytilin D | *M. galloprovincialis* | gb \|ACF21701 | n.a. | GCASRCKAKCAGRRCKGWASASFRRR CYCKCFRC | Mitta *et al.*, 2000 |
| Mytilin G1 | *M. galloprovincialis* | N.A. | n.a. | TCGSLCKAHCTFRKCGYFMSVLYHGRC YCRCLLC | Mitta *et al.*, 2000 |
| Myticin A | M. galloprovincialis | gb \|AAD47638 | 4438 | HSHACTSYWCGKFCGTAKMCACVHCS RVNNPFRVNQVAKSINDLDYTPIM | Mitta *et al.*, 2000 |
| Myticin B | *M. galloprovincialis* | gb \|AAD47639 | 4562 | HPHVCTSYYCSKFCGTAKLCFCLHCSRV KFPFGATQDAKSMNELEYTPIM | Mitta *et al.*, 2000 |
| Myticin 1 | *R. decussatus* | n.a. | n.a. | QSVACTSYYCSKFCGSAKICYCLHCRRA ESPLALSGSARNVNDKNNEMDNSPVM | Gestal *et al.*, 2007 |
| Myticin 2 | *R. decussatus* | n.a. | n.a. | VPCASTYCARFCGSAKICYCLHCRRAES PLALSGSARNVNDKNNEMDNSPVM | Gestal *et al.*, 2007 |
| Myticin 3 | *R. decussatus* | n.a. | n.a. | VPCASTLCSRFCGSAKICYCLHCRRAES PLALSGSARNVNDQNKEMDNSPVM | Gestal *et al.*, 2007 |
| Mytimycin | *M. edulis* | n.a. | 6233.5 | DCCRKPFRKHCWDCTAGTPYYGYSTRN IFGCTC | Charlet *et al.*, 1996 |

Aminoacidic sequences of the AMPs presented in the text with their accession number (from Swissprot and GenBank data bases), molecular weight and references.
*The amino acids different in GenBank database are highlighted in grey.
n.a.: not available.

peptides. The bacterial challenge caused the release of MGD-1 and MGD-2 from the stimulated hemocytes (Mitta *et al.*, 1999b, 2000).

The MGD-1 and MGD-2 antibacterial test at 10ul/100ul against *M. luteus,* using microtitration plates that were measured 24 h post incubation at 30 °C (OD 600 nm), showed inhibitory effects. Antifungal activity was monitored against spores from *F. oxysporum;* growth inhibition was observed microscopically after 24 h incubation at 30 °C with 10ul/80ul, and quantified by measurement of optical density at 600 nm after 48 h (Felhbaum *et al.*, 1994, Mitta *et al.*, 1999b).

Mytilin comprise isoforms A, B, C, D and G1, containing eight cysteines, represent the second group of AMPs described in mussels. Isoforms A and B were isolated from *M. edulis* plasma (Charlet et al, 1996); isoforms B, C, D and G1, on the other hand, were isolated from *M. galloprovincialis* hemocytes (Mitta *et al.*, 2000).

Mytilins A (3773.7 Da) and B (3974.3 Da) were isolated by conventional chromatographic methods. The concentration of mytilins in the blood of mussels may be estimated at approximately 2 µM. This is a range of the MIC determined for most of the tested bacteria. Mytilins appeared primarily effective against Gram-positive bacteria (0.6-1.2 uM) and less active against Gram-negative bacteria (2.5 - 10 uM). Minimal Inhibitory Concentration (MIC) values are expressed as an interval (a - b), where (a) represents the highest peptide concentration tested at which bacteria are still growing and (b) the lowest concentration that causes 100 % growth inhibition (Charlet *et al.*, 1996).

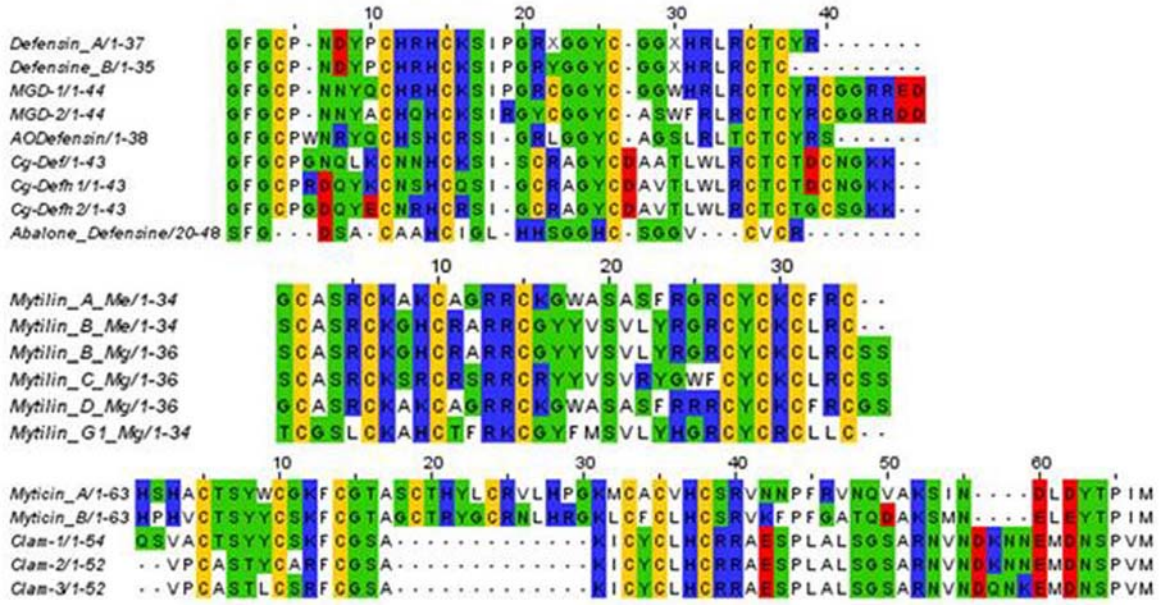


**Fig. 1** ClustalW alignment of the peptides sequences from Defensin, Mytilin and Myticin from *M. edulis, M. galloprovincialis, C,virginica*, *C. gigas, H. discus discus* and *R. decussatus.* The color code is basic blue, acidic red, polar without charge green and hydrophobic white, the Cys are highlighted in yellow. The figures were created with Jalview (Clam *et al.*, 2004)

Mytilins B, C, D, and G1 were isolated from *M. galloprovincialis* (Mitta *et al.*, 1999a). They were synthesized as precursors and processed as active peptides within the hemocytes. They were found, by confocal microscopy analysis in two subclasses of circulating granulocytes, one containing small granules and one with large clear granules (Mitta *et al.*, 2000).

The 3-D solution structure of synthetic peptides derived from the structure of Mytilin B (34-residue) was established by 1H NMR. This structure consists of the common cysteine-stabilized α β motif (CS αβ) closely related to the one observed in the mussel defensin MGD-1. The 8 cysteines formed four disulfide bonds (2 - 27, 6 - 29, 10 - 31, and 15 - 34) only involving the β-strand II. The percentage of the hydrophilic and hydrophobic areas from Mytilin and MGD-1 are closely related (63 % - 37 % and 64 % - 36 %, respectively). The C10C (AcCVCYRGRCYCNH2) mytilin fragment showed antiviral activities again with white spot syndrome virus (WSSV). This peptide was precipitated in salty water, although it was able to recover its original structure once the salt content was lowered, and appeared to be soluble in a high ionic strength environment. This fact anticipates its potential application as an antiviral agent in both aquatic and terrestrial animals and in humans. The small size and ease of synthesis of C10C enables its biotechnology development (Roch *et al.*, 2008).

Diversity of mRNAs from mytilin B in *M. galloprovincialis* has been studied from circulating hemocytes, thus defining 10 individual DGGE (denaturing gradient gel electrophoresis) patterns in untreated mussels (Parisi *et al.*, 2009). Further analysis is required on AMPs polymorphism to determine the role of the environment on the polymorphism of these molecules in molluscs.

Mytimycin isolated from the plasma of *M. edulis* (6233.5 Da) involves twelve cysteines engaging in the formation of six intra-molecular disulfide bridges. Mytimycin demonstrated to be strictly anti-fungal when tested against the strains *Neurospora crassa* and *F. culmorum* (Charlet *et al.*, 1996).

Myticin isolated from *M. galloprovincialis* is a cysteine-rich peptide produced in two isoforms, A and B. Myticins A and B were isolated from the hemocytes (A 4.438 Da and B of 4.562 Da); myticin A was also isolated from the plasma of the mussel. The mature molecule consists of 40 residues, with four intramolecular disulfide bridges and a cysteine array in the primary structure, which is different from that of previously characterized cysteine-rich antimicrobial peptides (Mitta *et al.*, 1999a). The sequence analysis of the cloned cDNAs revealed that myticin precursors comprise 96 amino acids.

Myticins A and B displayed antibacterial activity against gram-positive bacteria, and myticin B is active against the fungus *F. oxysporum* and the Gram-negative bacteria *E. coli* D31 (Mitta *et al.*, 2000).

Myticin C, a novel antimicrobial peptide from *M. galloprovincialis* (Pallavicini *et al.*, 2008), appears to be extremely polymorphic. Seventy four variants with nucleotide mutations were identified using DGGE (Costa *et al.*, 2009).

**Defensin from oyster**

For the summary of the AMPs described in oysters and the relative alignments see Table 1 and Fig. 1.

The first mollusc defensin isolated from an oyster species was named American oyster defensin (AOD) (Seo *et al.*, 2005). It was purified from a gill extract of *C. virginica* using classical chromatography for cationic peptides. Electrospray ionization mass spectroscopy (ESI-MS) of AOD evidenced a mass of 4265.0 Da. The AOD (38 amino acids) has common structural features with arthropod defensins: i.e., 1) six cysteine residues; 2) at least 4 basic amino acid residues; 3) a hydrophobic loop in the amino terminal; 4) a tetra-amino acid motif Gly-Gly-Tyr-Cys; and 5) a carboxy terminal penta amino acid motif Cys-Thr-Cys-Tyr-Arg.

AOD has high sequence homology (62 - 73 %) with the defensins from *M. edulis* and *M. galloprovincialis*, respectively (Charlet *et al.*, 1996; Hubert *et al.*, 1996; Mitta *et al.*, 1999b).

Sequence homology search of the purified peptide was performed using BLASTP 2.2.10 and TBLASTN 2.2.10 on Genome Net (http://www.ncbi.nlm.gov/BLAST). The theoretical isoelectric point (pI) and molecular mass were estimated by ExPASy (http://www.expasy.ch tools/peptide-mass.html). Sequence alignment was performed using the ClustalX program (Thompson *et al.*, 1997).

The antibacterial activity of the purified peptide was tested using a double-layer radial diffusion assay (Lehrer *et al.*, 1991). Using the minimal effective concentration (MEC) as a parameter, significant activity against the Gram-positive bacteria *Lactococcus lactis subsp.lactis* and *S. aureus* at 2.4 and 3.0 ug/ml, respectively, was detected,. On the other hand, a lower effect on the Gram-negative bacteria *E. coli* D31 and *Vibrio parahemolyticus*, MEC at 7.6 and 15.0 ug/ml, respectively, was observed. MEC was calculated as described (Zhao *et al.*, 2001).

Another defensin from an oyster was identified in the mantle of *C. gigas* (Cg-Def) (Gueguen *et al.*, 2006). The *Cg-def* gene is continuously expressed in the mantle. The structure of the recombinant peptide in *E. coli* is CS-αβ like arthropod defensins, but it includes an additional disulfide bond as the mussel defensin MGD-1. Nonetheless, the difference with MGD-1 is the size of their loops and the presence of two aspartic residues. The oyster *Cg-def* cDNA contained 323 bp. The 195 bp coding region encoded a 65 amino acid propeptide (GenBankTM CAJ19280) (Gueguen *et al.*, 2003). *Cg*-Def is not synthesized as a precursor.

The antimicrobial activity of the recombinant Cg-Def was determined against Gram-positive and Gram-negative bacteria and filamentous fungi, resulting mainly effective against the Gram-positive strains *Micrococcus lysodeikticus, S. aureus Brevibacterium stationi,* and *Microbacterium maritypicum* at 0.005 - 1.25 uM (MIC values). The activity of Cg-Def was retained *in vitro* at a salt concentration similar to that of seawater (Gueguen *et al.*, 2009).

It has been additionally established that the two isoforms of the defensin from the hemocytes of the oyster *C. gigas*, Cg-defh1 and Cg-defh2 (43 amino acids), have four disulfide bridges. This feature is also present in the defensins MGD-1 and MGD-2 from *M. galloprovincialis* hemocytes and Cg-Def from the *C. gigas* mantle (Gonzalez *et al.,* 2007).

A quantitative RT-PCR (qRT-PCR) analysis indicated that *Cg-defh*2 was continuously expressed in the hemocytes of *C. gigas*. In addition, the level of *Cg-defh*2 transcripts decreased drastically in the circulating hemocyte population after a bacterial challenge, thus suggesting a possible migration of the hemocytes towards the gill and mantle tissue. This fact may be correlated with an increase of *Cg-defh*2 transcripts in such tissues (González *et al.*, 2007).

**Rich cystein antimicrobial peptides from clams**

For the summary of the AMPs described in clams and the relative alignments see Table 1 and Fig. 1.

Myticin isoforms 1, 2 and 3 were identified and characterized for the first time in clams, *Ruditapes decussatus* (Gestal *et al.*, 2007), using the suppression subtractive hybridization technique (SSH). Suppression subtractive hybridization libraries may facilitate the identification of genes involved on bivalve immune response (Tanguy *et al.*, 2004).

Clam myticins (40 aa) and mytilin (34 aa) are similar to the myticins previously reported from *M. galloprovincialis*, as both conserved the cystein array with four intramolecular disulfide bridges, which are characteristic of the Myticin family (Gestal *et al.*, 2007).

Clams challenged with bacteria showed that clam myticin and clam mytilin increased the expression levels 48 h post-infection using qPCRs performed on hemocytes. During the challenge, specimens were injected 100 µl (containing $10^7$ cells/ml) of a dead bacteria mixture into the adductor muscle, which included *Micrococcus lysodeikticus, Vibrio splendidus* and *Vibrio anguillarum* (Gestal *et al.*, 2007).

Aminoacidic diversity of the clam myticin was found with the previously described mussel myticins A and B and with myticin C (Pallavicini *et al.*, 2008). The variation in aminoacidic residues of the different AMPs may occur as a response to the recognition of different pathogens in their environment (Gestal *et al.*, 2007).

**Defensins from gastropods**

For the summary of the AMPs described in abalone and the relative alignments see Table 1 and Fig. 1.

An abalone defensin, with 66 aa, lower molecular mass of 4.9 kDa, positive charge +5, hydrophobic residue ratio 46 %, α-helical structure and with an arrangement of six cysteine residues forming three disulfide linkages in C1-C4, C2-C5 and C3-C6, was characterized from the *H. discus discus*. The complete coding sequence of the defensin was obtained from the abalone cDNA library EST database (De Zoyza *et al.*, 2010). Hemocytes were collected for RNA extraction. A domain sequence was identified, 24 - 66 aa, exhibiting the same basic characteristics of the arthropod defensin family members. It is expressed constitutively in hemocytes, gills, muscle and digestive tract; however, the transcripts in tissues were significantly induced 48 h post-infection in abalones injected into the adductor muscle with 100 µl of a bacterial mixture containing *V. alginolyticus, V. parahemolyticus* and *Lysteria monocytogenes* ($5x10^7$ cells/ml).

**Conclusions**

Different research groups have focused on AMPs since they are molecules of the innate immune system of a wide range of organisms, displaying an efficient activity against pathogenic microorganisms without cytotoxic effects on eukaryotic cells. They were discovered in marine molluscs almost 20 years ago and they have been primarily identified in mussels mainly as defensins and cys-rich peptides. Since then, they have been purified from tissues and the genes associated to their expression have been identified; furthermore, the phylogenetic relationships with existing molecules from other invertebrates have also been established. All these aspects have contributed to the knowledge of the immune responses of this phylum.

The study of the structures and antimicrobial effects of the different AMPS, on the other hand, has allowed achieving significant progress in the elucidation of the different action mechanisms, thus suggesting specific models that have been integrated as part of a sequence of events of a more general mechanism. Various trends have also emerged where AMPs are regarded as alternative molecules to classical antibiotics due to their structural and physiological characteristics, stressing the fact that in vitro assays and clinical tests until phase III have confirmed that resistance evolution against antimicrobial peptides is less probable than that observed for conventional antibiotics, although the broad therapeutic use of AMPs is still unconsolidated and a long path must be covered to overcome the technical problems limiting such aspirations.

Although the diversity of marine molluscs is quite large, AMPs have been explored only in a few species; therefore, there is still a great potential to unveil new molecules in this phylum. The development of molecular biology, bioinformatics and genetic engineering techniques have allowed to produce AMPs in such quantities as to be used as drugs to curtail human, animal and plant diseases (Zasloff, 2002; Gordon *et al.*, 2005; Keymanesh *et al.*, 2009).

Genetic manipulation using antimicrobial peptide coding sequences to produce transgenic plants resistant to certain pathogens is presently an emerging research field. However, genetic engineering should also take into consideration obtaining stable recombinant genomes using genes from the same species and not different species. Nevertheless, such possibility still remains controversial.

Although the diversity of AMPs is astounding they still are not a solid alternative to conventional antibiotics. Therefore, there are ample opportunities for research in basic and applied aspects of antimicrobial peptide biology to fulfil expectations of their use in the control of infectious diseases.

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