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A prawn core histone 4: Derivation of N- and C-terminal peptides and their antimicrobial properties, molecular characterization and mRNA transcription

Mukesh Kumar Chaurasia[a], Rajesh Palanisamy[a], Prasanth Bhatt[a], Venkatesh Kumaresan[a], Annie J. Gnanam[b], Mukesh Pasupuleti[c], Marimuthu Kasi[d], Ramaswamy Harikrishnan[e], Jesu Arockiaraj[a,*]

[a] Division of Fisheries Biotechnology and Molecular Biology, Research Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur, Chennai 603203, Tamil Nadu, India
[b] Institute for Cellular and Molecular Biology, The University of Texas at Austin, 1 University Station A4800, Austin, TX 78712, USA
[c] Lab PCN 206, Microbiology Division, CSIR-Central Drug Research Institute, B.S. 10/1, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, Uttar Pradesh, India
[d] Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Semeling Bedong, 08100 Bedong, Kedah, Malaysia
[e] PG and Research Department of Biotechnology, Bharath College of Science and Management, Thanjavur 613 005, Tamil Nadu, India

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ABSTRACT

This study investigates the complete molecular characterization including bioinformatics characterization, gene expression, synthesis of N and C terminal peptides and their antimicrobial activity of the core histone 4 (H4) from freshwater giant prawn *Macrobrachium rosenbergii* (*Mr*). A cDNA encoding *Mr*H4 was identified from the constructed cDNA library of *M. rosenbergii* during screening and the sequence was obtained using internal sequencing primers. The *Mr*H4 coding region possesses a polypeptide of 103 amino acids with a calculated molecular weight of 11 kDa and an isoelectric point of 11.5. The bioinformatics analysis showed that the *Mr*H4 polypeptide contains a H4 signature at ^{15}GAKRH^{19}. Multiple sequence alignment of *Mr*H4 showed that the N-terminal (21–42) and C-terminal (87–101) antimicrobial peptide regions and the pentapeptide or H4 signature (15–19) are highly conserved including in humans. The phylogenetic tree formed two separate clades of vertebrate and invertebrate H4, wherein *Mr*H4 was located within the arthropod monophyletic clade of invertebrate H4 groups. Three-dimensional model of *Mr*H4 was established using I-TASSER program and the model was validated using Ramachandran plot analysis. Schiffer–Edmundson helical wheel modeling was used to predict the helix propensity of N (21–42) and C (87–101) terminal derived *Mr* peptides. The highest gene expression was observed in gills and is induced by viral [white spot syndrome baculovirus (WSBV) and *M. rosenbergii* nodovirus (*Mr*NV)] and bacterial (*Aeromonas hydrophila* and *Vibrio harveyi*) infections. The N and C terminal peptides were synthesized and their antimicrobial and hemolytic properties were examined. Both peptides showed activity against the tested Gram negative and Gram positive bacteria; however, the highest activity was noticed against Gram negative bacteria. Among the two peptides used in this study, C-terminal peptide yielded better results than the N-terminal peptide. Therefore, C terminal peptide can be recommended for the development of an antimicrobial agent.

1. Introduction

Unlike vertebrates, invertebrates rely highly on their innate immune components such as hemocytes and antimicrobial peptides for protection from pathogenic infections. Antimicrobial peptides (AMPs) are usually low-molecular weight, cationic, evolutionarily conserved amphipathic molecules (Mihajlovic and Lazaridis 2010; Pazgier et al. 2006) which show a multi-potent, rapid and non-specific response against pathogenic infections in

* Corresponding author at: Division of Fisheries Biotechnology and Molecular Biology, Research Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur 603 203, Chennai, Tamil Nadu, India.
Tel.: +91 44 27452270; fax: +91 44 27453903.
E-mail address: jesuaraj@hotmail.com (J. Arockiaraj).

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most of the organisms (Boman 2003; Guani-Guerra et al. 2010; Jenssen et al. 2006). They have broad-spectrum antimicrobial activity against a range of biotic agents, despite their evolutionarily conserved nature (Reddy et al. 2004). Even though AMPs vary in their amino acid composition, size and conformation, they show similarities in their antimicrobial function. These salient properties enable AMPs to interact with cell membranes and kill the invading pathogen by penetrating into the cell or by creating pores and collapsing the membrane equilibrium (Jenssen et al. 2006). AMPs particularly destroy the prokaryotic cells rather than eukaryotic cells (Zasloff 2002).

It is well established that histones play a key role in nucleosome structure formation and transcription regulation in eukaryotic cells. In eukaryotic cells, there are reports regarding existence of five types of histones namely H2A, H2B, H3, H4 and H1 linker histone which are rich in lysine and arginine residues (Tagai et al. 2011). However, in most vertebrate species (Brinkmann et al. 2004; Ng et al. 2013; Kawasaki and Iwamuro 2008; Zasloff 1992), histone whole protein and its derived peptides are also known to act against various microbial pathogens by themselves or releasing DNA to make extracellular traps (Anand et al. 2012). Recently, a few histone proteins that possessed antimicrobial property was also reported from various organisms including mollusk (Seo et al. 2011), fish (Noga et al. 2011), amphibian (Kawasaki et al. 2008), crustacean (Arockiaraj et al. 2013a,b) and human (Rose et al. 1998; Wang et al. 2011; Kai-Larsen et al. 2007). Apart from the above-mentioned peptides, many other AMPs have been derived from intact histone precursors through proteolytic cleavage in various organism (Fernandes et al. 2004; Lüders et al. 2005; Patrzykat et al. 2001; Nam et al. 2012; Jonathan et al. 2013; Cho et al. 2002, 2009; Park et al. 1998; Birkemo et al. 2003; De Zoysa et al. 2009; Sathyan et al. 2012a,b,c).

Interestingly, each type of histone protein has its own repertoire of variants that differ in their amino acid sequence mostly in the N-terminal region. In spite of sequence variations, H4 is the most conserved one with an ability to undergo post-translational modifications like acetylation, methylation, phosphorylation and can act as the docking site for other histones. The role of H4 is not well investigated, especially in terms of antimicrobial activity. Lee et al. (2009) isolated a peptide from H4 of human sebocytes and showed its antimicrobial property against *Staphylococcus aureus* and *Propionibacterium acnes*. Similarly, Kai-Larsen et al. (2007) extracted a peptide from H4 of human meconium and observed its antimicrobial potential. Dorrington et al. (2011) observed antimicrobial activity of recombinant H4 protein from *Xenopus laevis* against Gram negative and positive bacteria. But details on H4-derived peptide and its antimicrobial activity from prawn is very limited and especially H4 from freshwater giant prawn *Macrobrachium rosenbergii* is nil.

M. rosenbergii is an economically important cultivable crustacean species. But their culture system is affected due to virus infection such as white spot syndrome baculovirus (WSBV) and *M. rosenbergii* nodovirus (*Mr*NV) and bacteria such as *Vibrio harveyi* and *Aeromonas hydrophila* (Arockiaraj et al. 2013c). However, it is not clear whether the histones or histone-derived peptides could inhibit or kill the above-said pathogens. Hence, it is necessary to evaluate the antimicrobial property of *M. rosenbergii* histone 4-derived peptides. Therefore, we focused on characterization of *M. rosenbergii* histone 4 (named as *Mr*H4) using computational as well as biological tools. Moreover, we here report the difference in the *Mr*H4 mRNA transcription pattern due to viral and bacterial infections. In addition, two short peptides from *Mr*H4 protein were synthesized artificially and their antimicrobial property is demonstrated.

2. Materials and methods

2.1. Ethics statement

The use of human blood was approved by the ethics committee at SRM University (361/IEC/2012). Informed consent was obtained from the donors before collecting the blood.

2.2. Peptides

Peptides used in this work were synthesized by 1st Base (Malaysia). The purity (>95%) of these peptides were confirmed by mass spectral analysis (MALDI-ToF), provided by the suppliers. Peptides were diluted in endotoxin-free water and used as stock (3 mM) for subsequent experiment or stored at −20 °C until used. The stock solution was used for the subsequent experiment.

2.3. Microorganism

Gram negative (*A. hydrophila* ATCC 7966, *Escherichia coli* ATCC 25922, *Edwardsiella tarda* ATCC 15947, *Vibrio parahaemolyticus* ATCC 27969, *Vibrio alginolyticus* ATCC 17749 and *V. harveyi* BAA-1116) and Gram positive (*Bacillus subtilis* ATCC 23857, *Streptococcus iniae* ATCC 29178, *S. aureus* ATCC 29213, *Enterococcus faecium* ATCC 14934 and *Lactococcus lactis* ATCC 19434) were used for the minimum inhibitory concentration (MIC) assay. All the bacteria were maintained on 30% glycerol stock, except marine water strains *V. alginolyticus* and *V. harveyi* which were maintained on marine agar stabs (Difco™ Marine Agar 2216).

2.4. M. rosenbergii cDNA library construction and identification of MrH4

A normalized *M. rosenbergii* cDNA library was constructed using total RNA extracted from muscle, gills, haemocyte, hepatopancreas and brain of *M. rosenbergii*. Then, mRNA was isolated using mRNA extraction kit (Miltenyi Biotech, Germany). This was followed by the single-strand cDNA synthesis and normalization conducted using CloneMiner™ cDNA library construction kit (Invitrogen) and Trimmer Direct Kit: cDNA Normalization Kit (BioCat GmbH). Thereafter, genome sequencing FLX™ (GS-FLX™) technology was applied to obtain the cDNA sequences, after which the library was constructed. The detailed information on *M. rosenbergii* cDNA library construction was explained in our earlier reports (Arockiaraj et al. 2013c). Screening of the constructed *M. rosenbergii* cDNA library revealed a cDNA sequence homologous to H4. Further, the full-length *Mr*H4 cDNA was obtained by internal sequencing using ABI Prism-Bigdye Terminator Cycle Sequencing Ready Reaction kit and analyzed in ABI 3730 sequencer. The following forward and reverse primers were used for the internal sequencing: *Mr*H4 F1: ATG ACT GGC CGC GGC AAG GGA GGC AAG GGT CTC GG and *Mr*H4 R2: CGC CAA GGC CGT ACC CTG TAC GGT TTC GGT.

2.5. Bioinformatics analysis

The full-length *Mr*H4 cDNA was analyzed on DNAssist (ver. 2.2) to obtain its 5′ and 3′ untranslated region (UTR), coding region and polypeptide sequences (Patterton and Graves 2000). Homology of *Mr*H4 was searched using BLAST program (http://blast.ncbi.nlm.nih.gov/Blast). Domain and motif analysis of *Mr*H4 was carried out on PROSITE Database (http://prosite.expasy.org/scanprosite/). Multiple sequence alignment of *Mr*H4 was conducted using ClustalW program (ver. 2) (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Phylogenetic tree of *Mr*H4 was established using Neighbor-Joining Method at MEGA