Fish & Shellfish Immunology 48 (2016) 228–238

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Molecular importance of prawn large heat shock proteins 60, 70 and 90

Mukesh Kumar Chaurasia [a], Faizal Nizam [a], Gayathri Ravichandran [a, b], Mariadhas Valan Arasu [c], Naif Abdullah Al-Dhabi [c], Aziz Arshad [d], Preetham Elumalai [e], Jesu Arockiaraj [a, *]

[a] Division of Fisheries Biotechnology & Molecular Biology, Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur, 603 203, Chennai, Tamil Nadu, India
[b] SRM Research Institute, SRM University, Kattankulathur, 603 203, Chennai, Tamil Nadu, India
[c] Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University, P. O. Box 2455, Riyadh, 11451, Saudi Arabia
[d] Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400, UPM, Serdang, Selangor, Malaysia
[e] School of Aquatic Food Products and Technology, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi, 682 506, Kerala, India

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form
17 November 2015
Accepted 23 November 2015
Available online 26 November 2015

Keywords:
Heat shock proteins
Macrobrachium rosenbergii
Bioinformatics
Gene expression
Pathogen

ABSTRACT

Considering the importance of heat shock proteins (HSPs) in the innate immune system of prawn, a comparative molecular approach was proposed to study the crustacean large HSPs 60, 70 and 90. Three different large HSPs were identified from freshwater prawn *Macrobrachium rosenbergii* (*Mr*) cDNA library during screening. The structural and functional characteristic features of HSPs were studied using various bioinformatics tools. Also, their gene expression and mRNA regulation upon various pathogenic infections was studied by relative quantification using $2^{-\Delta\Delta CT}$ method. *Mr*HSP60 contains a long chaperonin 60 domain at 46–547 which carries a chaperonin 60 signature motif between 427 and 438, whereas *Mr*HSP70 contains a long HSP70 domain at 21–624 and *Mr*HSP90 carries a HSP90 domain at 188–719. The two dimensional analysis showed that *Mr*HSP60 contains more amino acids (52%) in helices, whereas *Mr*HSP70 (40.6%) and *Mr*HSP90 (51.8%) carried more residues in coils. Gene expression results showed significant ($P < 0.05$) expression of *Mr*HSP60, 70 and 90 in haemocyte, gill and hepatopancreas, respectively. Further, the expression level was up-regulated upon bacterial (*Aeromonas hydrophilla* and *Vibrio harveyi*) and viral [white spot syndrome virus (WSSV) and *M. rosenbergii* nodo virus (*Mr*NV)] infections during various time periods. The gene expression results exhibited the potential involvement of these three HSPs in the immune system of prawn. The study indicated the potentiality of these molecules, thereby protecting cells against pathogens as well as severe cellular and environmental stresses in crustaceans.

1. Introduction

Thermal fluctuations have been considered as one of the most important factors which affect the integrity of physiological system at various cellular and molecular levels [1]. When cells perceive oxidative stress due to heat, heavy metals, etc., it aggravates active response to return to their normal functional state. To withstand such thermal changes, individuals show a highly conserved set of immune pathways. The common well-known characteristic feature of a heat shock response is the induction of a highly conserved set of proteins known as heat shock proteins (HSPs) [2]. The induction of HSPs occur under various environmental stress situations including induction such as temperature, heavy metals, bacterial and viral infections, nutrient deprivation, hydrostatic pressure, pollutants, UV exposure, oxygen radicals, malignant agents and re-oxygenation or hypoxia [3]. Due to the stimulation under different stressful situations, HSPs are collectively known as stress proteins. Moreover, researchers have provided evidence that HSP family is also induced in normal or in un-stressful situation of cells [4], and the quantity of these proteins reduces with ageing protocol [5].

* Corresponding author.
E-mail address: jesuaraj@hotmail.com (J. Arockiaraj).

http://dx.doi.org/10.1016/j.fsi.2015.11.034
1050-4648/

HSPs are ubiquitously present in all living organisms including both vertebrates and invertebrates. Preliminary investigation on the discovery of HSPs was carried out by Ritossa during 1962 and was firstly used to describe *Drosophila melanogaster* protein expressed at elevated temperature [6]. Members of HSPs family are well known molecular chaperones (or chaperonins) which prevent the gathering of newly produced polypeptides manage protein abasement and help miss fold proteins to obtain their original states [7]. Generally HSPs are found in the molecular weight ranging between 27 and 110 kDa. Based on their size, molecular weight and functions in eukaryotes, HSPs are broadly classified into six major groups namely HSP60, HSP70, HSP90, HSP100, HSP110 and small HSPs [2].

Among the different families of HSPs, HSP60, HSP70 and HSP90 are highly conserved and most extensively studied. The members of HSP60, HSP70 and HSP90 family are stress inducible, multigenic, and are present in all organisms studied till date [8]. It has been observed by Parsell and Lindquist [2] that HSP60, HSP70 and HSP90 family play a significant role in cell survival, stress and thermal tolerance in response to various heat shocks. HSP60 is also known as phage growth E large (GroEL) in prokaryotes such as bacteria [9] which have a particular toroid quaternary structure or distinct ring-shape that are present in eukaryotic mitochondrial cells [10]. HSP70 is different in structure and it is a constitutively expressed cognate stress protein, thus denoted as HSC70 protein in eukaryotes [8].

HSP60, 70 and 90 play an important role as molecular chaperones in intracellular organelles and stabilize unstable proteins through regulating the correct folding by gathering of proteins into oligomeric configuration, and thereby inhibiting the natural qualities of proteins [11]. These proteins are also involved in many cellular processes including signal transduction, DNA replication, protein synthesis and protein trafficking [12]. The extracellular role of HSPs was proposed by a number of observations but it still demands more clarification. Such HSPs have been found to be released from various types of cells which include human neuroblastoma cell-line, vascular smooth muscle cells, rat embryo cells, rat glial cells and human islet cells [13]. However, the definite secretary mechanism of HSPs is still unknown.

Giant river prawn, *Macrobrachium rosenbergii*, is a commercially important shrimp species which is present all over the world especially in Southeast Asian countries [14]. *M. rosenbergii* is farmed in aquaculture industries for human consumption. Researchers have found that *M. rosenbergii* can tolerate different thermal conditions ranged between 15 and 35 °C. However, the optimum temperature for maximum growth was ranged between 27 and 29 °C [15,16]. Water temperature is an important environmental parameter for normal growth of this prawn and the high temperature significantly affects the metabolism of this species [17]. In recent years, *M. rosenbergii* hatcheries have been affected due to various viral and bacterial infections such as white tail disease (WTD), white spot syndrome disease (WSSD), tail rot disease, vibriosis, and so on [39] resulting in considerable amount of economic loss which runs to billions of dollars to aquaculture industry every year [19].

In recent years, researchers have investigated that the heat shock chaperonin are involved in autoimmune and innate immune response in several species including crustaceans [20]. The use of antibiotic resistant bacteria to control such severe diseases is a traditional approach and has been ineffective and unsustainable due to their potential risk to human health as well as the environment [21]. Few studies [22,23] have suggested some anti-infective strategies as an alternate to antibiotics. As explained by Joly et al. [24] Tasanand Gao [25] and Arockiaraj et al. [26,27], HSP plays protective immune response against many bacterial and viral diseases, although the report is very far from sufficient. Hence, research into freshwater prawn defense mechanism by treating with HSP could be imperative to develop better disease control strategies. In this study, we approached a comparative aspect on molecular characterization of three different large HSPs namely HSP60, 70 and 90 from *M. rosenbergii* (designated as *Mr*HSP60, *Mr*HSP70 and *Mr*HSP90). In detail, this study dealt with the similarities and differences among *Mr*HSP60, *Mr*HSP70 and *Mr*HSP90 by utilizing bioinformatics tools. We also reported the gene regulation patterns of these three heat shock molecules upon different viral and bacterial infections.

2. Materials and methods

2.1. cDNA library of freshwater giant prawn and HSPs identification

Three partial length cDNA sequences of large HSPs namely *Mr*HSP60, *Mr*HSP70 and *Mr*HSP90 were identified from the already developed normalized cDNA library of freshwater giant prawn [18,28,29] during Blast2GO (https://www.blast2go.com/) annotation program. Then, the full lengths of these three sequences were obtained using the methodology of internal sequencing using ABI Prism-Big dye Terminator Cycle Sequencing Ready Reaction kit. Following sequencing, the full length sequences were confirmed with the help of ABI 3730 sequencer. In Table 1, we presented the sense and antisense primers used for the sequencing. The physicochemical properties of the three HSPs including translated and non-translated sequences, polypeptides, isoelectric point and molecular weight were obtained from DNAssist as demonstrated by Patterton and Graves [30].

2.2. Analysis of HSPs bioinformatics features

To study the bioinformatics features of *Mr*HSP60, *Mr*HSP70 and *Mr*HSP90, the following online web server were used: http://blast.ncbi.nlm.nih.gov/Blast.cgi for homologous analysis, http://prosite.expasy.org/scanprosite/for domains and motifs analysis, http://www.cbs.dtu.dk for signal peptide region prediction, www.sbc.su.se/~miklos/DAS/for trans-membrane region prediction, http://www.ebi.ac.uk/Tools/msa/clustalw2/for multiple sequence alignment, http://www.megasoftware.net/for evolutionary analysis, https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html for two dimensional (2D) analysis and http://zhanglab.ccmb.med.umich.edu/I-TASSER for three dimensional (3D) analysis.

2.3. Freshwater giant prawn

Freshwater giant prawn (wet body weight = 30 ± 5 g) were collected from Sri Sai Aqua farm, Nellore, Andhra Pradesh, India. The collected prawns were transported in plastic pots (20 L) to the laboratory, SRM University. In the laboratory, the prawns were stocked in 20 fiber tanks (capacity of each tank = 0.15 m^3) for a week of acclimatization. Each tank was stocked with 10 prawns. The tanks were filled with freshwater which is supplied with additional aeration using motor pump. During the acclimatization period, the water quality parameters were noticed as follows: temperature, 30 ± 2 °C; dissolved oxygen, 6.0 ± 0.3 mg/L and pH, 7.1 ± 0.2. Moreover, during the acclimatization period, the prawns were fed two times (09.00 and 16.00 h) with a commercial pellet diet up to satiation level.

2.4. Microbial challenge in freshwater giant prawn

To study the immune involvement of the three HSPs, the freshwater giant prawn were challenged [31] with viral pathogens, white spot syndrome virus (WSSV) and *M. rosenbergii* nodovirus