Comparative Biochemistry and Physiology, Part C 156 (2012) 211–220

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Gene expression profiles of four heat shock proteins in response to different acute stresses in shrimp, *Litopenaeus vannamei*

Zhaoying Qian [a], Xiaolin Liu [a,*], Lijun Wang [a], Xianzong Wang [a], Yang Li [a], Jianhai Xiang [b], Ping Wang [c]

[a] College of Animal Science and Technology, Northwest A & F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Shaanxi Yangling 712100, China
[b] Experimental Marine Biology Laboratory, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[c] Nanjiang Marine Biotechnology Company Limited, Sanya, Hainan 572000, China

ARTICLE INFO

Article history:
Received 18 February 2012
Received in revised form 1 June 2012
Accepted 4 June 2012
Available online 12 June 2012

Keywords:
Acute stresses
Biomarkers
Gene expression profiles
Heat shock proteins
White shrimp

ABSTRACT

Heat shock proteins (HSPs) are a suite of highly conserved proteins well known for their quick responses to environmental stresses. However, the respective roles of different HSPs in response to a particular environmental stress have not received adequate scientific attentions to date. In this study, the expression profiles of four HSP genes (*Lvhsp60*, *Lvhsp70*, *Lvhsc70*, and *Lvhsp90*) of the Pacific white shrimp, *Litopenaeus vannamei* under acute thermal stress, pH challenge, and heavy metal exposure were investigated, respectively, using the quantitative real-time reverse transcription polymerase chain reaction technique. Results showed that the four genes exhibited quite different expression profiles when the shrimp were subjected to each of the above stressors. Under acute thermal stress, the messenger RNA (mRNA) levels of all the four genes were significantly induced, and the transcription level of *Lvhsp70* was the most sensitive to temperature fluctuations. Under acute pH challenge, the relative mRNA expression of the four genes was shown to be time and pH dependent, and the strongest response occurred in *Lvhsp60*. Under acute heavy metal exposure, transcripts of each of the four genes varied depending on metal type and exposure time. *Lvhsp60* displayed particularly high sensitivity to cadmium and manganese exposure, while *Lvhsp70* showed the most sensitive response to iron and zinc treatments. The results obtained suggest that different LvHSP genes may play different roles in mediating cell stress caused by a specific environmental stressor. Given the response sensitivity and intensity of LvHSP genes to environmental stresses, *Lvhsp70* may be most suitable to act as a biomarker indicating thermal stress, iron and zinc stimulation, while *Lvhsp60* may be a promising candidate marker of pH stress, cadmium and manganese exposure in shrimp.

1. Introduction

Heat shock proteins (HSPs), also known as stress proteins and extrinsic chaperones, are a suite of highly conserved, broadly distributed proteins in nature (Roberts et al., 2010). They serve as regulators of normal cell function, assisting in proper folding, assembly, and transport of nascent proteins (Gething and Sambrook, 1992), and also function as cellular defenses, preventing protein denaturation, and aiding in the refolding, and removal of proteins denatured by biotic and abiotic stresses (Sanders, 1993; Feder and Hofmann, 1999). In living organisms, the expression of HSP genes has been known to increase in response to a wide range of stresses, such as heat (Currie and Tufts, 1997; Piano et al., 2005), organic pollutants (Sanders et al., 1991), trace-metal exposure (Sanders et al., 1991; Schill et al., 2003), osmolarity (Kultz, 1996), anoxia (Myrmel et al., 1994), and *Vibrio* infection (Cellura et al., 2006). HSPs in eukaryotes are generally categorized into six major families: small HSPs, HSP60, HSP70, HSP90, HSP100 and HSP110 according to their molecular weights (Parsell and Lindquist, 1993; Feder and Hofmann, 1999). In eukaryotes, the HSP70 family consists of stress inducible (Hsp) and constitutively expressed (Hsc) forms that differ in their structures and levels of expression under changing environmental conditions.

The rapid development of fishery worldwide in recent years has been accompanied by the deterioration of aquatic environment caused by emerging stimuli, such as fluctuations in temperatures, salinity, oxygen, over-stocking, and heavy metals, which constitute the main reason that aquatic organisms become more susceptible to infectious disease than ever before (Wu et al., 2008b). In response, various defense mechanisms of organisms including detoxification of xenobiotics, regulation of oxidative stress, management of denatured molecules, DNA damage if the DNA repair fails, and regulations of the cell cycle may be activated. Clearly, significant increases in molecular levels of defense machinery demonstrate that cells respond to a stimulus (internal or external) and, interpreted with caution, may be regarded as very early and sensitive markers of stressing conditions (Farcy et al., 2008). As main stress proteins in cells, HSPs

* Corresponding author. Tel.: +86 29 87054333; fax: +86 29 87092164.
E-mail address: liuxiaolin@nwsuaf.edu.cn (X. Liu).

1532-0456/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2012.06.001

minimize the biochemical, physiological, and histological alterations of the host which were caused by environmental variations, and therefore are important factors for the maintenance of homeostasis across environmental regimes. Their effectiveness against different sources of cellular stress conditions is mainly a result of their chaperoning functions. Due to their unique sensitivities to a large number of environmental changes, HSPs have a long history of use in studies of organism stress response, and their use as cell stress markers has been well-recognized in many species (Wahid et al., 2007). In recent years, increasing interest has been paid to the study of gene expression profiles of HSPs in response to various environmental stresses in aquaculture species such as the tilapia, *Oreochromis mossambicus* (Molina et al., 2000), the bay scallop, *Argopecten irradians* (Song et al., 2006), *Mytilus galloprovincialis* (Cellura et al., 2006), the European flat oyster, *Ostrea edulis* (Piano et al., 2005), the abalone, *Haliotis tuberculata* (Farcy et al., 2007), the Chinese shrimp, *Fenneropenaeus chinensis* (Jiao et al., 2004), *Macrobrachium rosenbergii* (Liu et al., 2004), the tiger shrimp *Penaeus monodon* (Lo et al., 2004) etc. However, most of these studies focused on only one or two HSP genes, and rare comparative investigations have been carried out on gene expression profiles of HSPs in response to environmental stresses.

The Pacific white shrimp, *Litopenaeus vannamei*, native to the western Pacific coast of Latin America, with high commercial value and excellent characteristics for breeding has become one of the major species in Asian aquaculture, especially in China during these years. Because of its ubiquity and importance in aquaculture, and sensitivity to environmental variations, the white shrimp is an ideal animal model for studying the molecular mechanism of cell stress response. The full-length cDNA sequences of *Lvhsp60* (GenBank accession no. FJ710169.1), *Lvhsp70* (AY645906.1) *Lvhsc70* (EF495128.1) and *Lvhsp90* (HQ008268.1) have been characterized in the white shrimp to date (Wu et al., 2008b; Zhou et al., 2010b). Expression responses of LvHSP genes (*Lvhsp60*, *Lvhsp70*, and *Lvhsc70*) under different heat shock conditions and in different tissues were elucidated in previous studies (Wu et al., 2008b; Zhou et al., 2010a; Huang et al., 2011). However, no comprehensive information about the changes in the mRNA expression of HSP genes in the response to different environmental stressors is available in this organism up to now. In order to better understand the biological process by which shrimp cope with various environmental stresses, a comparative study on relative mRNA expression patterns of *Lvhsp60*, *Lvhsc70*, *Lvhsp70*, and *Lvhsp90* in response to a range of environmental stresses was carried out here. Meanwhile, a candidate biomarker of a specific environmental stress in shrimp was suggested based on the obtained results.

2. Materials and methods

2.1. Experimental animal sampling and maintenance

Three-month old (90 days after hatching) healthy Pacific white shrimp, *L. vannamei* juveniles, with 3.58 ± 0.43 g in average body mass, 7.48 ± 0.54 cm in average body length, were obtained from a local commercial farm in Dongfang (Hainan, China). The shrimp were sampled from the same pond and only intermolt juveniles were used for experiments. The molt stage was determined based on the previous study (De Oliveira Cesar et al., 2006). Prior to experimental use, animals were reared in tanks containing aerated seawater for seven days to acclimate to the laboratory conditions. During the acclimation, the shrimp were fed twice daily with commercial prawn pellets, and seawater was changed every day (Buikema et al., 1982). Feeding was stopped 24 h before treatment. Throughout the experiments, the physical and chemical characteristics of the seawater are given in Table 1. Except the temperature, each value was calculated with conventional methods (APHA et al., 1992).

2.2. Heat shock treatment experiment

One hundred shrimp were used for the experiment. After acclimation to seawater, six shrimp were randomly selected as 0 h samples prior to heat shock treatment. The remaining 94 shrimp were removed to another container with seawater maintained at a temperature of 36 ± 1 °C for 6 h. Six shrimp were randomly collected at 1, 2.5, 4, and 6 h separately during the heat shock treatment at 36 ± 1 °C. The remaining 70 shrimp were then put back to containers at 27 ± 1 °C for a continuous post-stress recovery of 12 h. During the recovery time, six shrimp were randomly collected at 6 and 12 h separately. The hepatopancreases of the six shrimp at each sampling time were separated and were used immediately for RNA extraction. During the whole experiment, no mortality was observed.

2.3. Acute pH challenge experiment

Five plastic aquaria (water volume, 100 L), containing test solutions of pH 6.1, pH 7.1, pH 8.1, pH 9.1 and pH 10.1, respectively, were prepared, and 60 shrimp after acclimation to seawater (pH 8.1 ± 0.1) were placed into each of the aquarium. pH values of 6.1, 7.1, 8.1, 9.1, and 10.1 were set by regulating seawater with 1.0 mol/L HCl and 1.0 mol/L NaOH and varied about ± 0.1. pH was measured using a pH electrode (228573-A01 Orion Research, Inc., Beverly, MA, USA) attached to an Orion320 pH meter and monitored over the whole experiment. Before pH challenge, the hepatopancreases of six shrimp from the seawater group (pH 8.1) were collected as 0 h samples and served as control, and hepatopancreases of six shrimp from each experimental group (pH 6.1, pH 7.1, pH 8.1, pH 9.1 and pH 10.1) were collected at 4, 8, 16, 32 h after the start of the experiment separately and all the hepatopancreas samples were used immediately for RNA extraction. During the whole experiment, no shrimp died.

2.4. Acute heavy metal exposure experiment

After the organisms had acclimated to the laboratory conditions, 100 shrimp sampled at random were placed in each of the four plastic containers (water volume, 200 L) with the respective test solutions. The metals chosen for the experiment were cadmium, iron, manganese and zinc. According to the previous study, the concentrations of heavy metals that do not affect the structure and function of natural ecological systems (safe level) are usually one percent of the respective 96-h median lethal concentrations (96-h LC50) values (Castillo, 2004). In our experiment, in order to get stressful response without any death of the shrimp, the concentrations of metals for test solutions were calculated using their respective 96-h LC50 values (Frias-Espericueta et al., 2001, 2003) multiplied by application factor (AF) 0.1. That is, the metal concentrations of test solutions were one tenth of their respective 96-h LC50. Since metals in solution occur in many forms, all values were reported as total concentrations of metals in micromolars (Table 2). Stock solutions of Cd, Fe, Mn and Zn in concentrations equivalent to 100 times of the respective 96-h

Table 1
Physical and chemical characteristics of seawater used in this study.

Parameter	Dissolved metals (nM)
Temperature: 27 ± 1 °C	As: 1.1–1.9
Total alkalinity: 80–120 mg L^{-1}	Cd: 3.6–4.5
Salinity: 32 ± 1‰	Cr: 1.9–3.8
pH: 8.1 ± 0.1	Cu: 140.6–234.4
Total ammonia-N: <0.5 mg L^{-1}	Fe: 32.1–53.6
Non-ionic ammonia-N: <0.1 mg L^{-1}	Hg: 0.5–1.0
Nitrate-N: <10.0 mg L^{-1}	Mn: 27.3–36.4
Nitrite-N: <0.1 mg L^{-1}	Pb: 5.8–8.7
Dissolved oxygen: >5.0 mg L^{-1}	Zn: 107.7–138.5
Hydrogen sulfide: <1.0 mg L^{-1}	