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Short communication

Co-expression of heat shock protein (HSP) 40 and HSP70 in *Pinctada martensii* response to thermal, low salinity and bacterial challenges

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ABSTRACT

Heat shock protein (HSP) 40 proteins are a family of molecular chaperones that bind to HSP70 through their J-domain and regulate the function of HSP70 by stimulating its adenosine triphosphatase activity. In the present study, a HSP40 homolog named PmHSP40 was cloned from the hemocytes of pearl oyster *Pinctada martensii* using EST and rapid amplification of cDNA ends (RACE) techniques. The full-length cDNA of *PmHSP40* was 1251 bp in length, which included a 5′ untranslated region (UTR) of 75 bp, an open reading frame (ORF) of a 663 bp, and a 3′ UTR of 513 bp. The deduced amino acid sequence of PmHSP40 contains a J domain in the N-terminus. In response to thermal and low salinity stress challenges, the expression of *PmHSP40* in hemocytes and the gill were inducible in a time-dependent manner. After bacterial challenge, *PmHSP40* transcripts in hemocytes increased and peaked at 6 h post injection. In the gill, *PmHSP40* expression increased, similar to expression in hemocytes; however, transcript expression of PmHSP40 was significantly up-regulated at 12 h post injection. Furthermore, the transcripts of *PmHSP70* showed similar kinetics as that of *PmHSP40*, with highest induction during thermal, low salinity stress and bacterial challenges. Altogether these results demonstrate that PmHSP40 is an inducible protein under thermal, low salinity and bacterial challenges, suggesting its involvement in both environmental and biological stresses, and in the innate immunity of the pearl oyster.

1. Introduction

Heat shock proteins (HSPs), are ubiquitous and evolutionarily conserved proteins found in all living organisms [1,2]. They are responsible for the maintenance of cellular viability under various conditions of cellular stress, such as heat shock, bacterial infection, ultraviolet radiation and salinity stress [1]. On the basis of their molecular weight and functions, HSPs have been categorized into different families, including HSP110, HSP90, HSP70, HSP60, HSP40 and several low molecular weight HSPs [3]. HSP40 proteins facilitate cellular recovery from proteotoxic stress primarily by regulating the ATPase activity of HSP70 proteins [4]. Recent study shows that HSP40 binds to HSP70 through a well conserved J domain [5]. They are categorized into three types according to their domain organization. Type I HSP40s possess J-domain, Gly/Phe-rich region (G/F domain), cysteine-rich region, and C-terminal domain. Type II HSP40s lack the cysteine-rich zinc binding domain, whereas type III HSP40s retain only the J-domain.

To date, since the first discovery of HSP40 in bacteria, an increasing number of HSP40 or HSP40-like genes have been identified in various species. The molecular features and function of HSP40s have been under intense investigation in vertebrates [6–8]. However, little information is available about the *HSP40* gene in mollusk. Thus far, the *HSP40* gene has only been studied in *Mytilus edulis* and *Venerupis philippinarum* [9,10]. Moreover, no molecular evidence is available on *HSP40* expression in pearl oyster at the transcript level. Therefore, in the present study, a full-length cDNA of *HSP40* was identified from pearl oyster and its expression in response to challenges from high temperature, low salinity and *Vibro alginolyticus* was investigated.

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2. Materials and methods

2.1. Cloning of full-length PmHSP40 cDNA

An EST sequence similar to known *HSP40* genes was identified through tBLASTn search in the pearl oyster EST database at NCBI. On the basis of this EST sequence, the GeneRacer™ Kit (Invitrogen, CA, USA) was used to obtain its 3′ and 5′ ends, according to manufacturer's instructions. The first PCR used primers pairs GR5P/HSP40R1 and GR3P/HSP40F1 for 5′ RACE and 3′ RACE, respectively, and GR5NP/HSP40R2 and GR3NP/HSP40F2 were used for nested PCR (listed in Table 1S). The target PCR products were cloned into pGEM-T Easy Vector (Promega, WI, USA). The plasmid DNA was sequenced with forward and reverse universal primers using the BigDye-Terminator Kit and an ABI Prism 3730 DNA sequencer (PerkinElmer, Wellesley, MA, USA). The full-length cDNA sequences were obtained by combining the 3'- and 5'-end sequences.

2.2. Experimental animals

Two-year-old pearl oysters, approximately 45–60 mm in shell length, were obtained from a pearl oyster culture farm in Zhanjiang, Guangdong Province, China, and kept in aerated seawater. All pearl oysters were acclimated to this environment for one week prior to the experiment.

For bacterial challenge, oysters were randomly distributed into two groups, a bacterial challenge group and a control group. Groups receiving an injection of 100 μL (1×10^8)of *V. alginolyticus* resuspended in PBS (pH 7.4) or the same volume of PBS were used as the challenged group and control group, respectively. Five individuals were randomly sampled for each group, each at 3, 6, 12, 24 and 48 h after injection. Hemocytes and gills from five individuals were collected at 3, 6, 12, 24 and 48 h after injection, and stored at −80 °C until further use.

For thermal treatment, oysters were placed in pre-warmed (35 °C) seawater and shocked for 1 h. Previous studies indicate that 1 h thermal treatment is stressful to this species [11,12]. The oysters were then returned to ambient seawater (25 °C). The control group was always kept in ambient seawater (25 °C). Hemocytes and gills from five individuals were randomly collected at 2, 4, 6, 12, 24 and 48 h after heat shock, and stored at −80 °C until further use.

For the low salinity challenge experiment, oysters were randomly distributed into two groups and placed in two tanks at salinity values of 10‰ (low salinity) and 30‰ (control) parts per thousand (ppt). The salinity gradients were obtained by mixing seawater (33‰) with fresh water. Five individuals were randomly sampled in each tank at 3, 6, 12, 24 and 48 h after a particular treatment began. Hemocytes and gills from five individuals were collected at 3, 6, 12, 24 and 48 h after low salinity challenge and stored at −80 °C until further use.

2.3. RNA isolation and cDNA synthesis

Total RNA was extracted from unchallenged oyster tissues (hemocytes, digestive gland, heart, gill, mantle, adductor muscle, and gonad) and challenged oyster (hemocytes, gill) with TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol. To synthesize cDNA, 1 μg of unchallenged and challenged total RNA

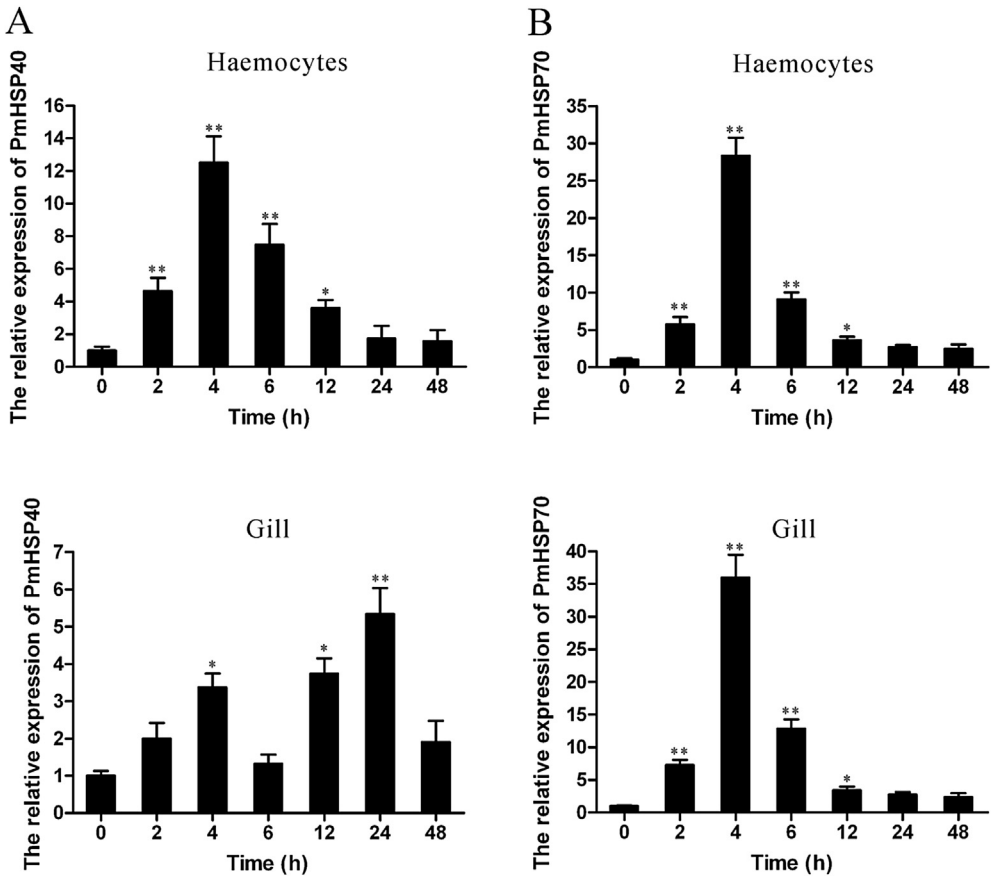

Fig. 1. Temporal expression of PmHSP40 (A) and PmHSP70 (B) in hemocytes and gill in response to thermal stress. Vertical bars represent the mean ± S.D. (N = 5). The values of control group were normalized to zero. An asterisk indicates a significant difference at $p < 0.05$ and two asterisks indicate a significant difference at $p < 0.01$ between the thermal stress and control group.