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Short communication

A small heat shock protein (sHSP) from *Sinonovacula constricta* against heavy metals stresses

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ABSTRACT

Small heat shock proteins (sHSPs) are ATP-independent molecular chaperones and involved into many physiological and stress processes. In the present study, the full-length cDNA of sHSP was cloned from razor clam *Sinonovacula constricta* (denoted as ScsHSP) through cDNA library and PCR approaches. Some feature motifs like the typical α-crystalline domain with six beta strands, three susceptible phosphorylation serines (S^{15}, S^{78}, and S^{82}) were conserved in the deduce amino acid of ScsHSP. Tissue distribution analysis of the ScsHSP revealed that the mRNA transcripts of ScsHSP were constitutively expressed in all examined tissues with the highest expressions in the haemocytes. The temporal expression of ScsHSP in gill and haemocytes after $PbCl_2$ and $CdCl_2$ exposure were recorded by qPCR. The suppressed expression patterns were detected in $CdCl_2$ stress at both tissues, and the minimum expression were detected at 36 h with 0.58-fold decrease in haemocytes and 0.30-fold in gill compared to each control group. During the $PbCl_2$ exposure experiment, the expression level of ScsHSP increased significantly with larger amplitude in haemocytes. As time progressed, the mRNA transcripts of ScsHSP recovered almost to the original level at 36 h. All our results indicated that ScsHSP was involved into mediating environmental pollutants exposure and considered to be a promising candidate bio-mark for heavy metals monitoring.

1. Introduction

A variety of proteins accumulate when razor clam *Sinonovacula constricta* is exposed to marine environmental pollution such as heavy metals, di(2-ethylhexyl) phthalate (DEPC) and polycyclic aromatic hydrocarbons (PAHs) [1], in which phylogenetically conserved proteins known as the heat shock proteins (HSPs) are the major group [2]. As one member of molecular chaperones that prevent aggregation of proteins [3], HSPs have attracted considerable interest among assembly, intracellular localization, secretion, regulation, degradation of other proteins and innate immunity [4,5]. There are five different categories of HSPs according to their approximate molecular weight, that are HSP90 (83–99 kDa), HSP70 (68–80 kDa), HSP60, HSP40 and a family of small HSPs (sHSPs, 15–30 kDa).

sHSPs first discovered as a set of proteins of small molecular weight induced after a heat shock [6]. The most striking feature of sHSP in structure is its highly conserved α-crystallin domain at the C-terminus, although the entire sequence shows much less similarity [7]. Functional studies in higher animals indicate that sHSPs were involved into cell protection, interaction with the cytoskeleton, apoptotic activity, oxidative stress and innate immunity [8]. Compared to other well-documented HSPs, sHSPs remains the least understood especially in functional aspect in invertebrates. In marine bivalves, its linkage with heavy metals and pathogen microorganisms is only investigated in scallop [9,10] and clams [2,11].

Nowadays, environmental issues are already at a critical level and they are getting worse, especially for global ocean. Heavy metals are popular for its extensive use in agricultural, chemical and industrial processes. Heavy metal pollution has the characteristics of long duration, not biological degradability and relative stability. These persistent pollutants ultimately uptake from water, sediments, and food sources by filter-feeding animals, including razor clams. It was widely accepted that heavy metal ions could interact with proteins and inhibited protein folding with chaperone-mediated protein disaggregation and refolding manners [12]. However, rare information is available regarding to molecular features and functions on sHSP towards heavy metals exposure in the species to our knowledge. The main purpose of the study are: (1) to clone the full-length cDNA of sHSP from *S. constricta* (ScsHSP); (2) to investigate the tissues expression patterns of

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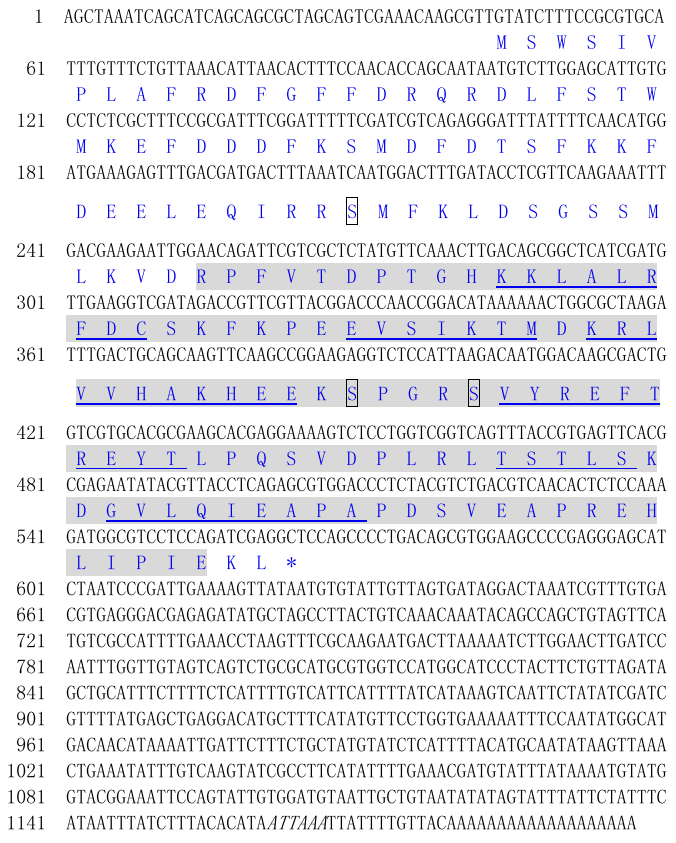

Fig. 1. Complete cDNA sequence of small heat shock protein from *Sinonovacula constricta* and its deduced amino acid sequence (upper). Nucleotides were numbered from the first base at the 5′ end. The asterisk indicated the stop codon. The polyadenylation signal sequence (ATTAAA) was italics. The typical α-crystalline domain was shadowed. Six beta strands were underlined. Three serines (S^{15}, S^{78}, and S^{82}) susceptible of phosphorylation were boxed.

ScsHSP; (3) to clarify the time-course expression profiles of ScsHSP in haemocytes and gill under heavy metals exposure.

2. Materials and methods

2.1. Clams, and challenge experiment

The razor clams *S. constricta* (3–4 cm in length) were collected from Ninghai, Ningbo, China in November, 2012. The clams were acclimated for three days before commencement of the experiment. The temperature was held at 21 ± 1 °C throughout the whole experiment. The salinity for the supplied sea water was kept at 30 ppt.

For heavy metals stresses experiment, the clams were randomly divided into five flat-bottomed rectangular tanks (60 cm × 45 cm - × 30 cm) with 60 clams each. One tank served as control. The others were exposed to two heavy metals of $CdCl_2$ and $PbCl_2$ with final concentration of 1 mg L^{-1} and 10 mg L^{-1} for 12 h and 36 h, respectively. The haemolymphs from the control and the treated groups were collected using a syringe individually and centrifuged at 2000 × g, 4 °C for 10 min to harvest the haemocytes. The gills were sheared and grinded in nitrogen for further use. There were five replicates for each treatment and the control group.

2.2. cDNA library construction and EST analysis

A SMART cDNA library was constructed from the whole bodies of *S. constricta* using SMART cDNA Library Construction Kit (Clontech) according to our previous work [13]. BLAST analysis of the EST sequences revealed that an EST of 884 bp was highly similar to the previously identified sHSPs. Therefore, the EST was selected for further cloning of the full-length cDNA of sHSP from *S. constricta*.

2.3. Cloning and analysis of the full-length cDNA of ScsHSP

Two sets of gene specific primers, P1 5′-ACAGATTCGTCGCTCTATGTTCA-3′ and P2 5′-ACACTCTCCAAAGATGGCGTCCT-3′, were designed based on the EST to clone the full-sequence cDNA of ScsHSP from cDNA libraries. The PCR conditions were the same as that described above [2], except for the annealing temperature (60 °C). The PCR products were cloned into the pMD18-T simple vector (TaKaRa) and sequenced by Invitrogen, Shanghai. The resulting sequences were assembled with the known EST and subjected to BLAST analysis.

The ScsHSP gene sequence was analyzed using the BLAST algorithm at NCBI web site (http://www.ncbi.nlm.nih.gov/blast), and the deduced amino acid sequence was analyzed with the Expert Protein Analysis System (http://www.expasy.org/). Sequence alignment of ScsHSP with human and other mollusk counterparts was performed with the ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/). Specific domain prediction was detected by SMART service (http://smart.emblheidelberg.de/). The predicted tertiary structure was established using the SWISS-MODEL algorithm (http://swissmodel.expasy.org/). The potential phosphorylation sites were forecasted by NetPhos 2.0 Server (http://www.cbs.dtu.dk/services/NetPhos/).

2.4. Spatial-course expression of ScsHSP mRNA

Total RNA was isolated from unchallenged clams' tissues of haemocytes, gill, gonad, mantle and water pipe using the TRIzol reagent (Invitrogen). First-strand cDNA synthesis was performed according to Promega M-MLV RT Usage information with the RQ1 RNase-Free DNase (Promega)-treated total RNA (1 μg) as template and oligo (dT) primer. The reactions were incubated at 42 °C for 1 h, terminated by heating at 95 °C for 5 min. The spatial expression levels of ScsHSP were measured by qPCR in Rotor-GeneTM 6000 real-time PCR detection system. Two ScsHSP gene specific primers, P3: 5′-GGACTAAATCGTTTGTGACGTGAGG-3′ and P4: 5′-ACAGAAGTAGGGATGCCATGGACC-3′, were designed to amplify a product of 192 bp. For normalizing the ScsHSP transcripts, *S. constricta* actin gene was amplified as an internal control with P5: 5′-GCCGCTTCTTCATCCTCAT-3′ and P6: 5′-GTCGGCAATACCTGGGAAC-3′. Melting curve analysis of the amplified products was performed at the end of each PCR to confirm that a single PCR product was produced and detected. The $2^{-\Delta\Delta CT}$ method was employed to analyze the expression level of ScsHSP.

2.5. Temporal expression profile of ScsHSP mRNA in haemocytes and gill post cadmium or lead exposure

Comparative qualification of ScsHSP transcript in haemocytes and gill against heavy metal stresses was measured by qPCR. PCR conditions, thermal profile and subsequent data analysis were according Section 2.4. All data were given in terms of relative mRNA expression as means ± S.E. The results were subjected to One-way Analysis of Variance (ANOVA) followed by multiple Duncan test to determine differences between challenged and control groups of each sampling time. The *P* values less than 0.05 were considered statistically significant.