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The link between selenium binding protein from *Sinonovacula constricta* and environmental pollutions exposure

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ABSTRACT

Selenium binding proteins (SeBPs) play a crucial role in controlling the oxidation/reduction in many physiological processes. Here we reported the isolation and characterization of a cDNA of SeBP gene from *Sinonovacula constricta* (denoted as ScSeBP). The full-length cDNA of ScSeBP was of 2345 bp, consisting of a 5′UTR of 246 bp, a 3′ UTR of 626 bp, and a complete ORF of 1473 bp encoding a polypeptide with 491 amino acid residues. The predicted molecular mass of deduced amino acid of ScSeBP was 54.85 kDa and the theoretical pI was 6.44. Tissue distribution analysis of the ScSeBP revealed that the mRNA transcripts of ScSeBP were constitutively expressed in all examined tissues with the higher expressions in gill, gonad and the haemocytes. The temporal expression of ScSeBP in gill and haemocytes after B[α]P and heavy metals exposure were recorded by qPCR. B[α]P exposure at 0.5 and 5 mg L^{-1} caused significant increase in mRNA expression of ScSeBP in haemocytes, but down-regulated ScSeBP mRNA expression in gill. Concerning heavy metals stresses, the suppressed expression patterns were detected in gill and haemocyte except lower concentration of $PbCl_2$ exposure in haemocytes at 12 h. All our results indicated that ScSeBP was one of key effectors in mediating B[α]P and heavy metals exposure.

1. Introduction

As an important oligo-element, selenium has been shown to play a central role in redox processes in the cell [1]. It is repoted that selenium depletion or deficiency are associated with development of a form of heart disease, hypothyroidism, Crohn's disease and a weakened immune system [2]. On the other hand, uncontrolled high level of selenium in living cells called selenosis can also seriously affect on organism's viability and increase the risk of cell damage. Symptoms of selenosis in human include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage [3]. Therefore, organisms have evolved to use some regulated proteins to tightly control selenium under optimal concentration, such as selenium-binding protein, glutathione peroxidases and the thioredoxin reductases [4].

Selenium-binding protein (SeBP), a ubiquitous and highly conserved protein, was originally discovered in the human liver in 1997 [5]. The protein could be covalently binded to selenium and tightly regulated by heavy metals [6]. In abalone *Haliotis discus hannai* Ino, dietary selenium and other metals could induce SeBP expression at mRNA level and the optimal concentration of selenium was 1 mg kg^{-1} [7]. Much attention was paid to the link of SeBP with the antioxidant defense system in reduction/oxidation modulation [1] [2], or as a scavenger of toxic oxidant species [8] [9]. The protein responsed to oxidative radical intracellular was tightly regulated by its antioxidan responsive element (ARE), which was located in the 5′ end of its mRNA [10,11]. In murine hippocampal HT22 cells, overexpression of SeBP prevented oxidative damage induced by hydrogen peroxide, and knock-down SeBP resulted in increased ROS as well as the differential activities of antioxidantenzymes, supporting that SeBP played an important function in ptrotecting cell from oxidant stress [12]. Jamba had also reported recently that SeBP affects the distribution of cadmium, when selenium was administered concurrently [13]. In lowere marine animals, 56-KDa SeBP from scallop *Chlamys farreri* haemocytes was shown to be involved into mediating the anti-oxidation mechanisms and immune response [4]. The connection of SeBP with innate immunity was observed in WSSV challenge shrimp [14] and AVNV challenged scallop [15] by transcriptome or proteomic analysis. However, physiological function of invertebrates SeBP was still largely unclear compared to the counterparts in higher animals.

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Environmental pollution is already at a critical level and they are worsening over time, especially for the global ocean. B[α]P are becoming more and more prevalent in marine ecosystems as a result of growing industrial activities and expanding urbanization [16]. Heavy metals are also popular for its extensive use in agricultural, chemical and industrial processes. These persistent pollutants ultimately uptake from water, sediments, and food sources by filter-feeding animals, including clams *Sinonovacula constricta*. However, rare information is available regarding to molecular features and functions on SeBP in the species. The purpose of the study are: (1) to clone the full-length cDNA of SeBP from *S. constricta* (ScSeBP); (2) to investigate the tissues expression patterns of ScSeBP; (3) to clarify the time-course expression profiles under heavy metals and B[α]P exposure.

2. Materials and methods

2.1. Animals and challenge experiment

S. constricta were purchased from Changjie, Ningbo city, Zhejiang province, China, and acclimated for three days before commencement of the experiment. The temperature was maintained at 20–22 °C throughout the whole experiment, and the salinity of the supplied seawater was maintained at 3%.

In the challenge experiment, around 500 clams *S. constricta* were randomly divided into eight glass tanks with 60 individuals each and were exposed to two different concentrations of B[α]P, $CdCl_2$ and $PbCl_2$. B[α]P (analytical purity >99.0%) was first dissolved in absolute acetone and subsequently diluted with an equal volume of seawater to achieve a stock solution of 50 mg L^{-1}. *S. constricta* from two glass tanks was exposed to two final concentrations of 0.5 and 5.0 mg L^{-1} B[α]P. The same volume of acetone and seawater were mixed into the third tanks to serve as acetone control group. The untreated individuals were selected as untreated control group. The tank was covered with glass to avoid evaporation of B[α]P in the whole experiment. For heavy metals challenge, *S. constricta* from four tanks were treated with $CdCl_2$ or $PbCl_2$ at two final concentration of 1 mg L^{-1} and 10 mg L^{-1}, respectively. Haemolymphs and gill from the control and the experimental groups were collected individually at 0, 12 and 36 h post each pollutant exposure. Haemolymphs were centrifuge at 1000 rpm for 5min to harvest the haemocytes. We performed five replicates for each of the experimental groups as well as the control group. Samples were immediately frozen in liquid nitrogen and stored at −80 °C for RNA extraction and cDNA synthesis.

2.2. cDNA library construction and EST analysis

An SMART cDNA library was constructed from the whole bodies of *S. constricta* using SMART™ cDNA Library Construction Kit (Clontech). Random sequencing of the library using T3 primer yielded 1220 successful sequencing reactions. BLAST analysis of the EST sequences revealed that two ESTs of 852 and 957 bp were highly similar to the known SeBPs. The sequences were then selected for further cloning of full-length cDNA of selenium binding protein gene from *S. constricta* (ScSeBP).

2.3. Cloning of the full-length cDNA of ScSeBP

Total RNA was isolated from the haemocytes and gill of clams 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 (3 μg) as template. The reactions were incubated at 42 °C for 1 h, terminated by heating at 70 °C for 10 min. Two specific primers, forward primers P1: 5′-GGTTTTGTCCTACTGGATGGCGA-3′ and reverse primers P2: 5′-TTTGATCTTTCTGTGGGGCGGCT-3′, were designed based on the two ESTs to obtain the gap sequence between them. For 3′RACE, the nested PCR strategy was applied with gene specific primer (P3: 5′-TTCAGCAACTGGCTACACGG-3′ or P4: 5′-CAACATCCCTGGTCTCGTCCTG-3′) and oligodT. The PCR products were cloned into the pMD18-T simple vector (TaKaRa) and sequenced bi-directionally with primers M13-47 and RV-M. The sequencing results were verified and subjected to cluster analysis.

2.4. Sequence and phlyogenetic analysis of ScSeBP

The ScSeBP cDNA sequence was analyzed by the BLAST algorithm at National Center for Biotechnology Information (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/). The percentages of similarity and identity of full length amino acid sequences between ScSeBP and SeBPs from other organisms were calculated by the Identity and Similarity Analysis program (http://www.biosoft.net/sms/index.html). An NJ tree was constructed with Mega4.0 software package (http://www.megasoftware.net/) and Clustal X (1.81). To derive the confidence value for the phylogeny analysis, bootstrap trials were replicated 1000 times.

2.5. Spatial and time-course analysis of ScSeBP mRNA by qPCR

Total RNA and first-strand cDNA were generated according to section 2.3. qPCR amplification was performed using a Rotor-Gene 6000 real-time PCR detection system. Two specific primers (P5: 5′-CGAGTGAGCCAATGTCATTCCT-3'and P6: 5′-TGTTGCTGCTGCTGATTTGTTGT-3′) were used to amplify an 274 bp fragment. Two β-actin specific primers (P7:5′-CGGGATCCATGGCTGAGACAATG-3′ and P8:5′-CCCAAGCTTCTAGCTAAGGAGTTTCTGGTC-3′) were used to amplify an 170 bp fragment as an internal control to verify successful reverse transcription and to calibrate the cDNA template. The real-time PCR amplifications were carried out in a total volume of 20 μL containing 10 μL 2 × SYBR Green Mix (Takara), 4 μL of the 1:50 diluted cDNA, 1 μL each primers (20 mM) and 4 μL PCR grade water. The qPCR parameter was denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 20 s, 72 °C for 20 s. Melting analysis of the amplified products was performed at the end of each PCR to confirm that a single PCR product was produced and detected.

2.6. Statistical analysis

The $2^{-\Delta\Delta CT}$ method was employed to analyse the expression level of ScSeBP, and the value obtained denoted the n-fold difference relative to the calibrator [17]. The data were presented as relative mRNA expression levels (means ± S.D., $n = 5$). 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. Significant differences between the treated group and the corresponding control group at each time point were indicated with one asterisk for $P < 0.05$ and two asterisks for $P < 0.01$.

3. Results

3.1. cDNA cloning and sequencing of ScSeBP

Two different ESTs from the cDNA library of *S. constricta* was homologous to the previously known SeBPs, in which one were matched known SeBPs from 1 to 200aa in N terminus, and the other was located from 253 to 410aa in its C ends by Blastx analysis. The 654 bp sequence representing the gap between the