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The response of glutathione peroxidase 1 and glutathione peroxidase 7 under different oxidative stresses in black tiger shrimp, *Penaeus monodon*

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ABSTRACT

Glutathione peroxidase (GPx) is part of the enzymatic antioxidant system that can eliminate the peroxides produced as effect of reactions of molecules with reactive oxygen species (ROS). We cloned two different GPx genes from *Penaeus monodon* (black tiger shrimp). Bioinformatics sequence analysis showed that PmGPx1 and PmGPx7 encoded polypeptides of 207 and 208 amino acids, respectively. PmGPx1 has a selenocysteine residue that is encoded by an opal codon 187TGA189. The PmGPx1 and PmGPx7 expression profiles were detected in the gills and hepatopancreas of the shrimps under osmotic stress, heavy metal exposure and bacterial infection, and the results suggested that PmGPx1 and PmGPx7 are involved in the responses to these stimuli. The recombinant PmGPx1 and PmGPx7 protein was expressed and purified through affinity chromatography and was refolded successfully using ion-exchange chromatography, which were used to measure antioxidant activity, and the results revealed that the rPmGPx1 and rPmGPx7 protein could catalyze the redox reaction between GSH and H_2O_2. This study provides useful information to help further understand the functional mechanism of the GPx family in the immunity of *P. monodon*.

1. Introduction

Reactive oxygen species (ROS) have a physiological function in signal transduction pathways and can induce enormous oxidative (Cesaratto et al., 2004). Oxidative stress, which results from an imbalance between the generation of ROS and the antioxidant capacity of the cell and causes direct or indirect damage to major cellular components such as lipids, proteins and DNA, could be induced by inflammation, stress and respiration (Huang, 2004). To eliminate peroxides, the organisms have developed protective mechanisms, including a non-enzymatic (ascorbic acid, β-carotene, glutathione (GSH), and α-tocopherol) and an enzymatic (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) antioxidant systems (Liu et al., 2007).

GPx not only protects biomembranes and other cellular components from oxidative damage, but also adjusts the intracellular signal transduction by catalyzing the reduction of H_2O_2 to water. As a family of enzymes that has been identified from various species including mammals, fish and insects, there are two kinds of GPx, one kind contains a redox-active cysteine (Cys), functionally identified as thioredoxin-dependent peroxidases, the other type contains selenocysteine at the active site that uses GSH as an obligate substrate (Wu et al., 2010; Lubos et al., 2011). In addition, selenium-dependent GPx genes are known to have the TGA codon for selenocysteine that interacts with the active site residues of tryptophan (Trp) and glutamate (Glu) (Ren et al., 2009). Four isoforms of Se-GPx have been identified in mammals, such as classical GPx (GPx1), gastrointestinal GPx (GPx2), and plasma GPx (GPx3), phospholipid hydroperoxide GPx (GPx4). GPx5 and GPx6 were also cloned from mammals, which were considered as the homologue of GPx3. Besides, phospholipid hydroperoxide GPx (GPx7), cloned in mammals, contains Cys instead of Se-Cys in the active site (Margis et al., 2008; Kryukov et al., 2003; Chu et al., 1993).

GPxs have been cloned from several crustaceans, including the *Metapenaeus ensis* (greasyback shrimp) (Wu and Chu, 2010), the *Scylla paramamosain* (mud crab) (Zeng et al., 2011), the *Exopalaemon carinicauda* (ridgetail white prawns) (Duan et al., 2013) and the *Procambarus clarkii* (crayfish) (Xia et al., 2013). Some previous studies also reported on a GPx gene from *P. monodon* (Liu et al., 2010; Tang et al.,

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2016). GPx expression and/or activity were most up-regulated when these crustaceans encounter pathogens, such as white spot baculovirus (WSSV), *Vibrio harveyi*, *Streptococcus agalactiae* (Liu et al., 2007; Zeng et al., 2011; Liu et al., 2010; Ren et al., 2015). The GPx expression of shrimp under different acute stresses was mostly up-regulated (Zeng et al., 2011; Ren et al., 2015). Increased GPx expression could contribute to metabolize ROS more effectively and help organisms overcome difficulties (Zeng et al., 2011).

P. monodon, an economically important crustacean aquaculture species in South China and Southeast Asia (Fu et al., 2015), has been shown to develop resistance to oxidative damage under multiple conditions, such as invading pathogens, environmental toxicity, or another change in environmental factors (Yingvilasprasert et al., 2014). To provide a theoretical basis for clarifying the antioxidant defense mechanism, we successfully cloned *GPx1* and *GPx7* full-length cDNA from *P. monodon*, and investigated the expression patterns of *PmGPx1* and *PmGPx7* transcripts exposed to osmotic stress, heavy metal and bacteria.

2. Materials and methods

2.1. Experimental animals and sample preparation

Experimental shrimps (15 ± 3 g body weight) specimens were collected from the Shenzhen base of South China Sea Fisheries Research Institute (Guangdong, China), and acclimated in aerated seawater (salinity 3.3%) for 3 days at 25 ± 1 °C before commencing the experiments. Approximately two thirds of the water in each tank was renewed daily. The shrimps were fed commercial diets during acclimation until 24 h before treatment.

2.2. Tissue distribution analysis

Ten tissue types, including the gills, heart, ovaries, stomach, haemolymph, intestines, hepatopancreas, testis, muscle, were collected from three shrimps to determine the tissue distribution of the *GPx1* and *GPx7* transcripts. All of the samples were kept in RNAlater (Ambion, CA, USA) and stored at − 80 °C until RNA extraction.

2.3. In vivo challenge of P. monodon with bacteria

Bacteria were stimulated with *V. harveyi* and *S. agalactiae*, both of which were Gram-negative and positive bacteria, and were able to observe the expression of the gene under the stimulation of these two bacteria. *V. harveyi* and *S. agalactiae* were respectively cultured in 2216E medium and Brain Heart Infusion (BHI) broth, with constant shaking (180 rpm) at 28 °C for 12 h, Bacteria were diluted in sterilized phosphate-buffered saline (PBS, pH 7.4) at adensity of 1.0×10^8 CFU/mL, according to previous studies (Shi et al., 2016). Three groups treated aerated seawater (salinity 3.3%) at 25 ± 1 °C were established, including a control group injected with 0.2 mL of PBS, and two other groups with 0.2 mL of *V. harveyi* (1×10^8 CFU/mL) or 0.2 mL of *S. agalactiae* (1×10^8 CFU/mL) injected that were used as the experimental groups, both of which were intramuscularly. Three individuals were collected at the indicated times post-infection, including 0, 6, 24, 48, 72, and 96 h. The hepatopancreas and gills were harvested in RNAlater and stored at − 80 °C until RNA extraction.

2.4. Osmotic stress challenge

Osmotic stress can disturb the redox balance of organisms and lead to oxidative stress. Glutathione peroxidase play an important role in the defense against ROS in crustaceans, and for this reason, we designed this experientment (Lushchak, 2011). Two different salinity conditions were used to challenge fifty shrimps, the first group was treated with low salinity (2.3%), and the second group of shrimps was maintained in 4.3% seawater, according to previous studies (Li et al., 2017). We adjusted salinity using fresh water and sea salt, and measure salinity of aquaculture water every day with salinity meter (WZ211, Shanghai JL Optics Instrument Co., Ltd., Shanghai, China). Three shrimps (control group) reared in normal seawater and three individuals exposed in two different salinity conditions were collected 0, 4, 8, 16, and 32 h. The hepatopancreas and gills were kept in RNAlater and stored at − 80 °C until they were needed.

2.5. Heavy metal exposure

Heavy metal pollution has become a serious problem in aquatic aquaculture ecosystems. Cadmium (Cd) is a widespread environmental pollutant of increasing worldwide concern. Exposure to high concentrations of cadmium can cause oxidative damage in cells, Zinc (Zn) has a physiological necessity for the normal development and growth of aquatic organisms, A certain concentration of copper (Cu) may affect the growth of shrimp (Qian et al., 2012; Wong et al., 1995). Cu, Zn and Cd, were selected as stressors. According to previous studies, the concentrations of Cu, Zn, and Cd were set to 2.8, 7.7, and 69 μM, respectively (Chen and Lin, 2001; Wang et al., 2001; Qian et al., 2012). Three kinds of heavy metal $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H_2O$ and $CdCl_2{\cdot}2.5H_2O$ were dissolved respectively in deionized water and equipped with 100 times working solution. The experiment was performed in 4 groups: control group, Cu treatment group, Cd treatment group and Zn treatment group. The gills and hepatopancreas of three individuals in the experiment groups were randomly collected at 0, 6, 12, 24, 48, and 96 h after heavy metal exposure. Six shrimps in normal seawater were collected as control samples. All of the samples were kept in RNAlater for further experiments.

2.6. RNA isolation and reverse transcription

The total RNA of the dissected tissues was extracted using Trizol reagent (Invitrogen, USA) following the manufacturer's protocol. RNA quantity, integrity and purity were verified by both native RNA electrophoresis on a 1.2% agarose gel and the ratio of UV absorbance was measured at 260 and 280 nm (NanoDrop Technologies, DE, USA). First-strand cDNA was synthesized from 1 μg of the total RNA by using a PrimeScript™ reverse transcriptase kit (TaKaRa, Dalian, China).

2.7. PmGpx1 and PmGpx7 cloning

Partial sequences of *PmGpx1* and *PmGPx7* were obtained from the transcriptome database of *P. monodon* in our laboratory (unpublished). According to blast sequence alignments of the National Center for Biotechnology Information (NCBI), we name the two genes *GPx1* and *GPx7* in this report. To obtain the full-length cDNA sequence of *PmGpx1* and *PmGpx7*, two pairs of primers (Table 1: GPx1-F, GPx1-R, GPx7-F, GPx7-R) were designed based on partial cDNA sequences to identify the *PmGpx1* and *PmGpx7* partial sequences. The full-length cDNA of *PmGpx1* and *PmGpx7* was amplified through the rapid amplification of cDNA ends (RACE) approach. RACE-PCR was performed using a SMART™ RACE cDNA amplification kit (Clontech, Takara, Dalian, China) with specific primers (Table 1: GPx1-5′GSP1, GPx1-5′GSP2, GPx1-3′GSP1, GPx1-3′GSP2, GPx7-5′GSP1, GPx7-5′GSP2, GPx7-3′GSP1, GPx7-3′GSP2).

2.8. Sequence analysis, multiple sequence alignment, and phylogenetic analysis

The full-length cDNA sequences of *PmGpx1* and *PmGPx7* were analyzed with the BLAST programs at the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The complete ORF regions and amino acid sequences were analyzed using an ORF finder (https://www.ncbi.nlm.nih.gov/