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Short communication

Response of selenium-dependent glutathione peroxidase in the freshwater bivalve *Anodonta woodiana* exposed to 2,4-dichlorophenol,2,4,6-trichlorophenol and pentachlorophenol

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ABSTRACT

2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP) pose a health risk to aquatic organism and humans, and are recognized as persistent priority pollutants. Selenium dependent glutathione peroxidase (Se-GPx) belongs to the family of selenoprotein, which acts mainly as an antioxidant role in the cellular defense system. In the current study, a Se-GPx full length cDNA was cloned from *Anodonta woodiana* and named as AwSeGPx. It had a characteristic codon at 165TGA167 that corresponds to selenocysteine(Sec) amino acid as U44. The full length cDNA consists of 870 bp, an open reading frame (ORF) of 585 bp encoded a polypeptide of 195 amino in which conserved domain (68LGFPCNQF75) and a glutathione peroxide-1 GPx active site (32GKVILVENVASLUGTT47) were observed. Additionally, the eukaryotic selenocysteine insertion sequence (SECIS) was conserved in the 3′UTR. The AwSeGPx amino acid sequence exhibited a high similarity with that of other Se-GPx. Real-time PCR analysis revealed that AwSeGPx mRNA had a widely distribution, but the highest level was observed in hepatopancreas. AwSeGPx mRNA expression was significantly up-regulated in hepatopancreas, gill and hemocytes after 2,4-DCP, 2,4,6-TCP and PCP exposure. Under similar environment, clams *A. woodiana* showed a more sensitive to PCP than that of 2,4-DCP and 2,4,6-TCP. These results indicate that AwSeGPx plays a protective role in eliminating oxidative stress derived from 2,4-DCP, 2,4,6-TCP and PCP treatment.

1. Introduction

Products chlorophenols (CPs) are ubiquitous globally in surface waters, groundwater, wastewater, sludge and drinking waters because they are widely used in agriculture and industry as pesticides, wood preservatives, and personal care formulations [1,2]. Furthermore, CPs are the most direct precursors of dioxin and polyaromatic compounds in incinerator gas emissions [3]. CPs have received worldwide concern due to their toxicity to aquatic life, persistence, and potential to bioaccumulation [4,5]. Among CPs, 2,4-dichlorophenol (2,4-DCP),2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol(PCP) are ubiquitous in significant quantities, which pose a health risk to aquatic organism and humans and are recognized as persistent priority pollutants in the United States, Europe and China [6,7]. At present, 2,4-DCP, 2,4,6-TCP and PCP exist widely in over 600 sites in the 7 major watersheds and 3 drainage areas of China [8]. They have been shown to induce reproductive toxicity, endocrine activity and oxidative stress in aquatic organism [9]. Previous studies indicate that principal mechanisms of action of PCP-induced toxicity are related to the uncoupling of oxidative phosphorylation in mitochondria and the generation of reactive oxygen species (ROS) [9,10].

In healthy organism, total ROS level stay a relatively low level and ROS will be rapidly eliminated by an array of anti-oxidant enzymes to maintain a balance between ROS level and anti-oxidant enzyme activity [11,12]. Moderate oxidative stress in a shorter

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time may function as an important player to induce different anti-oxidant enzyme expressions to deal with different ROS [13,14]. However, higher ROS concentrations exceed the scavenging capacity of anti-oxidant enzymes could bring about the risk of DNA damage, lipid peroxidation and protein carbonylation [15,16]. Orchestra of anti-oxidant enzymes is a key hallmark of organism homeostasis. Among of these enzymes, glutathione peroxidases (GPx) are one major component of the antioxidant system, which catalyze the reduction of hydrogen peroxide, two isoforms had been identified: selenium-dependent GPx (Se-GPx) and selenium independent GPx. SeGPx catalyzes the reduction of organic and inorganic peroxides like hydrogen peroxide (H_2O_2) while selenium-independent GPx reduces only organic peroxide [17].

Now, highlight of Se-GPx enzyme protecting organisms from oxidative damage gained widely attention. In mammals, four major members of Se-GPx (GPx-1 to GPx-4) have been identified, and show specificities for the hydroperoxide substrates. GPx-1 and GPx-2 reduce rapidly hydrogenperoxide or fatty acid hydroperoxides, GPx-4 can react with phospholipid hydroperoxides [18,19]. It has demonstrated that enhancement of Se-GPx activities contribute to attenuate oxidative injury in mammals and some fish species [20]. In mussel *Mytilus galloprovincialis*, inhibition of Se-GPx activity by Hg could promote a shift in the balance between oxidants and antioxidants in favor of oxidants, resulted in the enhancement of Hg induced oxidative and genotoxic effects [21]. In *Venerupis philippinarum*, administration of heavy metals, such as Cd and Cu, can results in a significant induction of VpSe-GPx expression [22].

Freshwater clams, *Anodonta woodiana* are widely distributed in the world and functions as a main criteria required for a bio-indicator organism. Earlier studies have revealed the ability of *A. woodiana* to accumulate trace elements and pesticides, as well as its potential to detect genotoxicity [23]. In addition, these mussels have important ecosystem functions such as particle filtration and processing, nutrient release, and sediment mixing [23,24]. Considering here, their decline of population can profoundly affect aquatic ecosystem. Take into consideration of great interest to investigate the effect of 2,4-DCP, 2,4,6-TCP and PCP on *A. Woodiana*, in the current study, one complete sequence of Se-GPx has been cloned and named AwSeGPx, and temporal expressions derived from 2,4-DCP, 2,4,6-TCP and PCA exposure were determined by quantitative real-time PCR.

2. Materials and methods

2.1. Ethics statement

All handling methods of clams were conducted in accordance with the guidelines on the care and use of animals for scientific purposes set up by the Institutional Animal Care and Use Committee of Nanyang Medicine College, Nanyang, China.

2.2. Materials

Approximately 1-year-old of clams *A. woodiana* (shell length, 6.5 ± 0.5 cm) were obtained from the Baihe River of Nanyang, Henan Province, China. Prior to experiment, animals were maintained in a recirculation system containing filtered freshwater at 24 °C for 2 weeks in laboratory. 2,4-DCP, 2,4,6-TCP and PCP were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO) and stock solutions were prepared by dissolution in dimethylsulfoxide (DMSO). The experiment was conducted in rectangular plastic boxes (40 cm × 25 cm; 10 cm height) containing 10 L artificial pond water and the concentration of DMSO in the water was not excess 0.01%. Clams *A. Woodiana* were randomly grouped into CPs treated groups in which animals were respectively administrated with 5 test concentrations of 2,4-DCP, 2,4,6-TCP and PCP, and control group with similar volume DMSO. Concentration ranges for viability studies were as follows: 60 μg/L, 120 μg/L, 240 μg/L, 480 μg/L, 960 μg/L for 2,4-DCP, 50 μg/L, 100 μg/L, 200 μg/L, 400 μg/L, 800 μg/L for 2,4,6-TCP, and 20 μg/L, 40 μg/L, 80 μg/L, 160 μg/L, 320 μg/L for PCP. Each group had triplicates per 10 clams and those were not fed during the study. Survival in each box was assessed at48 h and median lethal concentration (LC50) value was calculated using the log-probit method.

In order to determine the tissue distribution of AwSeGPx, several of tissues including foot, gill, hepatopancreas, adductor muscle, heart and mantle were sampled prior to the treatment. According to above mentioned way of animal treatment, 440 clams were selected to determine the expressions of AwSeGPx. Hepatopancreas, gill and hemocytes were pooled at 0, 6, 12, 24 and 48 h, then immediately frozen in liquid nitrogen and stored at −80 °C until total RNA extraction.

2.3. Total RNA isolation and reverse transcription

Total RNA was extracted using TRIzol (Invitrogen Life Technologies, USA) according to the manufacturer's protocol. Quality of RNA was monitored by 1.2% agarose gel electrophoresis and those with complete rRNA bands were selected to produce cDNA. First-strand cDNA was synthesized using M-MLV First-Strand cDNA synthesis Kit (Takara, China) according to the manufacturer's instructions and used as the template for PCR reaction.

2.4. Cloning of AwSeGPx cDNA

GPx fragment was amplified using two degenerate primers G1 and G2 (Table 1), which were designed according to conserved domains of SeGPx of other species including bivalve, gastropod, insect, crustacean and vertebrate. The PCR products were subcloned into the pMDT-19 (Takara, China), sequenced from both directions (Invitrogen Life Technologies, China) and identified SeGPx partial cDNA sequence. Highly stringent primers (Table 1) designed from the partial cDNA sequences were used to characterize the 5′ and 3′ regions of the AwSeGPx cDNA by rapid amplification of cDNA ends (RACE) approaches (Takara, China) according to the manufacturer's protocol. 5′ Raceouter primer and GPx5-1 (Table 1) were used for the first-round PCR of AwSeGPx 5′ RACE, 3′ Raceouter

Table 1
Description of the primes used in this study. G1 and G2 were degenerate primers and used to isolate partial cDNA of SeGPx. 5′ Race Outerprimer, 5′ Race Innerprimer, GPx5-1 and GPx5-2 were used to characterize the 5′ RACE of the AwSeGPx in the nest PCR, 3′ Race Outerprimer, 3′ Race Innerprimer, GPx3-1 and GPx3-1 for 3′ RACE. GPx-F and GPx-R as well as β-F and β-R were selected to isolate AwSeGPx and β-actin in real-time PCR, respectively.

Primer	Sequence (5′–3′)
G1	TTGTAGCCNGTCTNCCCAGG
G2	GTGTCGNCTGAGANGTAATAGAA
5′Race Innerprimer	CATGGCTACATGCTGACAGCCTA
5′Race Outerprimer	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
GPx5-1	TCCTTCTCATCGTGGTGCA
GPx5-2	ACCACGCCAAACTTGCCCT
3′Race Outerprimer	TACCGTCGTTCCACTAGTGATTT
3′Race Innerprimer	CGCGGATCCTCCACTAGTGATTTCACTATAGG
GPx3-1	CCTGAAATTCTCCAGCATATG
GPx3-2	ATCCTGCGACCCAGACACTG
GPx-F	CGTTGAAGTATGTGCGACCTGG
GPx-R	GCCATCAGGAGCAATCAGGAAC
β-F	CATCCCTTGCTCCTCCAACTATG
β-R	CTGGAAGGTAGAGAGAGAAGCCAAG