Fish and Shellfish Immunology 89 (2019) 595–602

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Response of a novel selenium-dependent glutathione peroxidase from thick shell mussel *Mytilus coruscus* exposed to lipopolysaccharide, copper and benzo[α]pyrene

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ARTICLE INFO

Keywords:
Mytilus coruscus
Selenium-dependent glutathione peroxidase
Environmental stressor
Enzyme activity

ABSTRACT

Glutathione peroxidase (GPx) plays an important antioxidant role in cellular defense against environmental stress. In the present study, a novel selenium-dependent glutathione peroxidase termed *Mc*SeGPx firstly identified in thick shell mussel *Mytilus coruscus*. *Mc*SeGPx consists of 197 amino acid residues, characterized with one selenocysteine residue encoded by an opal stop codon TGA, one selenocysteine insertion sequence (SECIS) in the 3′ untranslated region (UTR), two active site motifs and one signature sequence motif. *Mc*SeGPx transcripts were constitutively expressed in all examined tissues, and were significantly induced in gills and digestive glands with the stimulations of lipopolysaccharide (LPS), copper (Cu) and benzo[*α*]pyrene (B[*α*]P). Additionally, rough increases in *Mc*SeGPx activity were detected in both tissues under the challenge of LPS, Cu and B[*α*]P. Collectively, these results suggested that *Mc*SeGPx affiliate to selenocysteine dependent GPx (SeGPx) family and might play an important role in mediating the environmental stressors and antioxidant response in *M. coruscus*.

1. Introduction

Reactive oxygen species (ROS), generated as inevitable by-products during aerobic respiration and substrate oxidation [1], play crucial roles in regulating various physiological activities containing signal transduction, tissue and organ dysfunction, cell apoptosis and immune defense [2–4]. In the healthy organisms, ROS are continuously formed and scavenged in cells to keep them within a reasonable concentration range, and once ROS concentration exceed normal level, the accumulation of mass ROS will cause damage to cell structure, lipid, proteins and nucleic acids included, and even immune dysfunction [5]. Organisms have developed many scavenger mechanisms containing non-enzymatic antioxidant (melatonin, coenzyme Q, ascorbic acid, beta-carotene, glutathione, alpha-tocopherol, etc) and antioxidant proteins (glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT)) to eliminate excess ROS [6,7], among which antioxidant proteins were considered as the first line of antioxidant defense against peroxides, superoxide anion and hydrogen peroxide.

As an important component of antioxidant system, GPxs perform its antioxidant function by catalyzing the reduction of hydrogen peroxide and organic hydroperoxides to corresponding alcohols and water [8]. In general, GPxs are divided into two subfamilies: selenocysteine dependent GPx (SeGPx) and selenocysteine independent GPx (non-SeGPx), and the former contains one selenocysteine (SeC) residue encoded by TGA and the latter possesses cysteine instead of SeC [9]. Non-SeGPx could reduces organic peroxide while SeGPx catalyses the reduction both of organic and inorganic peroxides [10]. In mammals, eight isoforms containing GPx1-8 has been identified based on their cellular location, structure, and specific substrate [11], and among which GPx1-4 and GPx6 are SeGPx while GPx5 and GPx7-8 are non-SeGPx [12].

At present, the study of SeGPx enzyme which protects the body from oxidative damage has attracted extensive attention. The role of SeGPx in antioxidant defense and host innate immune has been proved in lower vertebrates such as fish. In sea bream and common carp, GPx was used as used as an indicator of oxidative stress under different environmental stimuli [13,14]. In *Alburnoides fasciatus*, GPx expression was significantly induced by cadmium of different concentrations [14]. In cells cultured in vitro from *Seriola lalandi* and *Oncorhynchus mykiss*, GPx expression changed in response to tert-butyl hydroquinone and selenium exposure, respectively [15,16]. Additionally, in large yellow

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https://doi.org/10.1016/j.fsi.2019.04.028
Received 22 February 2019; Received in revised form 1 April 2019; Accepted 10 April 2019
Available online 13 April 2019

croaker, GPx was proved to be involved in immune response to bacterial infection [17].

Despite of the crucial antioxidant function, fewer studies of SeGPx have been reported in mollusk. It has been demonstrated that SeGPx could be induced after toxic chemical exposure, bacterial and viral infections in some mollusk species including *Mizuhopecten yessoensis* [18], *Chlamys farreri* [19], *Venerupis philippinarum* [20], *Haliotis discus discus* [21,22], *Mytilus galloprovincialis* [23] and *Anodonta woodiana* [24]. However, there are very few reports on antioxidant responses of mollusk SeGPxs to heavy metal exposure. Aquatic organisms are facing increasing environmental stress including a wide range of biotic (bacterial and viral) and abiotic (dynamic variation in temperature, salinity, toxic chemicals, heavy metals) stresses [25]. The usage of biological indicators to monitor the environment change has been proved to be a effective measure. Bivalves, particularly Mytilus sp, representing the sedentary and sentinel species, have been frequently used to assess the possibility of anthropogenic contaminants in marine environment [26,27].

The thick shell mussel *M. coruscus,* described as an important economic shellfish, widely distributed in the eastern coast of East China Sea, especially Zhoushan coast. It has been demonstrated that adverse reactions caused by pathogenic bacterium and heavy metals have a serious impact on the cultivation of *M. coruscus* [28]. In previous study, we assessed the effects of low concentrations copper on antioxidant responses, DNA damage and genotoxicity in thick shell mussel *Mytilus coruscus*, and the results suggested that *M. coruscus* was susceptive to copper and deepen the mussels as a suitable model marine invertebrate species to study potential detrimental effects induced by possible toxicants [29]. In this study, we characterized a novel selenium-dependent glutathione peroxidase from *Mytilus coruscus*, meanwhile its response to LPS, Cu and B[α]P expose was analysed. These results would contribute to understand the regulatory mechanism of *Mc*SeGPx in oxidative stress and deepen the possibility of the SeGPx used as a novel biomarker.

2. Materials and methods

2.1. Experimental animals, challenge assays and sampling

Healthy thick shell mussels (weight 62.5 ± 5.6 g, length 9.1 ± 0.5 cm) were obtained from Dongji island, Zhoushan, Zhejiang province, China. Before treated, mussel individuals were acclimated in a plastic tank filled with air-pumped natural filtered sea water (salinity 30 ± 2‰, temperature 24 ± 1 °C) under the laboratory conditions for one week, and chlorella was fed daily.

The LPS and Cu^{2+} challenge were performed according to our previous research [30]. Briefly, for LPS challenge, mussel individuals were adductor injected with 200 μL LPS (0.5 mg/mL) while in the control group the same volume PBS was injected. For Cu^{2+} challenge, Cu^{2+} ($CuSO_4 \cdot 5H_2O$) was added to the seawater to a final concentration of 20 μg/L, whereas in the control group, nothing was added to the seawater. For B[α]P challenge assay, B[α]P (Sigma, America) was firstly diluted using dimethyl sulfoxide (DMSO) as the vehicle, then the B[α]P solution was added to the seawater to obtain a final concentration of 50 μg/L, and the final concentration of DMSO was 0.01%. In the control group, DMSO was added to seawater to obtain a final concentration 0.01%. Each group consisted of three biological duplications.

In LPS and B[α]P challenge assays, Gills and digestive glands were collected at 3, 6, 12, 24 and 36 h post induction (hpi) and immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction. In Cu^{2+} challenge assay, gills and digestive glands were sampled at 2, 5, 10, 16, 23 and 31 d post induction (dpi). Three individuals in each duplication were randomly sampled at every time point and pooled together to reduce individual variation.

For enzyme activity determination assay, moreover gills and digestive glands (100 mg) were collected and ground in liquid nitrogen, and then suspended in 0.9 mL phosphate buffer. The homogenate was centrifuged at 4 °C, 2500 rpm for 10 min and the resulting supernatant was collected for determination of the activities of *Mc*SeGPx.

Table 1
Description of the primers used in this study.

Primer	Sequence (5‘ to 3’)	Usage
GPx-ORF	ATGAACGAGTTGATCGACAAAT CTAAACTTTGTGTTTTTCTATTA	For ORF cloning
5′-RACE	GATTTCTTCACCGCTT CATTTTCCTGATGTCC TACAGGGAAATCCCAGGA	For 5′-UTR RACE
3′-RACE	CACTTCCAAGCGACGATGCTGTTA ATATGGGAGCCGGTGACAAGAACT	For 3′-UTR RACE
β-actin	ATGAAACCACCTACAACAGT TAGACCCACCAATCCAGACG	internal control
GPx-real	CATGTTCGACCAGGGAAAGGA GAAGCTAACAGCATCGTCGC	For *Mc*SeGPx qPCR

Six tissues, including the gills, digestive glands, hemocytes, muscle, gonads, and mantles were dissected from eight adult individuals to examine the tissue distribution of *Mc*SeGPx. All tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.2. Cloning of the McSeGPx cDNA

Total RNA was extracted using Trizol reagent (TaKaRa) and the First-strand cDNA synthesis was carried out based on Promega M-MLV reverse transcriptase using oligo (dT)-adaptor as primer. The cloning of *Mc*SeGPx cDNA was according to our previous study [31]. Briefly, through scanning the *M. coruscus* transcriptome [32], the open reading frame (ORF) region of *Mc*SeGPx was obtained, following specific primer pairs GPx-ORF as well as 5′- and 3′-RACE (Table 1) were designed to performed the gene cloning and rapid-amplification of cDNA ends (RACE) assays, finally, the ORF and 5′- and 3′- untranslated region (UTR) sequences were assembled to obtain the full length of *Mc*SeGPx cDNA using CAP3 software [33].

2.3. Bioinformatic analysis of McSeGPx

*Mc*SeGPx sequence were analysed and compared using the blastn program in NCBI (www.ncbi.nlm.nih.gov/blast). The signal peptide was predicted by signal program (http://www.cbs.dtu.dk/services/SignalP). Prediction of protein domain was carried out with the InterProScan (http://www.ebi.ac.uk/InterProScan/). The SECIS sequence and stem loop structure was predicted and generated by a SECISearch3 program (http://seblastian.crg.eu/). Multiple protein sequence alignments of SeGPx were aligned using the Clustalx2.0. Phylogenetic analysis were preformed by the Neighbor-joining method using MEGA6.0 software. Reliability of trees obtained was assessed by bootstrapping, using 1000 bootstrap replications.

2.4. Quantitative real time PCR of McSeGPx

TheR) was carried out to assess the temporal expression profile of *Mc*SeGPx transcripts according to our previous description [Qi 2018]. Briefly, qPCR was performed with one specific pair of primers GPx-real (Table 1) on 7500 Real-time PCR system (Applied Biosystems, USA). Amplifications were carried out in final volume of 10 μL, containing F and R primer 0.4 μL, respectively, SYBR® Premix Ex TaqTM II (TaKaRa) 5 μL, cDNA sample 0.4 μL, ROX Reference Dye II 0.2 μL, ddH_2O 3.6 μL. The relative expression levels were measured using the $2^{-\Delta\Delta Ct}$ method with β-actin as an internal reference [34].

2.5. Activity of McSeGPx enzyme

The *Mc*SeGPx activity was determined by the colorimetric method

using a GPx kit (Nanjing Jiancheng, China) based on the principle that oxidation of glutathione (GSH) and hydrogen peroxide (H_2O_2) could be catalyzed by GPx to produce oxidized glutathione (GSSG) and H_2O. In addition GSH reacts with 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) to produce stable yellow substances and the decrease of GSH at 412 nm during the reaction is indicative of GPx activity in tissues. One GPx unit of GPx activity (U) was calculated as the amounts of enzyme that will oxidize 1 μmol/L GSH in reaction system at 37 °C per minute in 1.0 g fresh tissue according to the assay kit. All of the enzymes were expressed as in U/g FW.

2.6. Statistical analysis

All data were expressed as means ± standard deviation (SD) of triplicate experiments (n = 3). Statistical analysis of the data were performed by one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test using SPSS 19.0 [35]. Differences were deemed significant at $P < 0.05$.

3. Results

3.1. In silico analysis of McSeGPx

The full length cDNA of a novel *SeGPx* (namely *Mc*SeGPx, accession number MH161185) was obtained through gene cloning and 5′, 3′ RACE. The *Mc*SeGPx cDNA of 970 bp consists of 156 bp 5′-UTR, 220 bp 3′-UTR and 594 bp ORF sequence coding a deduced amino acid sequence of 197 residues (Fig. 1A). The calculated molecular mass of *Mc*SeGPx is 22.6 kDa and the protein has a theoretical isoelectric point of 8.89. A stop codon ‘^{292}TGA^{294}’ encoded Sec amino acid as ‘U46’ was observed in *Mc*SeGPx putative amino acid sequence (Fig. 1A). There was no signal peptide was predicted in *Mc*SeGPx by online SignalP. In the N-terminal region, a signature sequence motif ‘^{70}LGFPCNQF^{77}’ and a glutathione peroxide-1 GPx active site ‘^{34}GKVILVENVASLUGTT^{49}’ were predicted (Fig. 1A). In addition, an extra active site motif ‘^{158}WNFEKF^{163}’, two arginine residues ‘R96’ and ‘R153’ involved in binding glutathione, two amino acids residues glutamine and tryptophan (‘Q80’ and ‘W158’) responded for fixation of Se in GPx were also predicted in *Mc*SeGPx (Fig. 1A). In addition, six amino residues ‘79H’, ‘82 N’, ‘84S’, ‘87E’, ‘90 N’, and ‘94H’ which were involved in dimer formation were observed (Fig. 1A). In the 3′-UTR, characteristic SECIS element (91bp) was identified by SECISearch3 program (Fig. 1A). Multiple alignment results revealed that above observed functional sites and motifs in *Mc*SeGPx were conserved from invertebrates to vertebrates (Fig. 1B).

3.2. SECIS element comparison and stem-loop structure of McSeGPx

As shown in Fig. 2A (ii), a 91-bp SECIS element which formed a stem-loop structure was predicted in the 3′-UTR of *Mc*SeGPx gene. The SECIS element of *Mc*SeGPx containing two helices separated by an internal loop, a quartet (SECIS core structure) and an apical loop surmounting helix II (Fig. 2A). Additionally, multiple alignment of SECIS elements showed that the almost completely conserved short sequences of ‘UGAU’, ‘AAA’ and ‘GGAU’ were predicted in the 5′ stem, apical loop and 3′ stem, respectively (Fig. 2B).

3.3. Temporal expressions of McSeGPx mRNA

The tissue distribution of *Mc*SeGPx transcripts was examined by qPCR. As shown in Fig. 3, *Mc*SeGPx mRNA was constitutively expressed in all examined tissues including digestive glands, gills, hemocytes, adductor muscle, mantles and gonads. However, it was different in the expression level of *Mc*SeGPx mRNA in various tissues, and the highest expression level was detected in hemocytes, followed by gills and digestive glands, weakest in gonads.

Under LPS, B[α]P and Cu^{2+} challenge, the expression of *Mc*SeGPx mRNA could be significantly induced in gills and digestive glands despite of the discrepant expression levels under these three challenges (Fig. 4). After LPS challenge, the expression of *Mc*SeGPx mRNA in gills was significantly induced at 3 hpi, after then the expression level gradually elevated and peaked at 24 hpi (an 18.7-fold increase), while in digestive glands, *Mc*SeGPx mRNA expression was moderately induced and reached the peak (an 3.9-fold increase) at 36 hpi when the assay finished (Fig. 4A and B). Under B[α]P challenge, the transcriptional expression of *Mc*SeGPx in digestive glands was significantly induced at 12 hpi and peaked at 24 hpi (an 7.2-fold increase), while in gills, *Mc*SeGPx mRNA level remarkably increased at 3 hpi and sharply decreased at 6 hpi, after then gradually increased to the peak value at 24 hpi (an 26.9-fold increase) (Fig. 4C and D). After Cu^{2+} challenge, the induction of *Mc*SeGPx mRNA showed an inverted ‘U’ profile in gills with the highest expression level observed at 10 dpi (an 6.9-fold increase), while in digestive glands, the mRNA expression of *Mc*SeGPx was observed to be significantly induced in all examined sampling nodes (Fig. 4E and F).

3.4. McSeGPx activities

As shown in Fig. 5, *Mc*SeGPx activities increased significantly in gills and digestive glands under LPS, B[α]P and Cu^{2+} stress and generally presented to be gradually increased and reached the peak value when the challenge assays finished. Remarkably, *Mc*SeGPx activity in digestive glands was significantly increased by almost 3.5-fold at 31 dpi in Cu^{2+} treatment which was the highest level of *Mc*SeGPx activity observed (Fig. 5C).

4. Discussion

Understanding the stress response mechanism of mussels may help to elucidate the large-scale mortality of mussels in the process of cultivation in essence, and then to establish a set of disease prevention and control measures [36]. High external stimuli could lead to the accumulation of ROS and cause a series of physiological disorders and the damage of organism. GPx, considered as a crucial member of antioxidant system, plays an effective role in the process of ROS scavenging, which arouse the interest of researchers in recent years. In the present study, a novel GPx homologue termed *Mc*SeGPx was identified in an economic improtant mussel *M. coruscus*. In the putative amino acid sequence of *Mc*SeGPx, a ‘U’ residue was encoded by ‘TGA’ which is the main characteristic of selenoprotein family [37], indicating that this novel GPx is SeGPx. The conserved SeGPx family elements such as two active site motifs, one signature sequence motif were also predicted in *Mc*SeGPx. Two important residues ‘Q’ and ‘W’, which are considered to play an important role in fixation of selenium during the progress of GPx catalytic activity [38], were also conserved in *Mc*SeGPx. Additionally, there existes two conserved ‘R’ residues in common SeGPxs, which are demonstrated to directs the donor substrate (glutathione) towards the catalytic center [39]. Here, two conserved ‘R’ residues were also identified in *Mc*SeGPx.

‘TGA’ is generally interpreted as a stop codon during translation, but SECIS, a secondary tertiary mRNA structure lying in the 3′UTR of eukaryote selenoprotein mRNAs, instead directed the specific binding of selenocysteine [39]. SECIS, a major characteristic feature of the SeGPxs mRNA sequence, interacts with SBP2, Sec-tRNA^{Sec}, eEFSec to directs the Sec into the UGA codon during the translation [39,40]. SECIS elements of McSeGPx had 91 bp which consisted of two helixes, internal loop and an apical loop, a SECIS core located between the top of internal loop and the base of helix II. According to the position of the conserved ‘A’ residues and the size of the apical loop in secondary structure, SECIS elements are divided into two main types, type 1 and type 2. In type 1, the AAA stretch is located at the apical loop, while in type 2, this stretch is transferred to an internal loop 2 [41]. Hence, the

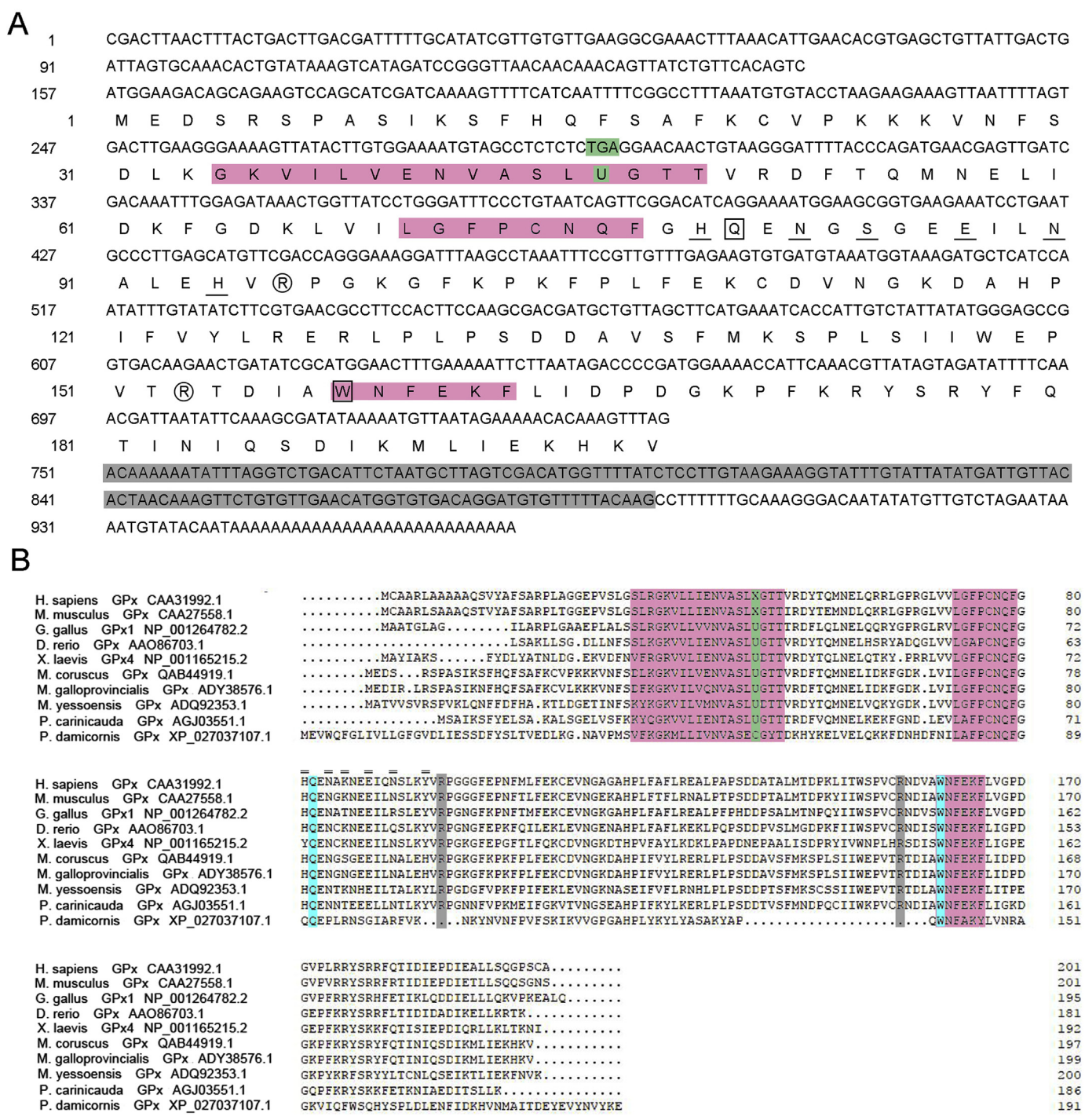

Fig. 1. Molecular characterization of *Mc*SeGPx. (A) The nucleotide sequences and the deduced amino acid sequences of *Mc*SeGPx. The complete sequence of *Mc*SeGPx cDNA is 970 bp, consists of 156 bp 5′-UTR, 220 bp 3′-UTR, an 594 bp ORF region coding the protein of 197 amino acid residues. The '^{292}TGA^{294}' coded selenocysteine 'U46' was shaded with light green. The SeGPx signature motif '^{70}LGFPCNQF^{77}' and two active site motifs '^{34}GKVILVENVASLUGTT^{49}', '^{158}WNFEKF^{163}' were highlighted with pink. Six residues involved in the dimmer formation were underlined. Glutathione binding involved arginine residues (R96, R153) were circled, and catalytically important residues glutamine (Q80) and tryptophan (W158) were boxed. The predicted SECIS element in the 3′-UTR was marked with light gray. (B) Multiple alignments of *Mc*SeGPx with GPx homologues that retrieved from NCBI database. GPx signature motif and two active site motifs were highlighted with pink. Six residues involved in the dimmer formation were doubly underlined. These conserved selenocysteine, two arginines, glutamine and tryptophan were shaded with light green, gray and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

SECIS elements of *Mc*SeGPx affiliates to type 1 SECIS elements with AAA located at the apical loop. However, additional experiments are needed to confirm the real potential function of the stem-loop structure of the SECIS element in *Mc*SeGPx in future study. Collectively, it could be speculated that the newly identified GPx congener *Mc*SeGPx belongs to the selenocysteine dependent GPx family, and might play the similar antioxidant role in the oxidative stress mechanism of action against external stimuli just as its counterparts play in mammals.

Generally speaking, molluscs live mostly in aquatic environments, and hence constantly facing to different environmental challenges

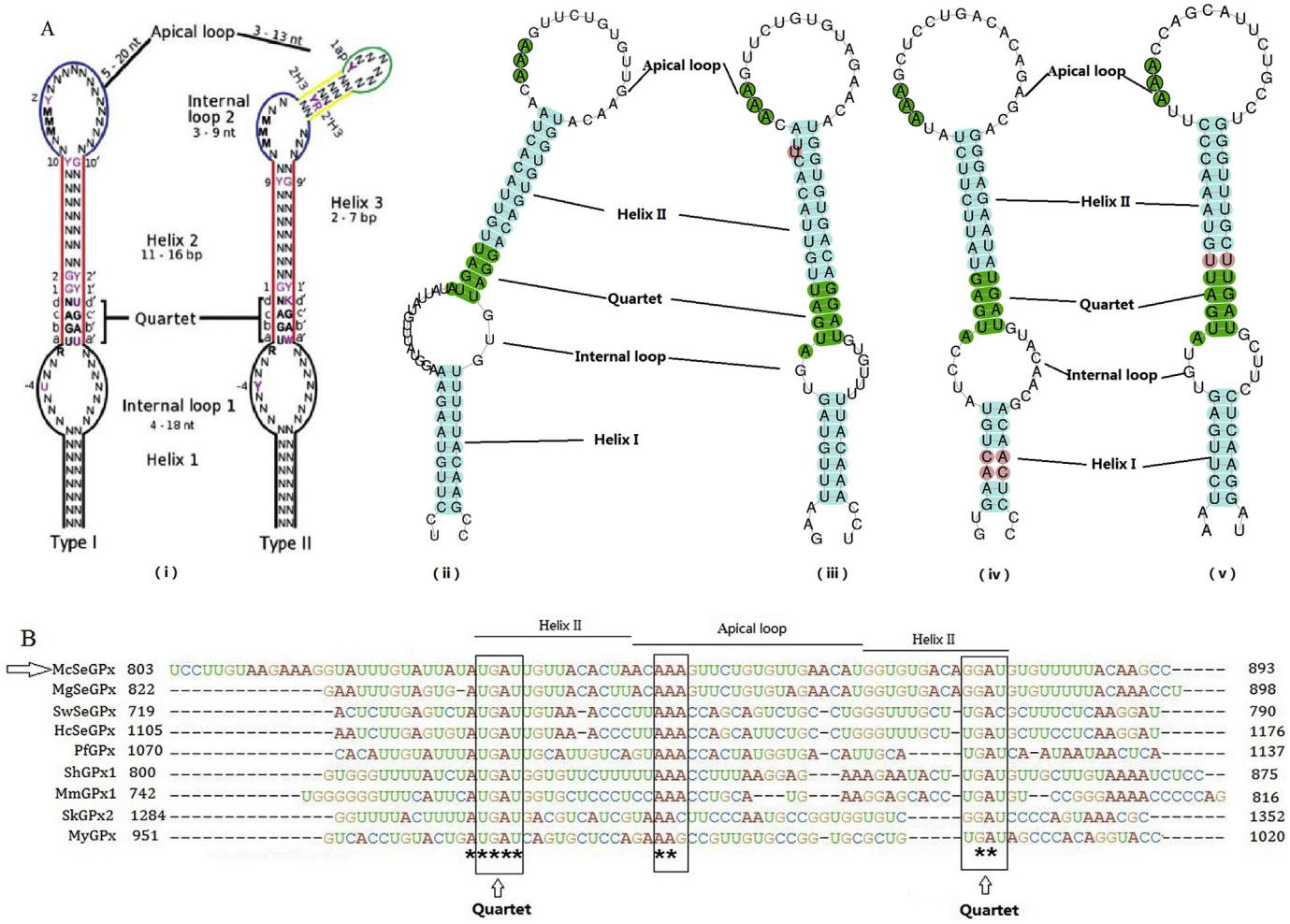

Fig. 2. Comparison of selenocysteine insertion sequence (SECIS) elements. (A) Schematic representations of SECIS structures of (i) type I and type II, (ii) *Mytilus coruscus*, (iii) *Mytilus galloprovincialis*, (iv) *Crassostrea gigas*,(v) *Sinanodonta woodiana*. (B) Mulitiple alignments of SECIS sequences of *Mc*SeGPx with other species. Mg: *Mytilus galloprovincialis*, Sw: *Sinanodonta woodiana*, My: *Mizuhopecten yessoensis*, Sh: *Sarcophilus harrisii*, Pf: *Pinctada fucata*, Mm: *Microcebus murinus*, Hc: *Hyriopsis cumingii*, Sk: *Microcebus murinus*.

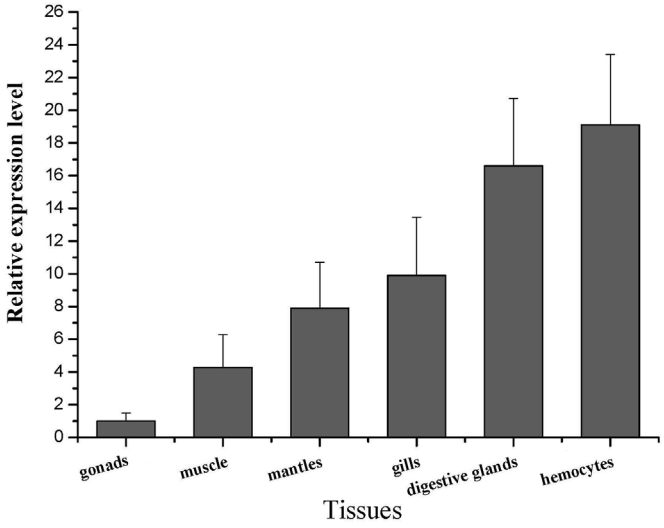

Fig. 3. Tissue distribution of *Mc*SeGPx transcripts. Relative expression of *Mc*SeGPx transcripts in different tissues including gills, digestive glands, hemocytes, muscle, gonads, mantles, respectively. Data were shown as mean ± S.D. (n = 8) with β-actin as internal control.

including pH, temperature, heavy metal content and salinity [42]. Aiming to elucidate the functional role of *Mc*SeGPx in the response mechanism against external stimuli, three different stimulators including LPS, B[α]P and Cu^{2+}, representing biological, organic and heavy metallic challenges, respectively, were chosen to treat healthy mussels in present research. In invertebrates, gills and digestive glands were speculated to be the main immune and antioxidant organs, which are extraordinary sensitive to environmental changes [43]. In previous reports, Shenaitirodkar et al. [44] assessed the antioxidant responses in gills and digestive gland of oyster *Crassostrea madrasensis* (Preston) under lead exposure. Here, these two tissues were chosen to assess the change of *Mc*SeGPx mRNA expession and enzyme activity under LPS, B[α]P and Cu^{2+} challenge.

Although metals, organics and pathogens have different damage mechanisms to biological organisms, they share the same result, causing ROS elevation and oxidative stresses [45]. LPS is an unique component of gram-negative bacteria cytoderm and deemed as a key inducing factor that triggers innate immune responses to local inflammation [46]. LPS could cause the release of cytokines, participates in the generation of free radical species, such as ROS, and further enhances oxidative stress through a prolonged production of inflammatory mediators [47]. Copper is assumed to be a transition metal and is widespread use in human society [48]. In aquatic arthropods and molluscs, copper is a component of the oxygen-carrier protein hemocyanin [49]. Although necessary, high concentrations of copper in

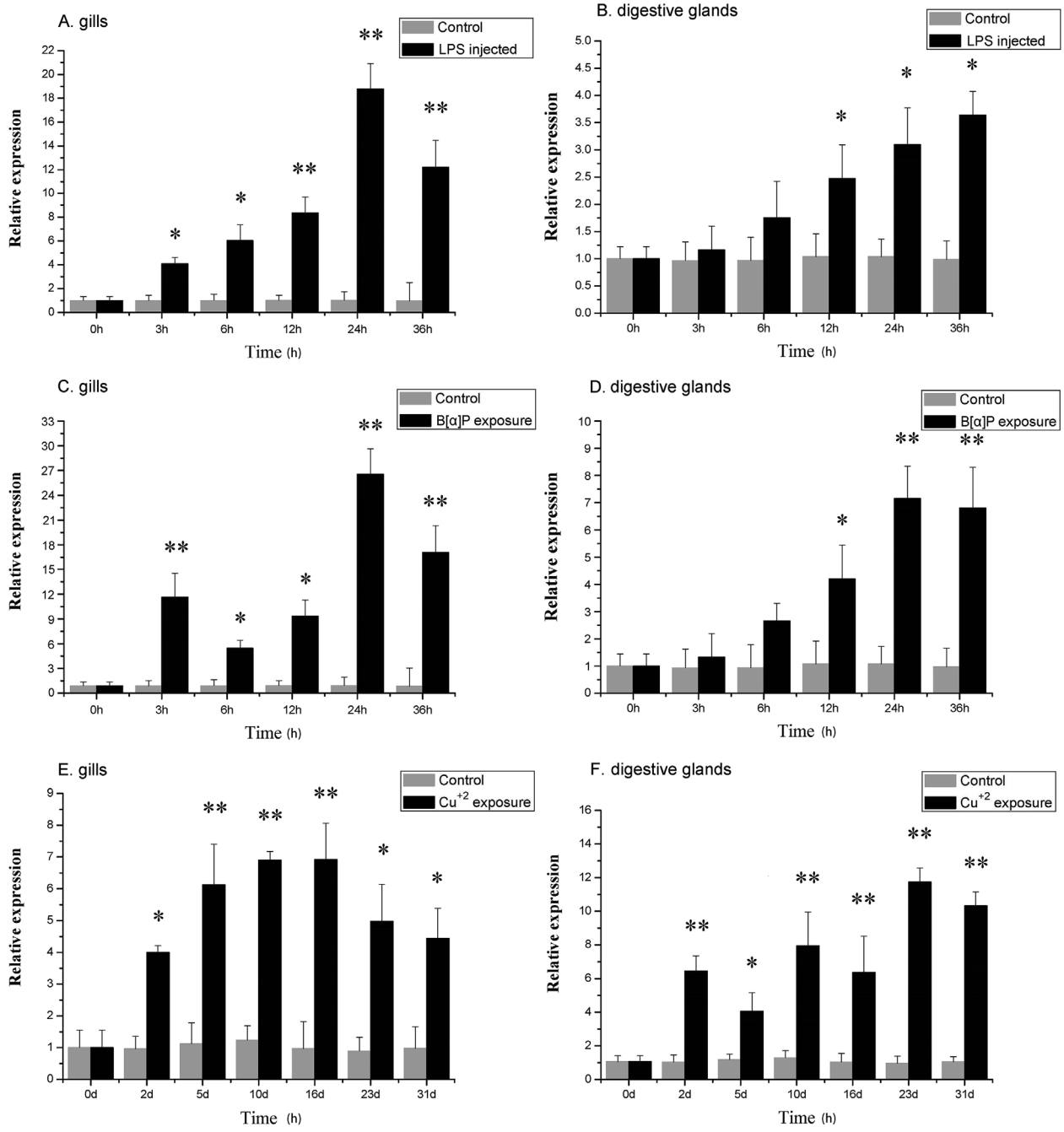

Fig. 4. Expression profile analysis of *Mc*SeGPx after the challenge of (A, B) lipopolysaccharide (LPS), (C, D) benzo[α]pyrene (B[α]P) and (E, F) copper (Cu^{2+}). Each bar represents the mean ± S.D. (n = 3). Significant difference from controls was marked by an asterisk'*'at $P < 0.05$ and'**'at $P < 0.01$.

water is a potent toxicant to aquatic animals [50–52], and could catalyses the generation of ROS [53]. B[α]P is generally regarded as the environmental indicator for Polycyclic aromatic hydrocarbons (PAHs), which could causes metabolism dysfunction, immune system destruction, reproductive damage and mutagenic response in marine lives [54]. Under challenges of LPS, B[α]P and Cu^{2+}, *Mc*SeGPx mRNA in gills and digestive glands was significantly induced, suggesting the effective effect on antioxidant response of the organism against all these three elements. In *Venerupis philippinarum*, SeGPx2 was found to be significantly down-regulated by low concentration of Cu^{2+} (10 μg/L) but up-regulated by high concentration of Cu^{2+} (40 μg/L of copper) [55]. In our previous reports, the low concentration of Cu^{2+} could induce antioxidant responses, DNA damage and genotoxicity in *M. coruscus* [28]. Additionally, Cu^{2+} could remarkably induce the expression of CAT gene which considered as another crucial antioxidant enzyme [56]. Most recently, Qu et al. [57] assessed the genotoxic damages of *M. coruscus* through comet assay, micronucleus test, and random amplified polymorphic DNA analysis, and a significant increase though transitory genomic damage was investigated after the B[α]P exposure. Basing on these scenarios, these results suggested that these three sources of stimuli could generate a mass of ROS, which need to be detoxified by *Mc*SeGPx protein translated by induced extra *Mc*SeGPx transcripts. The present results directly or indirectly suggested that *Mc*SeGPx might play a effective role in the antioxidant response to various environmental stimuli as their orthologues act in other animals.

For the aim to deep understand the function role of *Mc*SeGPx in mussel antioxidant course, the *Mc*SeGPx activity under LPS, B[*α*]P and Cu^{2+} challenge was assessed. A gradual elevation of *Mc*SeGPx activity

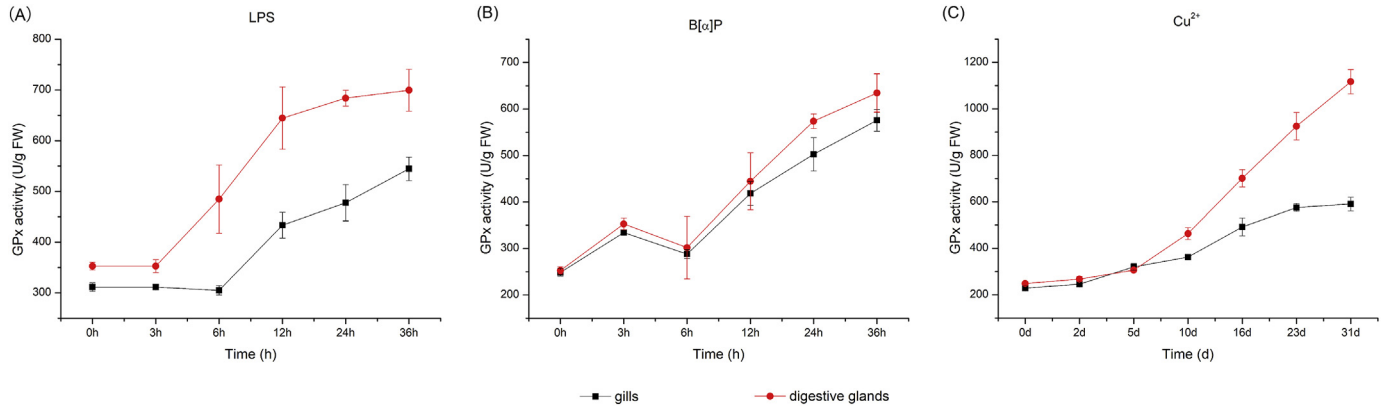

Fig. 5. *Mc*SeGPx activities in gill and digestive gland of *M.coruscus* with the challenge of (A) LPS and (B) B[α]P at 0 h, 3 h, 6 h, 12 h, 24 h, 36 h, (C) Cu^{2+} at 0d, 2d, 5d, 10d, 16d, 23d, 31d. Data were given as mean ± S.D. (n = 3).

was observed under three forms of challenges. Apparently, the enzyme activity results were not exactly matched to the mRNA expression results, because the mRNA levels showed a tendency to rise first and then decrease in several specific scenarios. It was speculated that in the later stage of infection, the production of antioxidant enzymes was sufficient to resist external stimuli, so the mRNA expression level decreased first, but the content of antioxidant enzymes was still accumulating. In short, these results of enzyme activity further confirms that *Mc*SeGPx was involved in the antioxidant response to various external stimuli including biotic, organic and metallic stressors.

In conclusion, a new molluscan GPx member affiliate to the selenocysteine dependent GPx family was identified from thick shell mussel *M. coruscus*. *Mc*SeGPx is constitutively expressed in different tissues and could be induced by LPS, B[*α*]P and Cu^{2+} in gills and digestive glands. In addition, *Mc*SeGPx activity also changed by three sources of external simuli. These results suggested that *Mc*SeGPx was involved in the antioxidant response to various environmental stimuli. It has been reported that gene expression based on the mRNA expression is frequently used in biomonitoring of contaminated sites, and it was possible to make estimate of chemical exposure and its effects on the exposed species [58]. Owing to the sensitive response of mRNA expression to diverse forms of stressors, GPx in aquatic organisms had been regarded as a crucial indicator and biomarker for environmental pollution [59,60]. The present results might be helpful to provide useful baseline for using *Mc*SeGPx as a potential biomarker for different pollutants.

Acknowledgments

This research was supported by the Natural Science Foundation of China (31671009), Zhejiang public welfare project (2017C32009), the Special Fund for the Key Research and Development Project of Zhejiang Province (2019C02056) and NSFC-Zhejiang Joint Fund for the Integration of Industrialization and Informatization (U1609204).

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