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Expression profiles of two small heat shock proteins and antioxidant enzyme activity in *Mytilus galloprovincialis* exposed to cadmium at environmentally relevant concentrations*

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Abstract Small heat shock proteins encompass a widespread but diverse class of proteins, which play key roles in protecting organisms from various stressors. In the present study, the full-length cDNAs of two small heat shock proteins (MgsHSP22 and MgsHSP24.1) were cloned from *Mytilus galloprovincialis*, which encoded peptides of 181 and 247 amino acids, respectively. Both MgsHSP22 and MgsHSP24.1 were detected in all tissues examined by real-time PCR, with the highest expression being observed in muscle and gonad tissues. The real-time PCR results revealed that Cd significantly inhibited MgsHSP22 expression at 24 h and MgsHSP24.1 at 24 and 48 h under 5 μg/L Cd^{2+} exposure. MgsHSP24.1 expression was also significantly inhibited after 50 μg/L Cd^{2+} exposure for 48 h. With regard to antioxidant enzymes, increased GPx and CAT activity were detected under Cd^{2+} stress (5 and 50 μg/L), while no significant difference in SOD activity was observed throughout the experiment. Overall, both MgsHsps and antioxidant enzymes revealed their potential as Cd stress biomarkers in *M. galloprovincialis*.

Keyword: *Mytilus galloprovincialis*; small heat shock protein; superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GPx); cadmium

1 INTRODUCTION

Over the past few decades, heavy metal contamination has been of great concern in marine and coastal ecotoxicology (Pipe and Coles, 1995; Haslbeck, 2002; Järup, 2003; Liu et al., 2011). Among the heavy metals, cadmium (Cd) is considered an important toxicant accumulated by many marine organisms (Roccheri et al., 2004). It can cause a series of detrimental effects to the organisms, such as production of reactive oxygen species (ROS), depletion of glutathione, and inhibition of enzymes involved in DNA synthesis and repair (Waisberg et al., 2003; Roccheri et al., 2004).

The rapid elimination of excessive ROS induced by Cd is essential for the organisms. The primary defense against oxidative damage consists of the major antioxidant enzymes, e.g. superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and some low-molecular weight compounds (vitamins A, C, and uric acid) (Orbea et al., 2000). These antioxidant enzymes can act as effective free radical scavengers in response to oxidative stress, and

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they have also been proposed as biomarkers of environmental contaminants (Richardson et al., 2008; Jena et al., 2009).

Living organisms usually synthesize a small number of highly conserved proteins called heat shock proteins (HSPs) in response to different environment stressors (Lindquist, 1986). HSPs are a family of proteins that enhance cell survival under the influence of various stressors including hyperthermia, heavy metals, and oxidants, among others. In ecotoxicology, HSPs have been widely used as biomarkers of environmental pollutants (Lewis et al., 1999). There are several HSP families based on their molecular mass and function, these include HSP100, HSP90, HSP70, HSP60, and the small HSPs (sHsps) (Trent, 1996). The sHsps are composed of a conserved α-crystallin domain with molecular weights ranging from 12–43 kDa (Franck et al., 2004; Sun et al., 2005). sHsps are essential in protecting cells from various stresses, facilitating the formation and structure of proteins, and maintaining the balance between cell survival and cell death (Gusev et al., 2002; Haslbeck, 2002). For example, the bay scallop, *Argopecten irradians*, sHSP22 transcript was up-regulated after Cd exposure for 10 days (Zhang et al., 2010). sHsps also participate in toxic (Cd) resistance mechanisms in amphipod species (Shatilina et al., 2010). However, sHsp responses to heat shock and contamination have been more extensively studied in mammals and insects than in marine organisms (Klemenz and Gehring, 1986; Benndorf et al., 2001; Chowdary et al., 2004). More recently, experimental evidence has suggested that an interrelationship between sHsps and redox status exists. For example, *Drosophila* HSP27 and murine HSP25 modulate oxidative stress-induced cell death (Arrigo, 2001). It was suggested that sHsps might protect against the deleterious effects induced by oxidative stress through modulating the intracellular redox state (Arrigo, 2002).

Mytilus galloprovincialis are ubiquitous coastal and estuarine filter-feeders and can accumulate higher levels of heavy metals than other species, and have thus been frequently used as bioindicators in environmental monitoring programs (Kavun et al., 2002). In the present study, we aimed to clone the full-length sHSP22 and sHSP24.1 cDNAs from *M. galloprovincialis*, and investigate their temporal expression and the variation of antioxidant enzyme activity in response to Cd exposure, to provide more information about the modulatory system that responds to heavy metal stress in *M. galloprovincialis*.

2 MATERIAL AND METHOD

2.1 Mussels and treatments

M. galloprovincialis (shell length 6.5–7.5 cm) were purchased from a local aquaculture farm and acclimated for 10 days prior to the experiment. The sea water was aerated continuously and salinity and temperature maintained at 33 and 17°C throughout the experiment. Mussels were fed with *Chrysophyta* and *Tetraselmis chuii*, and seawater was renewed daily. After 10 days' acclimatization, the mussels were divided into nine groups and cultivated in 30-L aquarium tanks, each containing 25 individuals. Six tanks of mussels (three replicates for each treatment) were exposed to environmentally relevant concentrations of Cd^{2+} (5 and 50 μg/L Cd^{2+}, prepared from $CdCl_2$). The Cd concentrations applied have been previously reported in some heavily polluted sites in the Bohai Sea (Zhang et al., 2001). Mussels cultured in filtered, uncontaminated seawater served as the control group. The toxicant-laden seawater was renewed daily and $CdCl_2$ was added. The hepatopancreas of six individuals from each treatment and the control were sampled at each time interval (0, 24, 72, and 96 h after Cd exposure), and then divided into two parts: one for antioxidant enzyme assay and the other for total RNA extraction. The tissues, including the hepatopancreas, hemocytes, muscle, gonad, gills, and mantle, were dissected from six individuals as parallel samples and subjected to total RNA extraction with TRIzol® reagent (Invitrogen).

2.2 Full-length MgsHSP22 and MgsHSP24.1 cDNA cloning

Two ESTs, highly similar to previously identified sHSP22 and sHSP24.1, were identified from the *M. galloprovincialis* cDNA library. The MgsHSP22 and MgsHSP24.1 3' and 5' ends were obtained by nested PCR. The PCR products were gel-purified and subcloned into the pMD18-T Simple Vector (TaKaRa, Japan). After being transformed into *Escherichia coli* Top10F' competent cells, three positive clones for each sHsp were sequenced bi-directionally with the primers M13-47 and RV-M (Table 1).

2.3 Sequence analysis

The nucleotide and protein sequence analyses were performed with the BLAST algorithm at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). The deduced amino acid

Table 1 Primers used in the present study

Primers	Sequence (5'-3')	Sequence information
F1 (forward)	CGAACTAAATAAGCCAGCA	RT primer for MgsHSP22
R1 (reverse)	TGCTGGCTTATTTAGTTCG	RT primer for MgsHSP22
F2 (forward)	GGTGCCTTAGTGTCAAGTGATAGT	RT primer for MgsHSP24.1
R2 (reverse)	AGATCGTGGAACTGTCATCTGAC	RT primer for MgsHSP24.1
Mg-F (forward)	GCTATCCAGGCCGTACTCT	β-actin primer
Mg-R (reverse)	GCGGTGGTTGTGAATGAG	β-actin primer
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	Sequencing primer
RV-M	GAGCGGATAACAATTTCACACAGG	Sequencing primer

sequences were analyzed with the Expert Protein Analysis System (http://www.expasy.org/). Multiple sHsp alignments were performed using the ClustalW Multiple Alignment Program (http://www.ebi.ac.uk/clustalw/), and signal peptides were predicted with SignalP 3.0. Two phylogenetic trees were constructed with Mega4.1 using the neighbor-joining method. Bootstrap trials were replicated 1 000 times to test the relative support for the phylogeny analysis.

2.4 Quantification analysis of mRNA expression post Cd exposure

MgsHSP22 and MgsHSP24.1 expression levels in the hepatopancreas after Cd exposure were measured by quantitative real-time RT-PCR. The RNA extraction and cDNA synthesis were conducted as per Zhang et al. (2011). The gene-specific primers, F1 and R1 for MgsHSP22 and F2 and R2 for MgsHSP24.1, and the internal control primers, Mg-F and Mg-R for β-actin (Wang et al., 2010), are listed in Table 1. The amplification was performed in a total volume of 50 μL, containing 25 μL of 2×SYBR® Green Master Mix (Applied Biosystems), 4 μL of the 50×diluted cDNA mix, 1 μL (each) of the forward and reverse primers (10 μmol/L), and 19 μL of DEPC-treated water. The thermal profile was 50°C for 2 min and 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. At the end of each PCR reaction, a dissociation curve analysis of the amplification products was performed to confirm only one PCR product amplified and was detected. To maintain consistency, the baseline was automatically set by SDS (SDS v. 2.3, Applied Biosystems). The relative expression level of both genes was analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.5 Antioxidant enzyme activity assay

To assay antioxidant enzyme activity, 0.1 g of hepatopancreas was homogenized in 10% (w/v) homogenate medium (10 mmol/L Tris-HCl, 0.1 mmol/L EDTA-Na_2, 10 mmol/L sucrose, and 0.8% NaCl solution, pH 7.4) using an IKA homogenizer (Ultra Turrax IKA T10 basic, Staufen, Germany). The homogenates were centrifuged (425×*g*) for 15 min at 4°C to harvest the supernatant and subjected to an antioxidant enzyme assay. GPx, SOD, and CAT activity were measured according to the manufacturer's protocols (Jiancheng, China). The total protein concentration of the supernatant was measured according to Bradford (1976) using bovine serum albumin (BSA) to normalize enzyme activity data. All enzyme activity is expressed in terms of units per milligram protein, where one unit represents the change in absorbance /(min·mg)/protein.

2.6 Statistical analysis

The data were normally distributed (Shapiro-Wilk test, $P>0.05$). One-way ANOVA with Tukey's test was performed on all data in SPSS 16.0, and *P*-values less than 0.05 were considered statistically significant. All data are given as means±SE ($n=6$).

3 RESULT

3.1 MgsHSP22 and MgsHSP24.1 sequence analysis

The complete MgsHSP22 and MgsHSP24.1 cDNA sequences were deposited in GenBank under the accession Nos. JF803804 and JF803805, respectively. The MgsHSP22 and MgsHSP24.1 nucleotide sequences were predicted to encode a polypeptide of 162 and 247 amino acids, respectively (Fig.1). No signal peptide was found in either MgsHSP22 or MgsHSP24.1 by SignalP analysis, which revealed that the deduced proteins were cytosolic sHsps. SMART program analysis indicated the existence of the α-crystallin domain in the deduced MgsHSP22

a

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   1 ACATTCTACAAACCGGATGCAGAAAGCAGACGATAGTTTTGAAATGTCCAGCCGTATTGT
   1               M  I  G  I  F  F  D  K  Q  M  D  M  F  P  S  F
  61 TCCAGTTAAGCGTTATGATTGGAATTTTTTTCGACAAGCAAATGGACATGTTCCCATCTT
  17  K  N  D  F  D  K  D  F  F  K  D  A  K  H  S  S  M  D  E  E
 121 TTAAAAATGATTTCGACAAAGATTTTTTCAAGGATGCCAAGCATTCAAGTATGGATGAAG
  37  I  A  R  M  K  R  E  M  F  S  L  T  T  P  E  S  S  L  K  V
 181 AAATAGCAAGGATGAAACGGGAGATGTTCAGCCTGACAACACCAGAGTCAAGTTTGAAAG
  57  D  Q  P  F  V  Q  D  F  T  G  D  K  K  M  A  L  R  F  D  C
 241 TAGATCAACCTTTTGTTCAGGATTTTACTGGAGATAAGAAAATGGCTCTACGGTTTGATT
  77  S  Q  F  N  P  E  E  I  Q  V  K  T  M  D  K  Q  L  T  V  H
 301 GCAGTCAGTTTAATCCTGAGGAAATTCAAGTAAAAACAATGGACAAACAATTAACTGTAC
  97  A  K  H  E  E  V  S  P  G  R  K  V  Y  R  E  F  T  K  S  Y
 361 ATGCCAAACATGAAGAAGTTTCACCGGGAAGGAAAGTGTACAGAGAATTCACCAAGTCCT
 117  T  L  P  Q  D  V  D  P  L  S  L  K  S  S  L  T  N  D  G  F
 421 ACACGTTACCACAGGATGTTGACCCTTTGTCATTGAAGTCCAGCCTGACCAACGACGGAT
 137  L  Q  V  E  A  P  A  P  K  T  C  V  A  R  K  E  I  F  I  P
 481 TTTTACAAGTTGAAGCTCCTGCACCTAAAACATGTGTCGCAAGGAAGGAAATATTTATTC
 157  I  E  K  M  L  K  *
 541 CAATTGAGAAAATGCTTAAGTAAATAATTCGAACTAAATAAGCCAGCAAAATGCCAATTA
 601 TATATAACATGCTGTGTTGGAAACCATCAGTGAGAAATTATTTTGACCGTTCCGTTTCGA
 661 TGAGGCATTACTATTTTGTACAATATCAGAAGAATTTCTTTTGACCGATTCTATGATGAA
 721 TTGACATCTATGTACACTTTCAGAGACTTTGGATAGTGCGCAAAATTTGACAATTAATTC
 781 AGTATTTTTTGCAGTTTTTAACTATTTTAACAATTTTACATATATGGTTTCGTACTAGCT
 841 TTGCATATGTTGTTGTGCATGTTTTGGACTAGCATTCCTGGATCGGTTTCCGTGACAAGA
 901 CAATTATTTGAATTCAGTTGTATATGAAAATAAAAAGGTATAAGCAAAAAAAAAAAAAAA
 961 AA
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b

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   1                                                           M
   1 GTGGGTGGGAATTAAACGAAATCAACAATACAGACGACTTTTAGCAGAACAGAGTAAAGA
   2  T  F  V  P  V  L  W  N  R  Y  N  Q  S  T  Q  H  D  F  D  D
  61 TGACGTTTGTCCCAGTGCTTTGGAACCGCTACAATCAGTCTACACAGCATGATTTTGATG
  22  M  F  H  F  M  D  D  W  E  P  M  S  V  G  Y  G  Y  G  R  H
 121 ACATGTTTCACTTTATGGATGACTGGGAGCCTATGTCCGTAGGTTATGGGTATGGTCGTC
  42  P  P  C  P  G  M  A  V  R  R  R  R  R  P  E  T  S  L  A  D
 181 ACCCACCATGCCCGGGAATGGCAGTGCGTAGGAGGCGCAGACCGGAGACTTCACTGGCGG
  62  K  K  W  T  Y  S  V  K  I  G  D  F  D  A  Q  H  V  K  V  K
 241 ATAAAAAGTGGACATACAGCGTAAAAATCGGAGACTTTGATGCACAGCATGTCAAAGTGA
  82  V  D  N  G  N  V  I  I  H  A  K  Y  T  D  G  N  D  E  W  G
 301 AAGTTGATAACGGTAATGTAATTATTCATGCCAAGTACACTGATGGTAATGATGAATGGG
 102  D  T  V  E  R  K  R  T  V  K  V  P  E  N  V  D  A  E  K  M
 361 GGGATACTGTTGAACGAAAAAGGACCGTCAAGGTTCCAGAGAATGTTGATGCTGAGAAAA
 122  H  S  F  M  R  S  D  G  T  M  V  L  E  A  P  Y  L  H  P  E
 421 TGCACAGCTTCATGAGATCAGATGGTACTATGGTATTAGAAGCCCCATACCTTCATCCAG
 142  E  R  Q  I  Q  V  V  P  H  E  G  G  A  L  V  S  S  D  S  H
 481 AGGAGAGACAAATACAAGTTGTACCACATGAAGGAGGTGCCTTAGTGTCAAGTGATAGTC
 162  S  N  L  M  K  F  N  V  E  N  F  R  P  E  E  V  K  V  S  C
 541 ACAGCAACTTGATGAAATTCAATGTTGAAAATTTCCGACCAGAGGAGGTTAAAGTGTCGT
 182  K  D  G  I  L  T  V  Q  G  E  K  Q  R  S  E  D  G  H  E  I
 601 GTAAGGATGGAATATTGACAGTCCAAGGAGAAAAACAGAGGTCTGAAGATGGTCATGAAA
 202  R  E  S  F  W  R  Q  M  T  V  P  R  S  V  D  G  K  N  I  E
 661 TACGAGAGTCATTCTGGCGTCAGATGACAGTTCCACGATCTGTGGATGGAAAGAATATTG
 222  C  L  K  D  E  E  G  N  L  T  I  R  A  P  V  V  E  D  V  E
 721 AATGCTTGAAAGATGAGGAAGGAAATCTTACTATTCGTGCACCAGTTGTTGAAGATGTTG
 242  M  E  Q  A  K  K  *
 781 AAATGGAACAAGCCAAAAAATAAATCCAGATTTGACTGTCCAAGTCACAATAGCGGATTT
 841 TAGACTATACTTTTGAACTTGATTACATTTTTGTAGTCAACATTCTGTCTATTGTGATTG
 901 TTTTTGCAATATTGACCAATCATTTGATGTTTCTCATACTGTCAGTAATTTAAAAAAAAA
 961 AAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig.1 The cDNA and deduced amino acid MgsHSP22 (a) and MgsHSP24.1 (b) sequences

The nucleotide and amino acids are numbered along the left margin. The start and stop codons are in bold.

and MgsHSP24.1 amino acid sequence C-termini.

The deduced MgsHSP22 amino acid sequence was highly homologous to that of *Venerupis philippinarum* (ACU83231.1, 61.7% identity), *Bombyx mori* (NP_001036985.1, 40.1% identity), *Pteromalus puparum* (ACO57620.1, 37.8% identity), and *Caenorhabditis elegans* (AAA68804.1, 31.2% identity) sHSP22s (Fig.S1a). However, the deduced MgsHSP24.1 amino acid sequence displayed relatively low identity with its counterparts in other species (Fig.S1b). For example, it shared 37% identity with *Crassostrea gigas* sHsp (EKC27011.1), 37% to *Danio rerio* sHSP25 (AAV97950.1), and 36% to *Carassius auratus* sHSP27 (BAE93468.1).

3.2 Phylogenetic analysis

To estimate the evolutionary relationship of the two MgsHsps, two phylogenetic trees were constructed by the neighbor-joining method (Fig.2). MgsHSP22 clustered with *Venerupis philippinarum* sHSP22, *Caenorhabditis elegans* sHSP25, *Pteromalus puparum* sHsp, and *Bombyx mori* sHSP21.4, forming the invertebrate group (Fig.2a). MgsHSP24.1 formed a common branch with *Crassostrea gigas* sHsp (99% bootstrap percentage) (Fig.2b). It then formed the invertebrate group with *Venerupis philippinarum* sHSP22, *Brachionus ibericus* sHSP27, *Gastrophysa atrocyanea* sHSP23, *Drosophila buzzatii* sHSP27, *Tribolium castaneum* sHSP21, and *Hydra magnipapillata* sHsp. The relationships displayed in the phylogenic trees were in good agreement with traditional taxonomy.

3.3 Tissue distribution of MgsHSP22 and MgsHSP24.1

The tissue distribution of the MgsHsps transcripts was analyzed by real-time RT-PCR. The MgsHSP22 transcript was predominantly detected in muscle tissue, and moderately expressed in the mantle and gonad, while much lower expression was observed in the gills, hepatopancreas, and hemocytes (Fig.3a). In regards to MgsHSP24.1, the highest expression level was observed in the gonad tissue, and the lowest in the hemocytes (Fig.3b).

3.4 MgsHSP22 and MgsHSP24.1 mRNA expression profiles in response to Cd exposure

MgsHSP22 and MgsHSP24.1 transcript expression patterns in the hepatopancreas of mussels exposed to

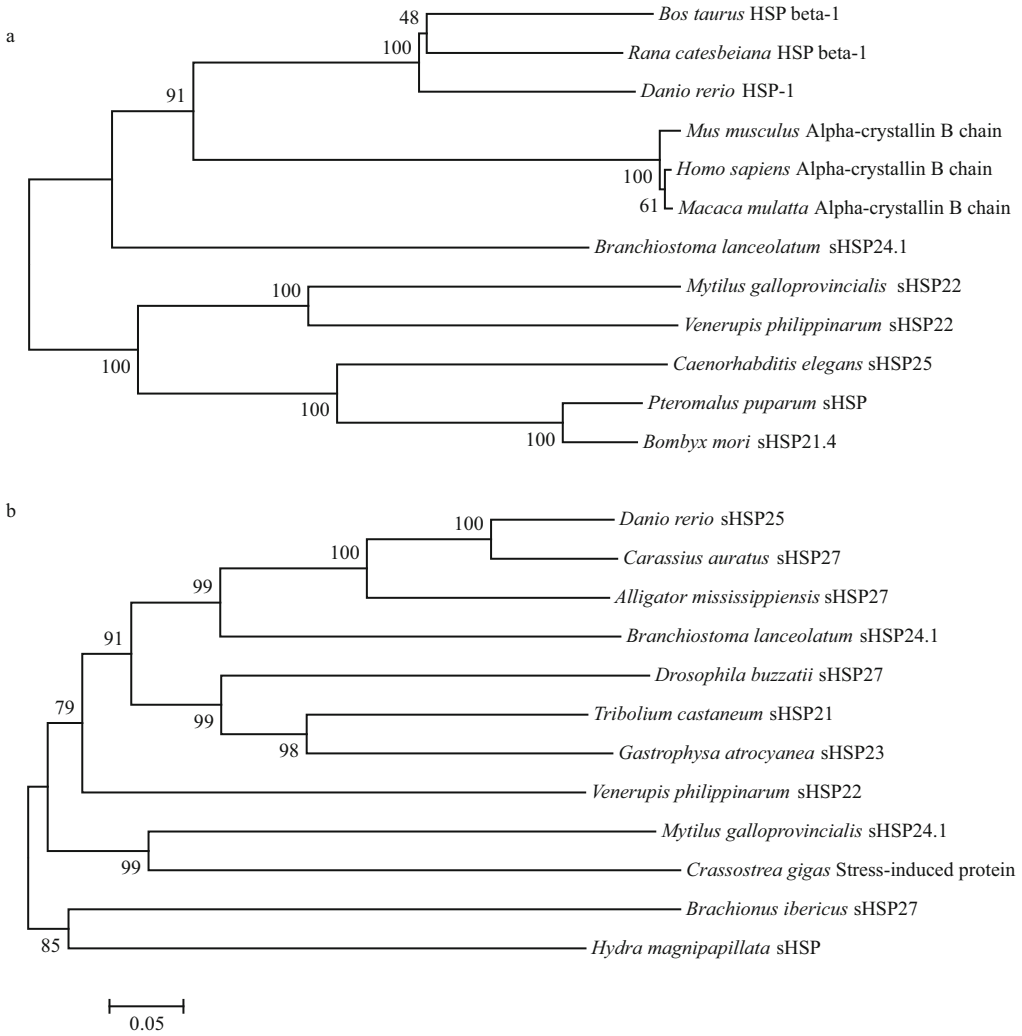

Fig.2 The sHSP22 (a) and sHSP24.1 (b) phylogenetic trees based on the amino acid sequences of sHsp family members by the neighbor-joining method

Bootstrap support values for the NJ tree are shown at the nodes (1 000 replicates). The sequences used here were retrieved from GenBank as follows: *Bos Taurus* HSP beta-1 (NP_001020740.1), *Rana catesbeiana* HSP beta-1 (ACO51783.1), *Danio rerio* HSP-1(CAM12245.1), *Mus musculus* alpha-crystallin B chain (NP_034094.1), *Homo sapiens* alpha-crystallin B chain (NP_001876.1), *Macaca mulatta* alpha-crystallin B chain (XP_001106498.1), *Branchiostoma lanceolatum* sHSP24.1 (CAE83570.1), *Venerupis philippinarum* sHSP22 (ACU83231.1), *Caenorhabditis elegans* sHSP25 (AAA68804.1), *Pteromalus puparum* sHsp (ACO57620.1), and *Bombyx mori* sHSP21.4 (NP_001036985.1) for MgsHSP22; *Danio rerio* sHSP25 (AAV97950.1), *Carassius auratus* sHSP27 (BAE93468.1), *Alligator mississippiensis* sHSP27 (BAF94137.1), *Branchiostoma lanceolatum* sHSP24.1 (CAE83570.1), *Drosophila buzzatii* sHSP27 (ABX80641.1), *Tribolium castaneum* sHSP21 (XP_973442.1), *Gastrophysa atrocyanea* sHSP23 (BAD91165.1), *Venerupis philippinarum* sHSP22 (ACU83232.1), *Crassostrea gigas* sHsp (EKC27011.1), *Brachionus ibericus* sHSP27 (ADR79277.1), and *Hydra magnipapillata* sHsp (XP_002156113.1) for MgsHSP24.1.

Cd are shown in Fig.4. MgsHSP22 mRNA expression was significantly down-regulated and dropped to 0.2-fold (P<0.01) that of the control group following exposure to 5 μg/L Cd^{2+} for 24 h. As time progressed, the expression level recovered to original levels at 24 h and 48 h post exposure. However, when Cd concentration was increased to 50 μg/L, no statistically significant fluctuation in MgsHSP22 expression (P>0.05) was detected during the exposure period.

As for MgsHSP24.1, a significant decrease in the expression level (0.5- and 0.6-fold that of the control, P<0.05) was observed at 24 h and 48 h in the 5 μg/L Cd^{2+} treated group. After that, the expression level almost recovered to the original level at 96 h. For the high dose (50 μg/L) Cd-exposed samples, the MgsHSP24.1 expression level decreased significantly to 0.4-fold that of the control group (P<0.01) at 48 h, and then rose slightly at 96 h.

3.5 Antioxidant enzyme activity in response to Cd stress

As shown in Fig.5, Cd had a significant effect on antioxidant enzyme activity in the *M. galloprovincialis*

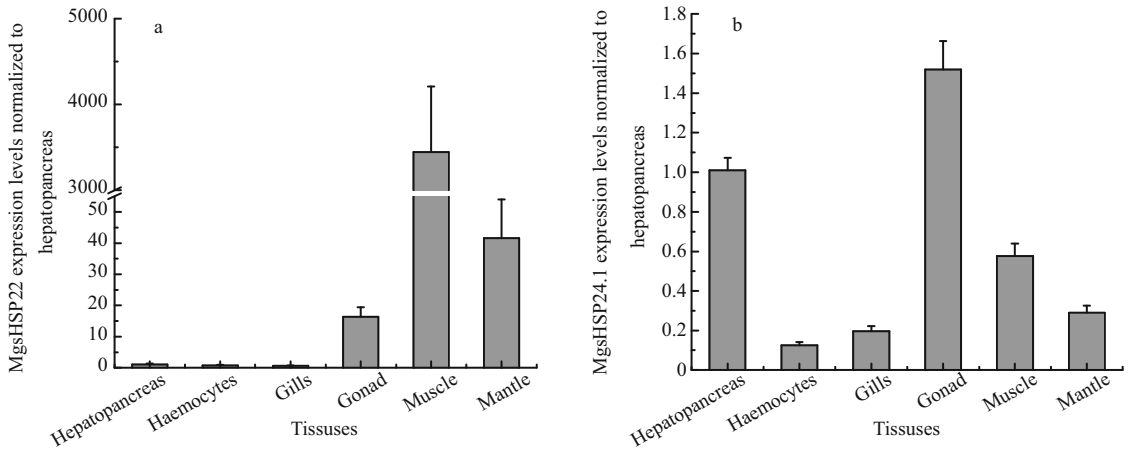

Fig.3 Tissue distribution of the MgsHSP22 (a) and MgsHSP24.1 (b) transcripts as measured by SYBR Green RT-PCR

Transcript levels in the hemocytes, gills, gonad, muscle, and mantle were normalized to that of hepatopancreas. Vertical bars represent the means±SE (n=6).

hepatopancreas. GPx activity attained its peak level after 24 h exposure to both Cd^{2+} concentrations (P<0.01 and 0.05, respectively) (Fig.5a). As for CAT, activity increased significantly (P<0.05) at both 24 and 48 h after 5 μg/L Cd^{2+} exposure. However, there was no significant alteration in CAT activity during the 50 μg/L Cd^{2+} exposure treatment (Fig.5c). Moreover, SOD activity was not significantly affected throughout the experiment (Fig.5b).

4 DISCUSSION

In this study, the cDNAs encoding MgsHSP22 and MgsHSP24.1 were identified from *M. galloprovincialis*. The α-crystallin domains in the MgsHSP22 and MgsHSP24.1 C-termini confirmed that they belong to the α-crystallin-sHsp superfamily. The high homology of MgsHSP22 and relatively low identity of MgsHSP24.1 with their counterparts in other species was in accordance with the inference that sHsp homology varies from between 20% in remote members to 60% in closely related members of the sHsp family (Berengian et al., 1999).

sHsp transcript tissue distribution has been reported in many vertebrates and invertebrates. In the present study, MgsHSP22 transcript expression was predominantly observed in *M. galloprovincialis* muscle tissue. This result is in agreement with that of Benndorf et al. (2001), who reported that most members of the mammalian sHsp family (e.g. HSP22), which are abundant in muscles, play a role in muscle function and the maintenance of muscle integrity. A similar result was also observed in sHsp tissue expression in other organisms. For example, HSP22 in *Argopecten irradians* was predominantly expressed in the heart and muscle (Zhang et al., 2010). In addition, the MgsHSP24.1 transcript was predominantly detected in *M. galloprovincialis* gonads, indicating that sHSP24.1 participates in development and differentiation regulation. Four *Drosophila* HSP27 isoforms are constitutively expressed in the testes and two others in the ovaries (Marin et al., 1996). Certain human sHsps are specifically expressed in the testes where they play a crucial role in sperm development (Kappé et al., 2003), and pig HspB9 and HspB10 are testis-specific (Verschuure et al., 2003).

Evidence accumulated to date indicates that environmental contaminants can adversely affect the normal physiological functions in marine organisms. As a major toxic metal in seawater, Cd^{2+} is responsible for many toxic effects. For example, Cd^{2+} can induce the production of ROS, e.g., superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^{-}), and singlet oxygen ($^1O_2$), and promote oxidative stress in various organisms (Almeida et al., 2002; Basha and Rani, 2003; Gao et al., 2007).

As the first line of defense against ROS, SOD eliminates the superoxide radical (O^{-2}) by catalyzing it to hydrogen peroxide (H_2O_2), then GPx and CAT catalyze the conversion of hydrogen peroxide (H_2O_2) to water (Basha and Rani, 2003; Pan and Zhang, 2006). Increased SOD, CAT, and GPx activity have been reported in *Charybdis japonica* and *Pinctada fucata* gills hepatopancreas tissues after cadmium and copper exposure, respectively (Jing et al., 2006; Pan and Zhang, 2006). In the present study, no significant difference in SOD activity after Cd exposure was

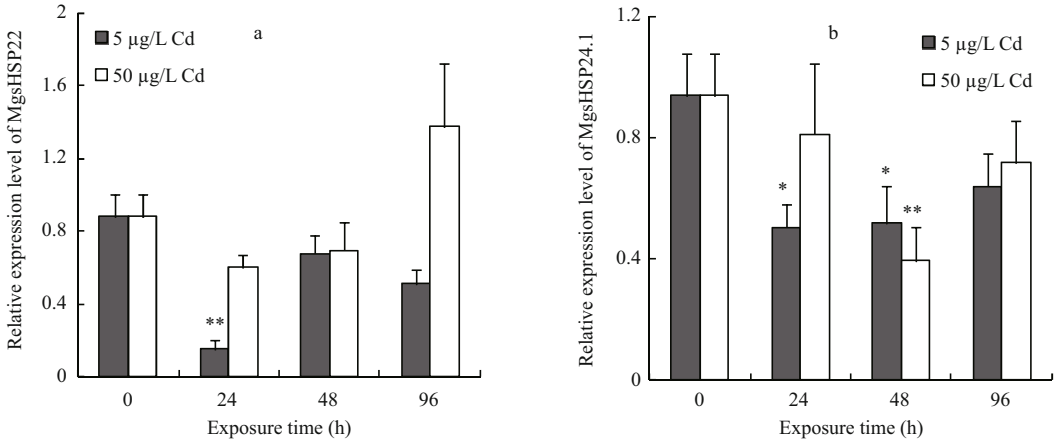

Fig.4 The MgsHSP22 (a) and MgsHSP24.1 (b) mRNA expression levels in mussels exposed to 5 μg/L and 50 μg/L Cd^{2+}

The values shown are means±SE (n=6). * Significant differences (P<0.05) and ** highly significant differences (P<0.01).

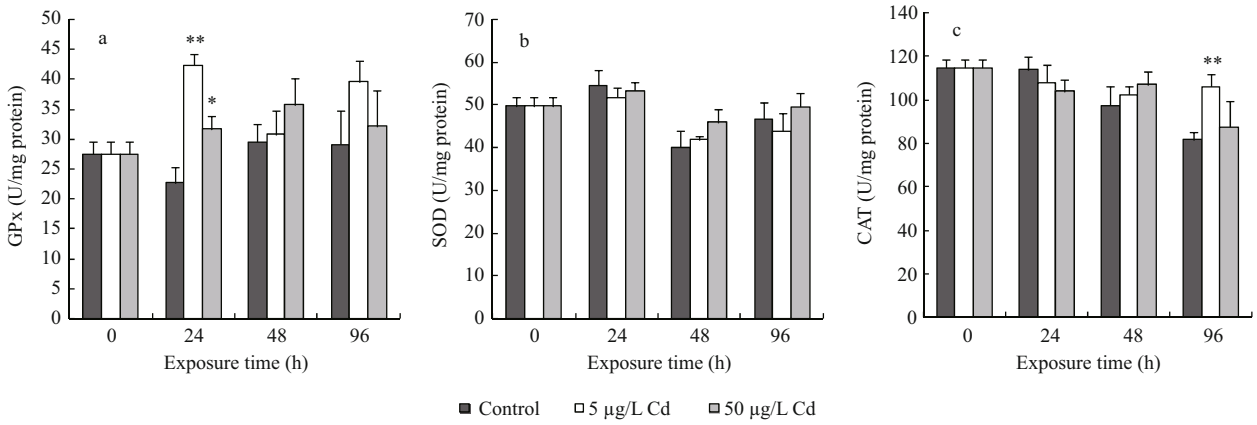

Fig.5 Effects of Cd^{2+} exposure on GPx (a), CAT (b), and SOD (c) activity in the *M. galloprovincialis* hepatopancreas

Vertical bars represent the means±SE (n=6). * Significant differences (P<0.05) and ** highly significant differences (P<0.01).

observed; this might be because the initial sampling time was delayed. We propose that SOD activity in mussels is mobilized to scavenge the superoxide radical (O^{-2}) during the early stages of exposure, and is then restored to normal levels after an exposure time of 24 h. Significant increases in GPx activity at 24 h and CAT activity at 24 and 48 h were recorded. This might be the result of the production of H_2O_2 from O^{-2} dismutated by SOD activity in the hepatopancreas. Overall, the three antioxidant enzymes (SOD, GPx, and CAT) in the *M. galloprovincialis* hepatopancreas performed differently after exposure to two Cd concentrations at different exposure times, and could therefore be used as biomarkers in evaluating the toxicity of Cd to *M. galloprovincialis*. However, further study is required to validate this.

The cytoprotective effect of sHsp expression has been observed in cells exposed to oxidative stress (Huot et al., 1996; Mehlen et al., 1996; Preville et al., 1999; Zhang et al., 2010). For example, HSP32 expression was regulated by cellular glutathione against free radical-induced cell damage during neurodegeneration (Calabrese et al., 2008). HSP27 expression can modulate the expression and/or the activity of enzymes involved in the ROS-glutathione pathway (Arrigo et al., 2002). In RPE (retinal pigment epithelium) cells, α-crystallin overexpression with increased GSH levels protects cells from apoptosis induced by oxidative stress (Parameswaran et al., 2009). Furthermore, a direct positive correlation between αA-crystallin and GSH was detected in lens epithelial cells, where the absence of αA-crystallin was associated with decreased GSH levels (Kannan et al., 2001). In the present study, MgsHSP22 and MgsHSP24.1 transcript expression both decreased after Cd^{2+} exposure, while increased GPx activity was observed after 24 h of Cd^{2+} exposure. This phenomenon

might be related to the reduction of GSH, which was used by GPx to detoxify H_2O_2 (Saito et al., 2006). Relatively higher GPx activity might lead to lower GSH levels in the 5 μg/L Cd^{2+}-groups compared with that in 50 μg/L Cd^{2+}-groups (Saito et al., 2006), which could explain the relatively higher sHsps in the 50 μg/L Cd-groups (Kannan et al., 2001). In addition, this phenomenon was in line with the inverted U-shaped model (a low dose of stimulation and high dose of inhibition) in toxicology (Calabrese, 2003; Calabrese, 2004). Zhang et al. (2011) reported a similar hormesis phenomenon in *Venerupis philippinarum* treated with Cu and Cd at concentrations of 10, 20, and 40 μg/L. Further research is required to evaluate MgsHSP22 and 24.1 as biomarkers for oxidative stress induced by Cd^{2+}-exposure in *M. galloprovincialis*.

5 CONCLUSION

In this study, the full-length cDNAs of two small heat shock proteins were identified from *M. galloprovincialis*. Both transcripts were down-regulated in the hepatopancreas after Cd^{2+} exposure, and increased GPx and CAT activity was observed in response to Cd^{2+} stress. These results suggest that MgsHSP22 and MgsHSP24.1 transcript expression and GPx and CAT activity could potentially be used as biomarkers in response to Cd stress in *M. galloprovincialis*; however, further evaluation is required.

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