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Short communication

Molecular characterization of a manganese superoxide dismutase and copper/zinc superoxide dismutase from the mussel *Mytilus galloprovincialis*

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ABSTRACT

The full-length cDNA sequences coding respectively for a manganese superoxide dismutase (Mg−MnSOD) and copper/zinc superoxide dismutase (Mg−CuZnSOD) were cloned from *Mytilus galloprovincialis*. Mg−MnSOD and Mg−CuZnSOD cDNAs encoded a polypeptide of 228 and 211 amino acids, respectively. Sequence analysis indicated Mg−MnSOD was a mitochondrial MnSOD and Mg−CuZnSOD was an intracellular CuZnSOD. Multiple alignment analysis showed that both Mg−MnSOD and Mg−CuZnSOD sequences had the common features conserved in MnSODs and CuZnSODs, respectively. Phylogenetic analysis revealed that Mg−MnSOD clustered together with MnSODs from other mollusks, whereas Mg−CuZnSOD clustered with other mollusk intracellular CuZnSODs with a wider phylogenetic distance. By quantitative real-time RT-PCR (qPCR) analysis, both Mg−MnSOD and Mg−CuZnSOD transcripts were detected in all tissues examined with the highest expression level in hepatopancreas. Following bacterial challenge, the expression level of Mg−MnSOD and Mg−CuZnSOD increased first and subsequently decreased to the original level in hemocytes. In hepatopancreas, Mg−CuZnSOD mRNA was up-regulated significantly at 72 h and 96 h post challenge, while the level of Mg−MnSOD transcript had no significant change. Therefore, Mg−MnSOD and Mg−CuZnSOD expressions were inducible and they were probably involved in the immune response against bacterial challenge. These results suggest that these SODs may play important roles in the immune defense system of *M. galloprovincialis* and perhaps contribute to the protective effects against oxidative stress in this mussel.

1. Introduction

The bivalves can accumulate a large number of bacteria from the surrounding waters due to their filter-feeding habit. Therefore, they have developed an efficient immune system to maintain balance with commensal and pathogenic bacteria, especially with the Gram-negative *Vibrio* spp. [1]. In many bivalve species, phagocytic cells can be activated by foreign particles, or organisms and their products, to release reactive oxygen species (ROS) including hydrogen peroxide, hydroxyl radical and singlet oxygen [2]. The production of ROS is essential for the elimination of invading viruses, bacteria, fungi and protozoa through the activation of the respiratory burst [3,4]. In addition, ROS particularly hydrogen peroxide, may serve as second messengers in signal transduction pathway by activating the NF-κB transcription factor [5,6]. However, excessive ROS tends to cause oxidative damage to cellular components such as lipids, proteins, and nucleic acids [7]. The aerobic organisms have evolved various non-enzymatic and enzymatic antioxidant systems to eliminate the redundant ROS and maintain redox homeostasis [8]. The specific enzymatic antioxidants include superoxide dismutase, catalase and glutathione peroxidases [9].

The superoxide dismutases (SODs, EC 1.15.1.1) are metalloenzymes that represent one important line of defenses against ROS and in particular superoxide anion. The SODs can catalyze the dismutation of superoxide into molecular oxygen and hydrogen peroxide [10]. The SOD family proteins are usually classified into four groups based on their structures, cellular localization and the metal cofactors in active sites: manganese SOD (MnSOD), copper/zinc SOD (CuZnSOD), iron SOD (FeSOD) and nickel SOD (NiSOD)

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[10,11]. MnSOD and CuZnSOD have been shown to be involved in the innate immune responses of marine invertebrate animals, as evidenced by the rapid modulation of MnSOD transcription during challenges with bacteria [12–15] or viruses [16,17], and up-regulation of CuZnSOD transcription after bacterial challenge [18,19]. However, little information is available about the molecular features of SODs and their immune response against pathogen infection in the mussel *Mytilus galloprovincialis*.

At present, several SOD genes have been cloned in bivalves, including scallops [13,14,18,19], oysters [20,21] and clams [15,22–24]. To our knowledge, there is no report about MnSOD gene in mussels including *M. galloprovincialis*. Although a CuZnSOD gene has been reported in *M. galloprovincialis* [25], its transcriptional response against bacterial infection has not been investigated. Therefore, the objective of this study was to isolate and characterize SOD genes in order to understand the transcriptional response of these genes against bacterial challenge in *M. galloprovincialis*.

2. Materials and methods

2.1. Animal culture

The mussels *M. galloprovincialis* (shell length: 4–6 cm) were purchased from a local mussel culture farm and acclimatized for 10 days at 25 °C before bacterial challenge. During acclimation, the mussels were fed with *Isochrysis galbana* and *Platymonas helgolandica* and they were cultured in seawater (32 psu) which was completely changed once every day.

2.2. Total RNA extraction and full-length cDNA cloning

The soft tissues of six individuals were quickly dissected and frozen in liquid nitrogen. Frozen tissues were pulverized under liquid nitrogen, and subjected to total RNA extraction using the TRIzol Reagent (Invitrogen, USA). The extracted RNA was then treated with RQ1 RNase-Free DNase (Promega, USA) to remove DNA contamination. Single-stranded cDNA was synthesized from the total RNA with M-MLV reverse transcriptase (Promega, USA).

BLAST analysis of all expressed sequence tag (EST) sequences from a cDNA library of *M. galloprovincialis* revealed that two ESTs were highly similar to the previously identified MnSOD and CuZnSOD, respectively. The 5′ and 3′ ends of Mg–MnSOD and Mg–CuZnSOD were obtained by rapid amplification of cDNA ends (RACE) using the SMART RACE cDNA Amplification Kit (Clontech, USA) according to manufacturer's instruction. The PCR products were purified using agarose gel electrophoresis and subcloned into the pMD18-T simple vector (Takara). After transformed into the competent cells of *Escherichia coli* Top10F′, three positive clones were sequenced by the Chinese National Human Genome Center (SinoGenoMax, Beijing, China).

Table 1
Primers used in this study.

Primer name	Primer sequence (5′–3′)	Sequence information
P1 (forward)	TGTAACAAACTGGGACGATA	Real time primer for β-actin
P2 (reverse)	AGCATGAGGAAGGGCATAAC	Real time primer for β-actin
P3 (forward)	GATGCAGCAGTAGCAGTCCA	Real time primer for Mg–MnSOD
P4 (reverse)	GTAGGCATGCTCCCAGACAT	Real time primer for Mg–MnSOD
P5 (forward)	AGGCGCAATCCATTTGTTAC	Real time primer for Mg–CuZnSOD
P6 (reverse)	CATGCCTTGTGTGAGCATCT	Real time primer for Mg–CuZnSOD

Table 2
Sequences used for multiple alignments and phylogenetic analysis. "IcCuZnSOD" means cytoplasmic CuZnSODs and "EcCuZnSOD" means extracellular CuZnSODs.

Species	Accession numbers	Sequence size	Abbreviation
Mytilus galloprovincialis	JN863295	228aa	Mg–MnSOD
Argopecten irradians	ABW98672	226aa	Ai–MnSOD
Mizuhopecten yessoensis	BAE78580	226aa	My–MnSOD
Tegillarca granosa	ADC34695	227aa	Tg–MnSOD
Crassostrea gigas	EKC27985	225aa	Cg–MnSOD
Danio rerio	AAP34300	224aa	Dr–MnSOD
Gallus gallus	AAK97214	224aa	Gg–MnSOD
Mus musculus	CAA28645	222aa	Mm–MnSOD
Homo sapiens	AAP34407	213aa	Hs–MnSOD
Salmo salar	ACN10263	226aa	Ss–MnSOD
Xenopus laevis	AAQ63483	224aa	Xl–MnSOD
Mytilus galloprovincialis	JN863296	211aa	Mg–CuZnSOD
Mytilus galloprovincialis	CAQ68509	157aa	Mg–icCuZnSOD
Mytilus edulis	CAE46443	158aa	Me–icCuZnSOD
Crassostrea gigas	CAD42722	156aa	Cg–icCuZnSOD
Haliotis discus discus	ABG88844	154aa	Hd–icCuZnSOD
Oncorhynchus mykiss	NP_001117801	154aa	Om–icCuZnSOD
Bos taurus	AAA73164	152aa	Bt–icCuZnSOD
Mus musculus	NP_035564	154aa	Mm–icCuZnSOD
Argopecten irradians	EU563958	152aa	Ai–icCuZnSOD
Chlamys farreri	ABD58974	153aa	Cf–icCuZnSOD
Litopenaeus vannamei	ADM64316	178aa	Lv–icCuZnSOD
Argopecten irradians	EU683812	218aa	Ai–ecCuZnSOD
Macrobrachium rosenbergii	AAZ29240	201aa	Mr–ecCuZnSOD
Salmo salar	ACI66718	215aa	Ss–ecCuZnSOD
Homo sapiens	AAA66000	240aa	Hs–ecCuZnSOD
Mus musculus	NP_035565	251aa	Mm–ecCuZnSOD

2.3. Sequence analysis of Mg–MnSOD and Mg–CuZnSOD

The searches for nucleotide and protein sequence similarities were performed with the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast). The deduced protein sequences were analyzed with ExPASy (http://www.expasy.org/). Characteristic domains or signature motifs were identified using the SMART software (http://smart.embl-heidelberg.de/). Probability to be exported to mitochondria was predicted by the Predotar Server (http://urgi.versailles.inra.fr/predotar/predotar.html). Signal peptide was predicted by SignalP 4.0 server (http://www.cbs.dtu.dk/services/SignalP/). Multiple alignments were performed with the ClustalW program (http://www.ebi.ac.uk/clustalw/). A phylogenetic tree was constructed with Mega4.1 software using the neighbor-joining (NJ) method and a Poisson correction model based on the alignment of amino acids. Bootstrap analysis was used with 1000 replicates to test the relative support for the branches produced by the NJ analysis [26].

2.4. Tissue-specific expressions of Mg–MnSOD and Mg–CuZnSOD mRNAs

Hemocytes, gill, hepatopancreas, mantle and adductor muscle were taken aseptically from six clams and subjected to total RNA extraction. One microgram of total RNA was used for cDNA synthesis according to the manufacture's protocol (Promega, USA). qPCR was carried out in an ABI 7500 Real-time Detection System by using the SYBR ExScript qPCR Kit (Takara, Japan) as described

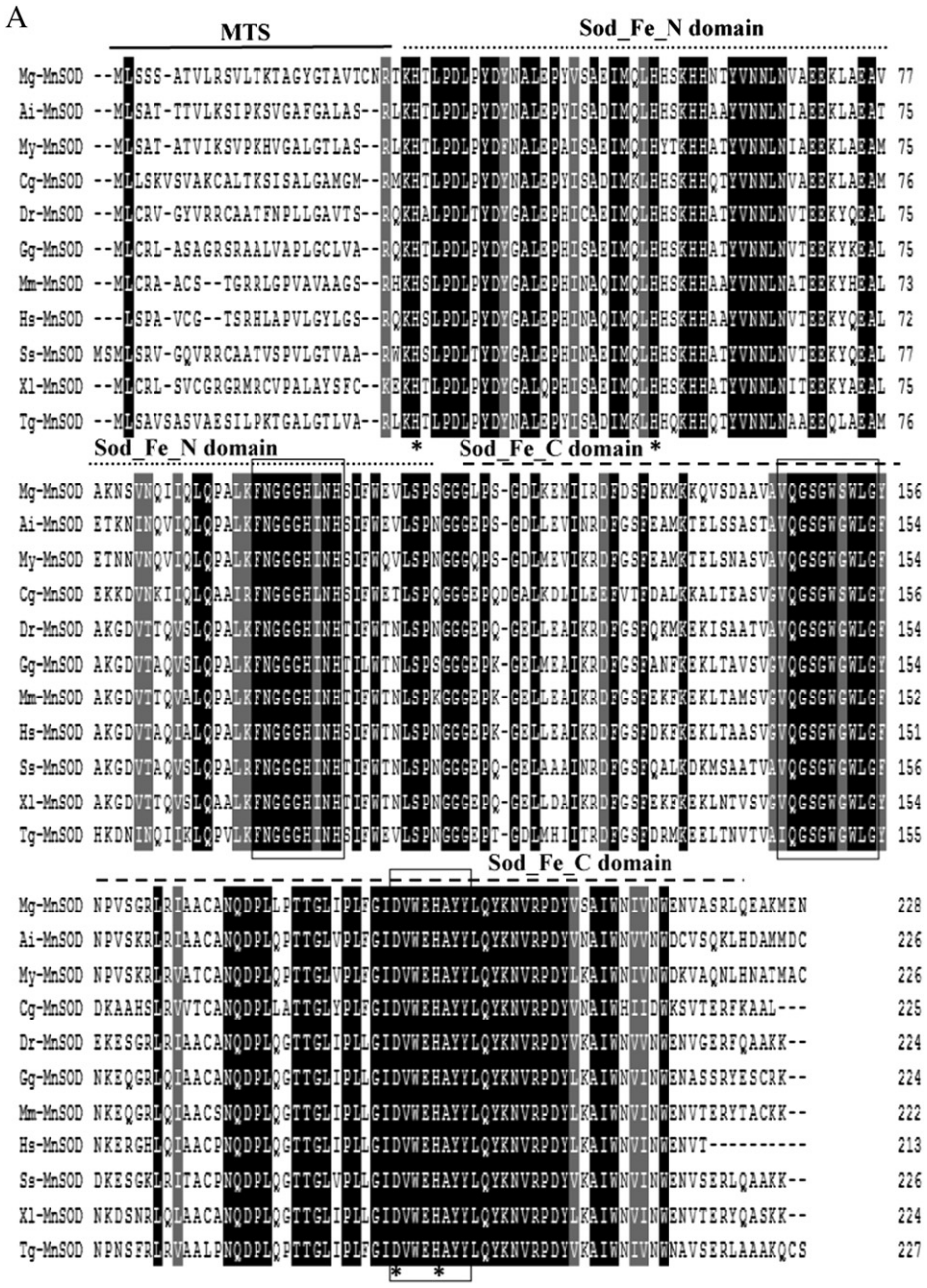

Fig. 1. Multiple sequence alignments for MnSOD (A) and CuZnSOD (B) proteins. The black shadow region indicates positions where all sequences share the same amino acid residue. Gaps are indicated by dashes to improve the alignment. The GenBank accession numbers are shown in Table 2. The mitochondrial targeting sequence (MTS, solid line), Sod_Fe_N domain (dot line), Sod_Fe_C domain (dashed line), Sod_Cu domain (dot line) are marked above the SOD sequences. The signatures are marked by frame. The amino acids responsible for combination with the metal are indicated with asterisks.

previously [27]. The PCR amplification was carried out in a total volume of 50 μL, containing 25 μL of 2 × SYBR Green PCR Master Mix, 20 μL of the diluted cDNA, 1 μL of each of primers (10 μmol/L), and 3 μL of DEPC-treated water. The thermal profile for qPCR was 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All reactions were run in triplicate. Dissociation curve analysis of amplicons was performed at the end of each PCR reaction to confirm that only one PCR product was amplified and detected. The expression of Mg–MnSOD and Mg–CuZnSOD was analyzed using the $2^{-\Delta\Delta CT}$ method [28] with β-actin gene as the internal control [27,29]. The primers used to quantify the expression of Mg–MnSOD and Mg–CuZnSOD were listed in Table 1.

2.5. Bacterial challenge and temporal expression profiles of Mg–MnSOD and Mg–CuZnSOD mRNAs

Vibrio anguillarum was grown in marine broth 2216E at 28 °C to exponential phase, and then the cultures were centrifuged at 2000 × *g* for 5 min to harvest the bacteria. The bacterial pellet was resuspended in filtered seawater and diluted to 1×10^7 CFU mL^{-1}. After acclimatization, the mussels were randomly divided into three experimental groups with three replicates each containing 30 individuals. For the challenge group, 50 μL live *V. anguillarum* (1×10^7 CFU/mL) resuspended in sterilized seawater was injected into the adductor muscle of each mussel. For the control group, the mussels were injected with an equal volume of sterilized seawater

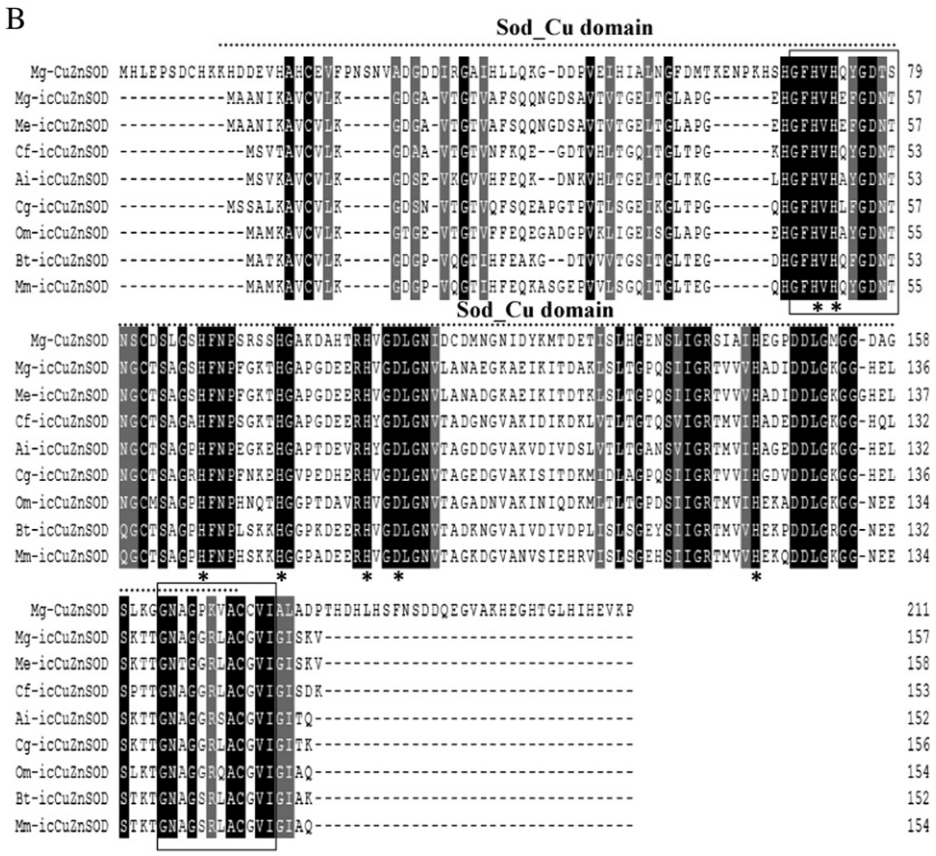

Fig. 1. (*continued*).

respectively. The untreated mussels were employed as the blank group. At 24, 48, 72 and 96 h intervals following the challenge, the hemolymph of six mussels in each group was collected with syringes, and then the hemocytes were harvested (1000 × *g*, 4 °C, 10 min) for subsequent mRNA extraction. Meanwhile, the hepatopancreas of six individuals from each group were also sampled. The RNA extraction, cDNA synthesis and qPCR thermal profile were conducted as described above.

2.6. Statistical analysis

SPSS 16.0 software (SPSS Inc., USA) was used for statistical analysis. All data were given in terms of relative mRNA expression as means ± SE ($n = 6$). One-way analysis of variance (ANOVA) was performed on all data and $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Sequence and homology analysis of Mg–MnSOD and Mg–CuZnSOD

Two nucleotide sequences of 907 bp and 935 bp representing the complete cDNA sequence of Mg–MnSOD and Mg–CuZnSOD were obtained by RACE and deposited in GenBank under accession no. JN863295 and JN863296, respectively. The opening reading frame (ORF) of Mg–MnSOD cDNA encoded a polypeptide of 228 amino acids with the predicted molecular weight (mw) of 25.31 kDa and the theoretical isoelectric point of 6.44. The mw of Mg–MnSOD was similar to the molecular masses of MnSODs from other animals [12,13,16,21–24]. SMART program analysis revealed that Mg–MnSOD contained a Sod_Fe_N domain ranging from Lys^{29} to Ser^{110}, and a Sod_Fe_C domain ranging from Gly^{115} to Leu^{211}. SignalP program analysis revealed that no putative signal peptide existed in Mg–MnSOD. Besides, a 27 amino acid long mitochondrial targeting sequence (MTS) at N-terminal suggesting its localization to the mitochondria was found in Mg–MnSOD by the Predotar Server.

The ORF of Mg–CuZnSOD encoded a polypeptide of 211 amino acids with the predicted molecular weight of 22.7 kDa and the theoretical isoelectric point of 5.48. SMART program analysis revealed that Mg–CuZnSOD contained a SOD_Cu domain ranging from Lys^{10} to Ile^{174}. SignalP program analysis revealed that no putative signal peptide existed in Mg–CuZnSOD. CuZnSOD is usually classified into two isoforms according to its localization. Extracellular CuZnSOD has an N-terminal signal cleavage peptide, whereas intracellular CuZnSOD has no signal peptide [30]. For Mg–CuZnSOD, no signal peptide was found, indicating that it belongs to a member of the intracellular CuZnSOD family. Alignment of Mg–CuZnSOD showed that only 63 identical amino acid residues with previous reported intracellular CuZnSOD from *M. galloprovincialis* [25]. However, Mg–CuZnSOD encoded a polypeptide of 211 amino acids, which was much longer than previous reported intracellular CuZnSODs from mollusks. Remarkably, Mg–CuZnSOD had a longer C-terminal tails than those of other CuZnSOD orthologs.

Blast analysis revealed that Mg–MnSOD shared high homology with MnSODs from the bivalves *Argopecten irradians* (ABW98672, 73% identity), *Mizuhopecten yessoensis* (BAE78580.1, 73% identity) and *Tegillarca granosa* (ADC34695.1, 73% identity). Multiple alignment indicated the potential metal binding sites for manganese (His^{30}, His^{54}, Asp^{187}, His^{191}) and three MnSOD signatures -FNGGGHLNH- (from 94 to 102), -VQGSGWSWLG- (from 146 to

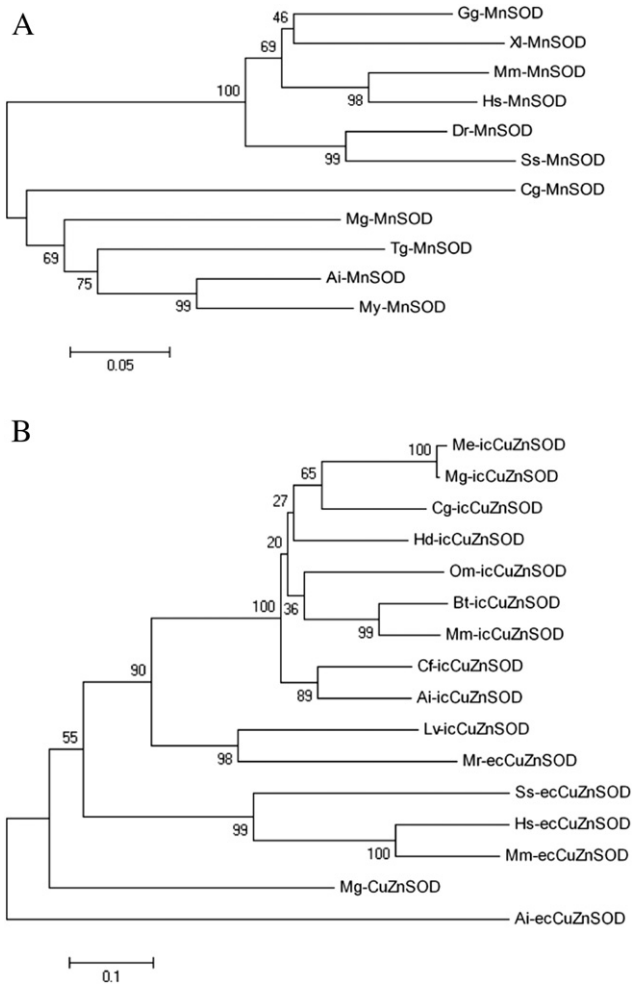

Fig. 2. Phylogenetic trees for MnSOD (A) and CuZnSOD (B) constructed by neighbor-joining method based on the sequences from different animals. The GenBank accession numbers are shown in Table 2.Numbers at the forks indicate the bootstrap values (in %) out of 1000 replicates.

155), and -DVWEHAYY- (from 187 to 194) were conserved in Mg–MnSOD (Fig. 1A). Thus, Mg–MnSOD had high similarity with other MnSODs from mollusks and also contained all the conserved motifs of MnSOD in its nucleotide sequences. All these results indicated that the cloned Mg–MnSOD was a MnSOD homolog.

For Mg–CuZnSOD, several characteristic motifs and SOD signature sequences could be identified (Fig. 1B). For example, two cysteine residues (C^{82} and C^{171}) involved in internal disulfide bond formation were totally conserved in Mg–CuZnSOD. Additionally, the Cu, Zn binding sites (H^{71}, H^{73}, H^{88}, H^{145} for Cu binding, and H^{88}, H^{96}, H^{105}, D^{108} for Zn binding) and two CuZnSOD signatures (GFHVHQYGDTS) from 69 to 79 and (GNAGPKVACCVI) from 163 to 174 were also identified in Mg–CuZnSOD.

3.2. Phylogenetic analysis

The evolutionary trees for different types of SODs were constructed based on protein sequences of SODs orthologs. In the phylogenetic tree of MnSOD (Fig. 2A), Mg–MnSOD was firstly clustered with MnSODs from bay scallop *A. irradians*, Japanese scallop *M. yessoensis* and blood clam *T. granosa*, and then formed a bivalve branch with MnSOD of *Crassostrea gigas*. The bivalve branch clustered with MnSODs from the vertebrate animals. According to the phylogenetic tree of CuZnSOD (Fig. 2B), the CuZnSOD orthologs from animals were mainly clustered into two groups. In the group of intracellular CuZnSOD, the intracellular CuZnSODs from bivalves including *M. galloprovincialis* formed as a subgroup and then clustered with CuZnSODs from vertebrates. However, Mg–CuZnSOD clustered closely with extracellular CuZnSODs other than intracellular CuZnSODs. Mg–CuZnSOD first grouped with extracellular CuZnSOD from bay scallop and then clustered with extracellular CuZnSODs from arthropods and vertebrates.

In this study, Mg–MnSOD showed strong relationship to MnSODs from other bivalves. The topology of MnSOD approximately reflected the taxonomic classification of the corresponding species. However, Mg–CuZnSOD was more closely related to extracellular CuZnSODs other than intracellular CuZnSODs. Similarly, intracellular CuZnSOD from *Litopenaeus vannamei* (Lv–CuZnSOD) was also more closely clustered with the extracellular CuZnSOD from *Macrobrachium rosenbergii* other than the other intracellular CuZnSODs. In the phylogeny tree, the MnSODs showed a strong relationship among different species, whereas CuZnSOD orthologs had a relatively weak relationship among species. This difference probably results from the erratic evolutionary changes of CuZnSODs, compared to MnSOD which showed a relatively constant rate of evolution [31].

3.3. Tissue-specific expression of Mg–MnSOD and Mg–CuZnSOD

The tissue distribution of Mg–MnSOD and Mg–CuZnSOD mRNA were investigated by quantitative Real-time PCR with β-actin as internal control. During the qPCR assays, there was only one peak at the corresponding melting temperature in the dissociation curve analysis, indicating that the PCR was specifically amplified. The mRNA transcripts of Mg–MnSOD and Mg–CuZnSOD were detected in all the tissues examined, including hepatopancreas, hemocytes, muscle, mantle and gills. The Mg–MnSOD transcript was dominantly expressed in hepatopancreas, moderately expressed in muscle and gonad (Fig. 3A). A similar tissue-specific expression pattern was also observed for Mg–CuZnSOD transcript with the highest expression level in hepatopancreas, and moderate level in gonad, muscle and mantle (Fig. 3B).

In bivalves, the tissue-distribution profiles of SODs are species-dependent. Our study indicated both the transcripts of Mg–MnSOD and Mg–CuZnSOD were dominantly expressed in hepatopancreas. The tissue-distribution profile of Mg–MnSOD was similar to that reported for the MnSOD in blood clam *T. granosa*, with the highest expression in hepatopancreas [23]. In *A. irradians* and *C. gigas*, the expression levels of MnSODs were highest in gills, while the predominant expression of MnSOD from *Ruditapes philippinarum* was detected in hemocytes [13,21,24]. The variable

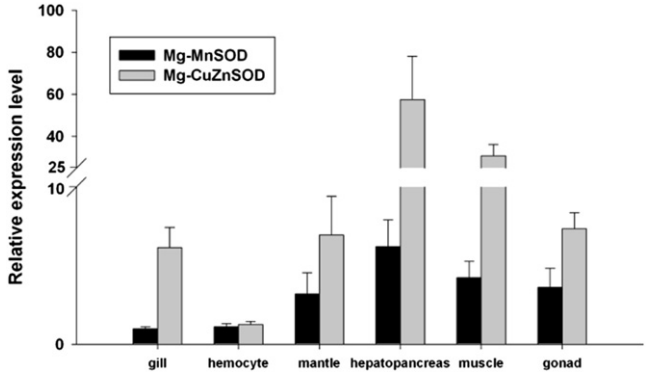

Fig. 3. Tissue-specific expression of Mg–MnSOD and Mg–CuZnSOD mRNA measured by quantitative real-time RT-PCR. The mRNA expression level is calculated relative to actin expression. Each symbol and vertical bar represents the mean ± SE ($n = 6$).

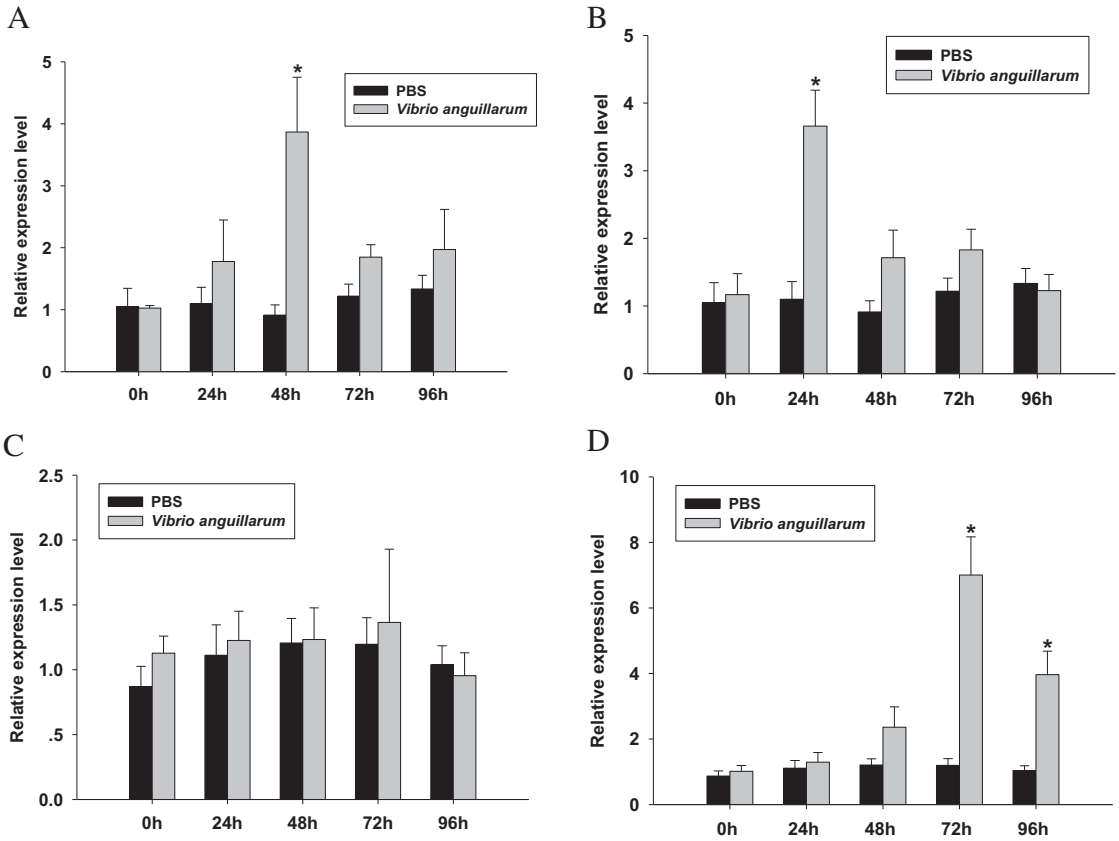

Fig. 4. Temporal expression profiles of Mg–MnSOD and Mg–CuZnSOD in hemocytes (A: Mg–MnSOD, B: Mg–CuZnSOD) and hepatopancreas (C: Mg–MnSOD, D: Mg–CuZnSOD) post *V. anguillarum* challenge. The mRNA expression level is calculated relative to actin expression. Each symbol and vertical bar represents the mean ± SE ($n = 6$). Significant difference from control is indicated with an asterisk at $P < 0.05$.

expression level of MnSOD was speculated to be related with tissue-dependent mitochondrial content and oxidative load [23]. For CuZnSOD, the transcript was dominantly detected in gills of *Chlamys farreri* and *A. irradians*, whereas extracellular CuZnSOD of *A. irradians* and intracellular CuZnSOD of *R. philippinarum* transcripts were mainly detected in hemocytes [14,18,19,24]. The differences in the tissue-distribution of CuZnSOD expression may be attributed to the balance of various biological processes acting on the production of ROS in different environments [18].

3.4. Temporal expression profile of Mg–MnSOD and Mg–CuZnSOD mRNA in hemocytes and hepatopancreas post bacterial challenge

Following bacterial challenge, the transcripts of Mg–MnSOD in hemocytes (Fig. 4A) increased significantly at 48 h post challenge ($P < 0.05$), and then returned to the original level at 72–96 h post challenge. For Mg–CuZnSOD, the expression level increased significantly at 24 h following challenge in hemocytes ($P < 0.05$), and then declined to the original value at 48–96 h (Fig. 4B) post challenge. In hepatopancreas, the expression level of Mg–MnSOD had no significant change during the challenge (Fig. 4C), while Mg–CuZnSOD (Fig. 4D) transcripts was up-regulated significantly at 72 h and 96 h post challenge ($P < 0.05$).

Both CuZnSODs and MnSODs are considered to be stress-responsive elements of defense system of which mRNA expression and enzyme activity could be influenced by various stressors and stimulators including environmental changes, pollutants and biological stimuli (pathogens) [12,32–35]. When pathogens invade, mollusks activate a series of immune defense responses. Phagocytosis is a major protective mechanism of hemocytes which leads to an increased oxygen consumption and ROS production [36]. In addition, other immune responses also require more ATP to support the high energy demand leading to an elevated ROS production and resulting mitochondrial oxidative load [9].

In the present study, we found that the expression of CuZnSOD and MnSOD were modulated by bacterial challenge. In hemocytes, the expression of MnSOD and CuZnSOD increased significantly at 48 h and 24 h post bacterial challenge, respectively. The transcripts of MnSOD and CuZnSOD were up-regulated to eliminate excessive ROS and protect the host against oxidative damage caused by bacterial stimulation. Similar expression profile was also found in bay scallop *A. irradians*, significant increase of MnSOD and extracellular CuZnSOD transcripts in hemocytes occurred at 3 h and 6 h post *V. anguillarum* challenge, respectively [13,14]. However, no significant change occurred in the intracellular CuZnSOD expression level of *A. irradians* in hemcoytes post *V. anguillarum* challenge [19]. In *C. farreri*, the expression level of intracellular CuZnSOD decreased first at 6 h and then recovered at 32 h post *V. anguillarum* challenge [18]. The different expression profiles of the MnSOD and CuZnSOD in hemocytes of different bivalves might be due to different challenge level and immuno-reaction in these species [13]. In hepatopancreas, Mg–CuZnSOD mRNA was up-regulated significantly at 72 h and 96 h post bacterial challenge ($P < 0.05$), while no significant change had been observed for the expression of Mg–MnSOD transcript during the challenge. This might be explained by the fact that as the challenge process proceeds, the level of ROS gradually increased in hepatopancreas, resulting in the up-regulated expression of Mg–CuZnSOD. For Mg–MnSOD, bacterial challenge probably posed no obvious oxidative stress on mitochondria in hepatopancreas.

In conclusion, a manganese and copper/zinc superoxide dismutases were cloned from *M. galloprovincialis*. Sequence analysis indicated Mg–MnSOD was a mitochondrial MnSOD and Mg–CuZnSOD was an intracellular CuZnSOD. Both Mg–MnSOD and Mg–CuZnSOD sequences had the common features conserved in MnSODs and CuZnSODs, respectively. Both Mg–MnSOD and Mg–CuZnSOD transcripts were detected in all tissues examined with the highest expression in hepatopancreas. The expression of Mg–MnSOD and Mg–CuZnSOD in hemocytes increased significantly in response to bacterial challenge, which indicated that these genes were inducible and might be involved in mussel innate immune responses. These results indicated both these SOD genes may play important roles in the immune defense system of *M. galloprovincialis*.

Acknowledgments

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