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ORIGINAL ARTICLE

Molecular cloning and oxidative-stress responses of a novel manganese superoxide dismutase (*MnSOD*) gene in the dinoflagellate *Prorocentrum minimum*

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Abstract

Dinoflagellate algae are microeukaryotes that have distinct genomes and gene regulation systems, making them an interesting model for studying protist evolution and genomics. In the present study, we discovered a novel manganese superoxide dismutase (*PmMnSOD*) gene from the marine dinoflagellate *Prorocentrum minimum*, examined its molecular characteristics, and evaluated its transcriptional responses to the oxidative stress-inducing contaminants, $CuSO_4$ and NaOCl. Its cDNA was 1238 bp and contained a dinoflagellate spliced leader sequence, a 906 bp open reading frame (301 amino acids), and a poly (A) tail. The gene was coded on the nuclear genome with one 174 bp intron; signal peptide analysis showed that it might be localized to the mitochondria. Real-time PCR analysis revealed an increase in gene expression of *MnSOD* and SOD activity when *P. minimum* cells were separately exposed to $CuSO_4$ and NaOCl. In addition, both contaminants considerably decreased chlorophyll autofluorescence, and increased intracellular reactive oxygen species. These results suggest that dinoflagellate *MnSOD* may be involved in protecting cells against oxidative damage.

Keywords *Prorocentrum minimum* · MnSOD · ROS · Genomic DNA · Gene expression

Introduction

Dinoflagellate algae are widely distributed in aquatic systems and present as autotrophic, heterotrophic, mixotrophic, or live in a symbiotic life style [1, 2]. As microeukaryotes, they possess distinct chromosomal, genomic, and transcriptomic features, including large nuclear genome size, dinoflagellate spliced leader (dinoSL) *trans*-splicing, high copy gene numbers, and post-transcriptional regulation [3–7]. In addition, some dinoflagellates are easy to culture and are sensitive to environmental contaminants [8–10]. For these reasons, they have been widely employed in genomics, protist evolution, and eco-toxicological research. Recently, molecular studies have shown that environmental toxicants can cause oxidative stress and cellular damage [8, 11], and that dinoflagellates use specific stress-related genes and antioxidant enzymes as defence mechanisms against such stresses [10–12]. Several antioxidant genes, such as catalase-peroxidase (*KatG*) and glutathione *S*-transferase (*GST*), have been discovered and characterized [11, 13]; however, many other genes remain to be elucidated.

Superoxide dismutases (SODs) are a group of metalloenzymes that catalyse the dismutation of the superoxide radical to molecular oxygen and hydrogen peroxide [14]. They are classified into three distinct families depending on their metal cofactors: Cu/ZnSOD, NiSOD, and Fe/MnSOD [15–17]. Among SODs, MnSOD is the only known SOD present within the mitochondria [18], and it has been considered a unique tumour suppressor protein with a pivotal role in regulating cell death events [19]. Previous studies reported that MnSOD levels respond to changes in oxygen tension, type of substrate, redox active compounds, the nature of the terminal oxidant, and the redox potential of

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the medium [20]. To date, SOD genes have been isolated and studied in many different species [18, 21–23]. However, the genetic structure and stress response of SODs have been poorly investigated in dinoflagellates.

Dinoflagellate *FeSOD* was the first *SOD* isolated from *Lingulodinium polyedrum* and its gene expression was examined under metal ion exposure [24]. Since then, seventeen *FeSODs* have been identified from *Crypthecodinium cohnii,* which may have been acquired via horizontal gene transfer [16]. Recently, the full ORF sequence of *CuZn-SOD* was examined from *Prorocentrum minimum*, and its gene expression was found to be regulated by intracellular reactive oxygen species (ROS) [25]. In addition, several partial *SOD* sequences from dinoflagellates (i.e., *Cochlodinium polykrikoides*, *Karenia brevis*, *Symbiodinium* sp.) have been recorded in the public Expressed Sequence Tags (EST) database [26–28], but their functions have been insufficiently studied. Even though the MnSOD enzyme has been identified from *Karenia brevis* using western blotting [26], the dinoflagellate *MnSODs* have not been sufficiently characterized with respect to their gene structure, phylogenetic relationships, and responses to environmental oxidative stress.

In the present study, we firstly determined the full-length sequence of the marine dinoflagellate *P. minimum MnSOD* gene (*PmMnSOD*), and characterized its structural features (e.g., conserved motifs and genomic coding region) and phylogenetic relationships. In addition, we examined the transcriptional responses of *PmMnSOD* and SOD activity in cells exposed to the environmental chemicals, copper sulphate ($CuSO_4$) and sodium hypochlorite (NaOCl), as they are well-known to induce intracellular ROS and thereby cause cell death [8]. In recent years, *P. minimum* has been commonly used in genomics and evolutionary studies [10, 29, 30] as it is easily cultured in the laboratory and has unique genomic features [5, 10].

Materials and methods

Algae culture

A strain (D-127) of *P. minimum* was obtained from the Korea Marine Microalgae Culture Center (Pukyong National University, Busan, Korea). The cells were cultured in f/2 medium at 20 °C, with 12:12 h light–dark cycle and a photon flux density of ~ 65 µmol photons/m^2/s.

RNA extraction, cDNA synthesis, and DNA extraction

To extract RNA from *P. minimum* cells, cultures were harvested, frozen immediately in liquid nitrogen, and then stored at −80 °C until RNA extraction. Preserved cells were physically broken by freeze-thawing in liquid nitrogen, and further homogenization was performed using zirconium beads (diameter 0.1 mm) with a Mini-bead beater (BioSpec Products Inc., Bartlesville, OK). Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and further purified on Mini Spin Columns of RNeasy Mini Kit (Qiagen, Valencia, CA). RNA quality and quantity were measured with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). Reverse transcription (RT) was carried out using a TOPscript™ cDNA Synthesis Kit (Enzynomics, Daejeon, Korea). Total genomic DNA was extracted from *P. minimum* following the cetyltrimethylammonium bromide (CTAB) protocol as described by Murray and Thompson [31].

Gene sequences determination

Full-length cDNA sequence of *PmMnSOD* was cloned by using the primers (Supplementary Table 1) designed according to the partial sequence from an EST database of *P. minimum*. The 3′ and 5′-UTR of *PmMnSOD* transcript were determined using the 3′- and 5′-RACE. Primary and the following nested PCRs were carried out using specific primers (Supplementary Table 1). Reaction conditions for the primary and secondary PCRs were as follows: pre-denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C/58 °C (primary/secondary, respectively) for 30 s, 72 °C for 2 min, and extension at 72 °C for 10 min. Positive core PCR products were purified, cloned into pMD20-T vector (Takara, Shiga, Japan), transformed into *E. coli* competent cells, and subjected to DNA sequencing. Genomic coding regions were determined by long PCR with specific primers (Supplementary Table 1).

PmMnSOD characterization and phylogenetic analysis

The 3′-end, partial sequences and 5′-end cDNA sequences of *PmMnSOD* were properly assembled by Sequencher v5.1. Protein motifs and conserved domains of the PmMnSOD protein were analyzed with online servers and public database, including the PROSITE (http://prosite.expasy.org/), Compute pI/Mw tool (http://web.expasy.org/compute_pi/), and NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Signal peptide and transmembrane structure of PmMnSOD protein was separately predicted with SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) and TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Multiple sequences alignment was performed with BioEdit 5.0.6 [32]. Phylogenetic analysis was performed with MEGA6 [33], using the neighbor-joining (NJ)

algorithm. A bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed [34]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. In the sequences analysis, all positions containing gaps and missing data were eliminated.

Toxicant treatments and gene expression

Exponential phase cells were treated by two different environmental toxicants, $CuSO_4$ (Cat. No. C1297, Sigma) and NaOCl (Cat. No. 425,044, Sigma). To test the doses effect of $CuSO_4$ and NaOCl on *PmMnSOD* transcriptional expressions, a series of concentrations of each toxicant were added in the *P. minimum* cultures (with final concentration of $CuSO_4$: 0.1, 0.2, 0.5, 1.0 and 2.0 mg/L; NaOCl: 0.1, 0.2, 0.5, 1.0 and 2.0 mg/L). All samples were performed in duplicate and all working dilutions were prepared from standard stock solutions. Treated and untreated cells were harvested for gene expression analysis at indicated time points. RNA extraction and reverse transcription were performed using the same protocol described above. All quantitative real-time (qRT)-PCRs were performed with the TOPreal™ qPCR 2X PreMIX SYBR Green Kit (TOP, Enzynomics, Daejeon, Korea) in a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The qRT-PCR conditions were as follows: 4 min at 50 °C; 10 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 15 s at 60 °C, and 15 s at 72 °C. All reactions were performed in triplicate, and the mean value was calculated. Specificity of amplification was verified through analysis of a melting curve generated by gradually heating the sample from 65 to 95 °C. Alpha tubulin was used as internal control [10]. Cycle threshold (Ct) values were obtained by using the software provided with the CFX96 Real-Time machine (Bio-Rad), and the fold-change relative to the control was calculated according to the method described by Pfaffl [35].

Measurements of ROS production and SOD activity

Dihydroxyrhodamine 123 (DHR123-D1054; Sigma) staining was employed to measure the production of ROS. In brief, DHR123 can be oxidized by ROS and emit green fluorescence [36]. Cells were treated with various concentrations of $CuSO_4$ and NaOCl after 24 h incubation. The cells were stained with DHR123 for 1 h at a final concentration of 10 µM, then harvested by centrifugation and washed twice with fresh f/2 medium. The cultures were resuspended in fresh f/2 medium, mounted onto a slide, and sealed. The stained cells were observed by using a fluorescence microscope (Carl Zeiss Axioskop, Oberkochen, Germany) to be determined the ROS production. The relative ROS levels were quantified with ImageJ software (NIH, Bethesda, MD) from the fluorescence microscopic images.

SOD activity was measured according to Beauchamp and Fridovich [37]. Algal cells were harvested by centrifugation at 4217×*g* for 10 min, and then 5 mL of 100 mM phosphate buffered saline (PBS, pH 7.8) was added to the algal cell pellet. The cells were homogenized using a Teflon pestle tissue homogenizer in ice. Next, the tube was placed in a water bath at 40 °C for 5 min. The mixture was centrifuged at 4217×*g* for 10 min. To the supernatant, 2.6 mL of the reaction mixture (0.05 M PBS, 130 mM methionine, 750 µM nitroblue tetrazolium, 100 µM Na_2EDTA, and 20 µM riboflavin) were added. The tubes were incubated in light (~65 µmol photons/m^2/s) for 30 min. The absorbance was read at 560 nm by using UV–visible spectrophotometer (Model V-730, Jasco, Tokyo, Japan). One unit of SOD (U) was defined as the amount of enzyme resulting in 50% inhibition of photochemical reduction of nitroblue tetrazolium (NBT). SOD levels were represented as U per 10^4 cells (U/10^4 cells).

Statistical analysis

All data presented are mean values of triplicates. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparison test was done for statistical comparisons between non- and treated samples, using Graphpad InStat (Graphpad Software, Inc., CA). $p < 0.05$ was accepted as significant.

Results and discussion

Characterization of *MnSOD* in *P. minimum*

The full-length cDNA sequence of the *MnSOD* gene was determined from the dinoflagellate *P. minimum* (GenBank No. MK625099). It contained the dinoSL sequence (5′-TCC GTA GCC ATT TTG GCT CAA G-3′) at the start of the 5′-UTR (untranslated region) and a poly (A) tail at the end of 3′-UTR (Fig. 1). *PmMnSOD* cDNA was determined to be 1238 bp long, comprising a 906 bp ORF, 104 bp 5′-UTR, and 228 bp 3′-UTR. It encoded a protein of 301 amino acids with a predicted molecular mass of 32.2 kDa and an estimated isoelectric point of 5.89. Four putative manganese binding site residues (His89, His132, Asp223, and His227) and five specific active site residues (Gly127, Gly128, Phe135, Gln203, and Asp204) were detected (Fig. 1a). This is in

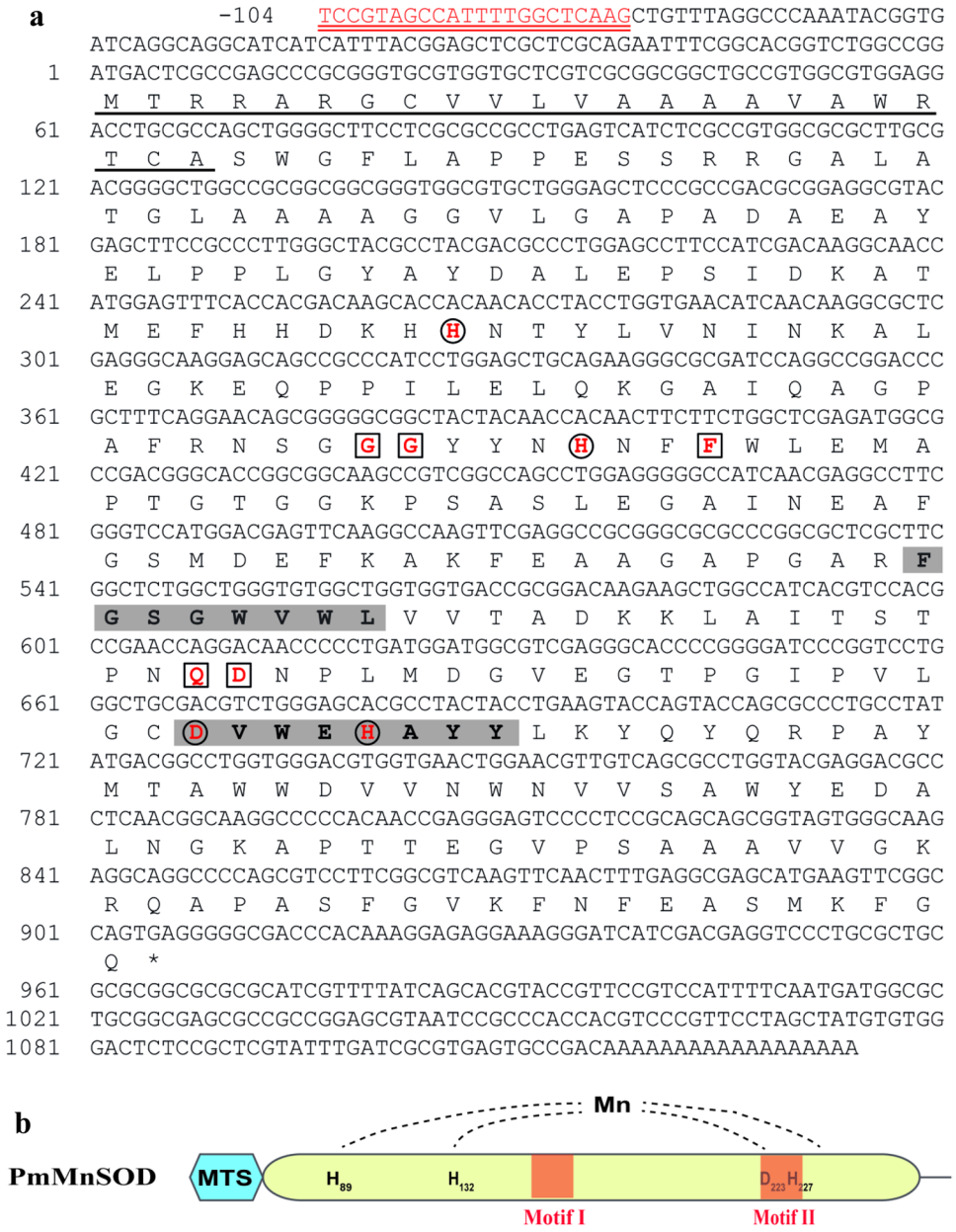


Fig. 1 The nucleotide and amino acid sequences of *PmMnSOD* (**a**) and signature motifs position (**b**). DinoSL sequence is marked in red and double underlines; two MnSOD signature motifs of PmMnSOD are highlighted as shaded gray and amino acids in bold; the signal peptide is underlined. The amino acids required for manganese (His89, His132, Asp223, and His227) in red and circle, and specific binding sites (Gly127, Gly128, Phe135, Gln303, and Asp304) binding are in red and box

accordance with the results of the green algae *Dunaliella salina* [20] and *Haematococcus pluvialis* MnSODs [22].

The MnSOD contains two conserved motifs defined as the Fe/MnSOD signature motifs [20, 23]; these two signature motifs, FGSGWVWL (from amino acids 180 to 187) and DVWEHAYY (from amino acids 223 to 230), were also identified in PmMnSOD. In addition, no putative transmembrane domain was found in the protein. However, we detected a putative mitochondrial targeting sequence (or transit peptide) of 23 amino acids, which can translocate the MnSOD protein into the mitochondrial matrix [19]. The amino acid composition of the transit peptide agrees with reports for other species [23, 38], in that it contains many basic, hydrophobic, and hydroxide-containing amino acids [39].

Sequence similarity and phylogenetic analysis

At the amino acid level, BLAST searches showed that PmMnSOD shared a high sequence similarity (71–73% identity and 88–99% coverage) with those of the dinoflagellate *Symbiodinium* sp., followed by the diatom *Fragilariopsis cylindrus* with 57% identity and 80% coverage (OEU17064). These demonstrate that the amino

acid sequences of MnSOD family are highly conserved (Fig. 2a), and that they may have similar functions in response to stress [20, 40].

In addition, the phylogenetic tree showed that PmMnSOD was clustered into one clade with those of the dinoflagellate *Symbiodinium* sp. (Fig. 2b). The dinoflagellate clade has a sister relationship with a group of diatoms (e.g., *Fragilariopsis cylindrus*, *Fistulifera solaris*, and *Thalassiosira oceanica*). These results showed that the amino acid sequences of MnSOD are highly similar (see Fig. 2a), suggesting that the protein are relatively well conserved.

The genomic coding structure of *PmMnSOD*

The genomic DNA sequence of *PmMnSOD* (MK625100) had a single intron in the coding genome which was 174 bp in length (Fig. 3). Our previous works have shown that dinoflagellates have few or no introns in the coding genome of antioxidant genes, including glutathione S-transferase [11], catalase-peroxidase [13], and *CuZnSOD* [25]. In addition, the dinoflagellate *C. polykrikoides* has a single intron in *Hsp90* coding genome [12]. This is in line with previous reports showing that many dinoflagellate genes possess very few or even lack introns [41, 42]. However, humans have four introns and the green algae *Spirogyra* contains five introns in the *MnSODs* [38, 43]. These results suggest that the gene structure in an organism's genome may vary distinctly among species.

Highly duplicated and/or sequence-conserved genes in dinoflagellates are present in tandem arrays [44, 45]. This might be a suitable mechanism to increase transcript and protein levels of certain genes in response to environmental changes [46]. In the case of dinoflagellates, actin in *Amphidinium carterae* contains at least two copies [42] and *HSP70* in *C. polykrikoides* has five copies in the coding genome [12]. Hence, we investigated *PmMnSOD* gene structure by using long PCR [12]; however, no intergenic region was found. This demonstrated that *PmMnSOD* might be present as a single copy or at different loci in chromosomes rather than in a tandem manner, such as human *MnSOD* [43].

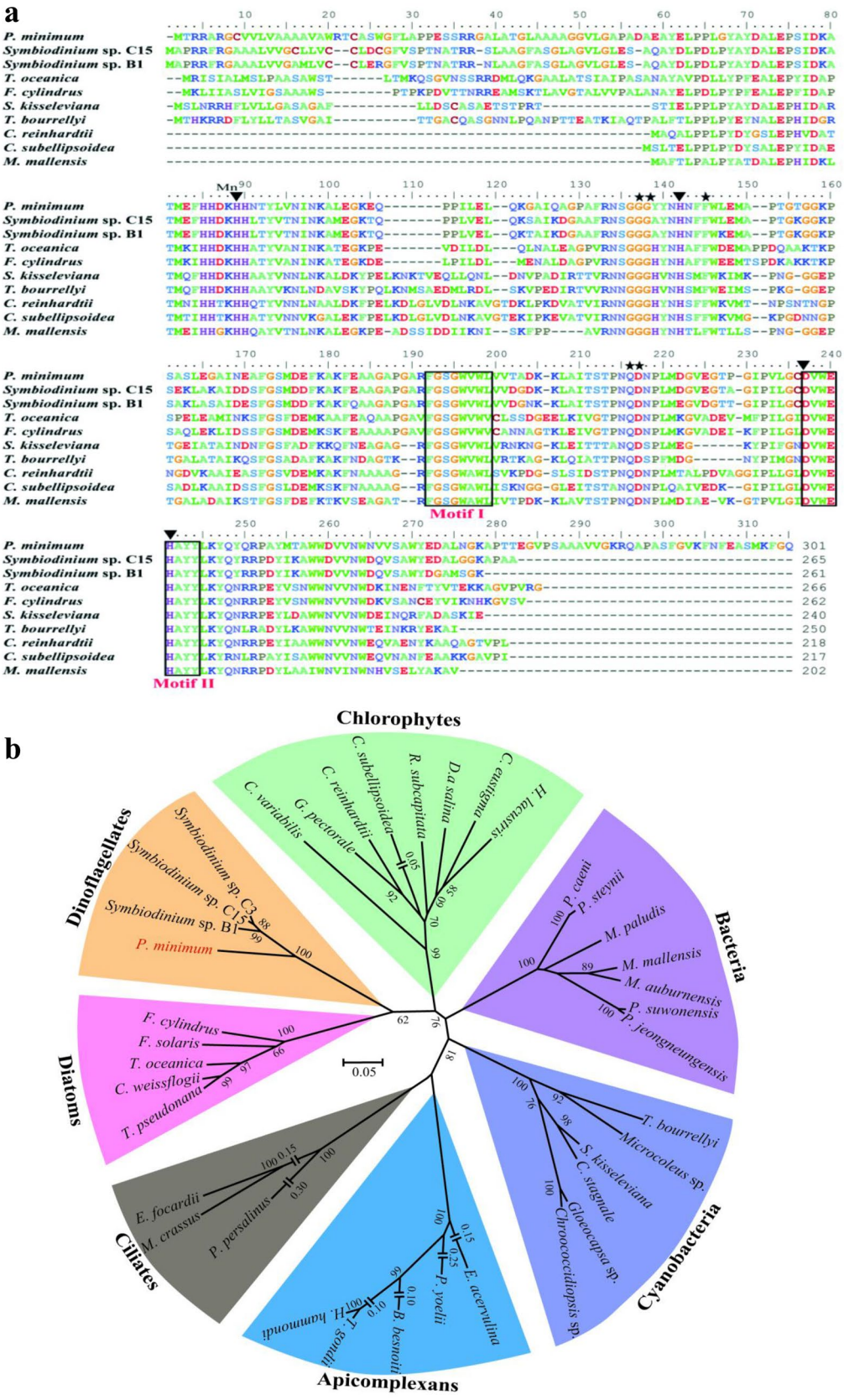
Effect of $CuSO_4$ and NaOCl on *PmMnSOD* transcription

MnSODs play crucial roles in antioxidant responses to environmental stress [20, 23]. However, there is little information about their responses in dinoflagellates. Hence, we investigated the transcriptional response of *PmMnSOD* to the ROS-inducing chemicals, $CuSO_4$ and NaOCl. As a result, the gene expression level of *PmMnSOD* initially increased, and then decreased with increasing $CuSO_4$ concentration (Fig. 4a). Transcript expression was induced at a low concentration of $CuSO_4$ (0.1 mg/L), while the maximum level of *PmMnSOD* expression was 2.8-fold higher at 1.0 mg/L. This up-regulated pattern was in accordance with previous studies on the chlorophyte *Chlamydomonas reinhardtii* [47], cyanobacteria *Microcystis aeruginosa* [48], and seagrass *Zostera muelleri* [49]. However, the expression patterns of *MnSOD* genes were different among these species when exposed to $CuSO_4$. *PmMnSOD* expression increased gradually up to high doses (2.0 mg/L of $CuSO_4$), whereas *C. reinhardtii MnSOD* expression increased and reached its highest levels at relatively low concentrations [47]. These results suggest that *MnSOD* is commonly involved in copper-induced gene regulation in dinoflagellates and other algae, but its expression pattern may depend on exposed doses and the species tested.

NaOCl, a disinfectant, affects the physiological and biochemical processes of organisms by damaging their intracellular molecules [50]. Overall, the expression pattern showed that the *PmMnSOD* response depended on the concentration of NaOCl (Fig. 4b). Transcript expression was not induced at lower doses (0.1–0.2 mg/L) of NaOCl, but significantly increased at higher doses (0.5–1.0 mg/L), with approximately 2.8- and 3.2-fold increases at 0.5 and 1.0 mg/L, respectively. Like the above results for copper exposure, the expression dropped at the highest concentration (2.0 mg/L), as lower doses of chlorine (i.e., NaOCl) can induce SOD expression but higher levels lead to RNA damage and cell death [51, 52]. Similarly, it has been observed that lower NaOCl concentrations activate higher gene expression in the marine dinoflagellate *C. polykrikoides* [12, 53, 54]. In addition, *MnSOD* expression was induced following exposure to elevated temperatures, hydrogen peroxide, or lead [16]. Taken together, *MnSODs* respond to a wide range of environmental stimuli and protect cells against oxidative stress by increasing its expression.

ROS accumulation, SOD activity and SOD roles in *P. minimum*

The physiological effects of $CuSO_4$ and NaOCl on the photosystems were assessed by measuring chlorophyll autofluorescence (CAF), which is an effective way to measure the efficiency of the photosynthetic apparatus in situ and photosynthetic responses to various stresses [55]. The CAF signal (red) exhibited similar results for both $CuSO_4$ and NaOCl treatment, decreasing with increasing dose (Fig. 5a, c). In addition, we evaluated oxidative stress by measuring ROS accumulation. The ROS levels (green signal) initially


a
P. minimum
Symbiodinium sp. C15
Symbiodinium sp. B1
T. oceanica
F. cylindrus
S. kisseleviana
T. bourrellyi
C. reinhardtii
C. subellipsoidea
M. mallensis
Mn
Motif I
Motif II
b
Chlorophytes
Dinoflagellates
Bacteria
Diatoms
Cyanobacteria
Ciliates
Apicomplexans
C. subellipsoidea
R. subcapitata
D.a salina
C. eustigma
H. lacustris
C. reinhardtii
G. pectorale
C. variabilis
Symbiodinium sp. C3
Symbiodinium sp. C15
Symbiodinium sp. B1
P. minimum
P. caeni
P. steynii
M. paludis
M. mallensis
M. aubrunensis
P. suwonensis
P. jeongneungensis
F. cylindrus
F. solaris
T. oceanica
C. weissflogii
T. pseudonana
T. bourrellyi
Microcoleus sp.
S. kisseleviana
C. stagnale
Gloeocapsa sp.
Chroococcidiopsis sp.
E. focardii
M. crassus
P. persalinus
E. acervulina
P. yoelii
B. besnoiti
T. gondii
H. hammondi
0.05

◂**Fig. 2** Multiple sequence alignment (**a**) and neighbor-joining tree (**b**) of PmMnSOD with other MnSOD proteins. The signature motifs were marked with black box. The highly conserved manganese binding residues are labeled by (filled inverted triangle) and specific active sites are labeled by (filled star). The location of present *P. minimum* is marked with a red color. Proteins used here were taken from GenBank database, and their accession numbers are provided in Supplementary Table 2. (Color figure online)

increased dramatically with increasing concentration, but decreased at higher doses of $CuSO_4$ (Fig. 5b) and NaOCl (Fig. 5d). Our observations are congruent with those in the green algae *C. ehrenbergii* when cells were exposed to $CuSO_4$ [56] and chlorine [57]. These results indicate that toxicants both stimulate oxidative stress within microalgae cells and damage intracellular components (e.g., chlorophyll content).

When adapting to increased oxidative stress, SOD levels (e.g., activity and protein amount) in plants and microalgae typically increase with the degree of stress [23, 47]. In the present study, we observed that SOD activity initially increased to its maximum level (~ 1.1 U/10^4 cells) and then decreased (Fig. 6), which corresponds to the production of ROS inside the cells following exposure to $CuSO_4$ and NaOCl. Similar to the present study, high SOD activity induced by copper has been well-documented [48, 58, 59]. However, there is little information about the effect of NaOCl on SOD activity in microalgae. A previous study by Ebenezer and Ki [60] reported an increase in SOD activity when the marine dinoflagellate *C. polykrikoides* was exposed to NaOCl. These results clearly showed that *P. minimum* was sensitive to oxidative stress caused by $CuSO_4$ and NaOCl, up-regulating SOD levels to scavenge the ROS.

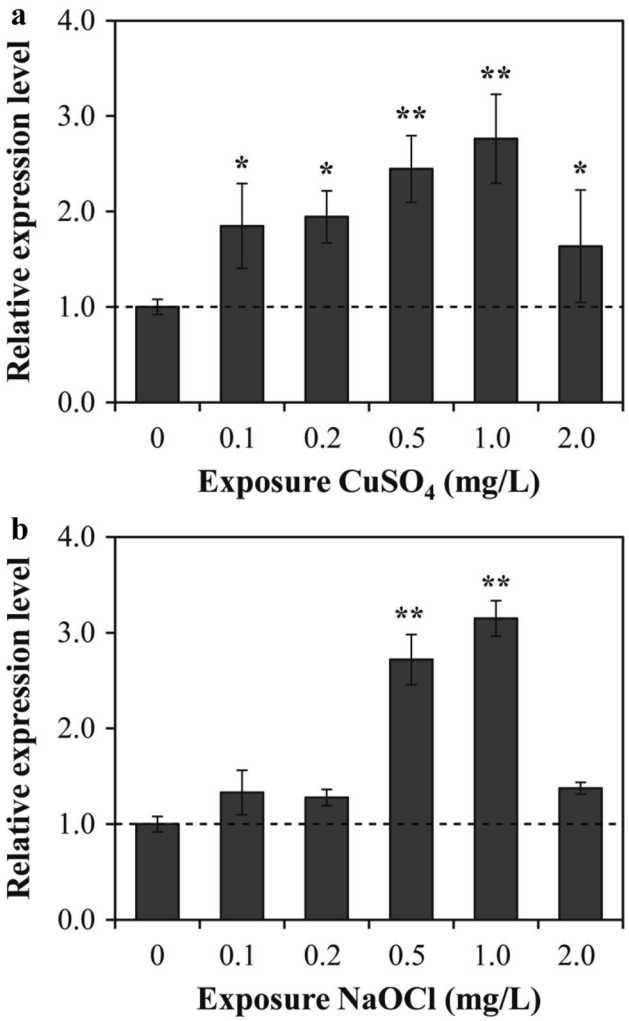


Fig. 4 Effects of $CuSO_4$ (**a**) and NaOCl (**b**) on the expression of *PmMnSOD*. Significant differences between the control and treated sample, as determined by one-way ANOVA, are highlighted $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

Implications of PmMnSOD in *P. minimum*

Figure 7 represents putative roles and the location of intracellular SOD in *P. minimum*, which is the general pathway reported by Sheshadri and Kumar [61]. The genetic structure of SODs varies according to their different cellular localizations [20]. In general, most MnSODs are located in the mitochondria or cytosol, but newly synthesized MnSODs stay in the nucleus in a precursor state and are translocated into the mitochondria or cytosol using signal peptides [23, 62], where they carry out their functions. In the present study, with stress induced by $CuSO_4$ and NaOCl, *P. minimum* produced free oxygen radicals, such as H_2O_2 and O^2-, which were toxic to *P. minimum* itself. This resulted in the production of ROS, destroying the

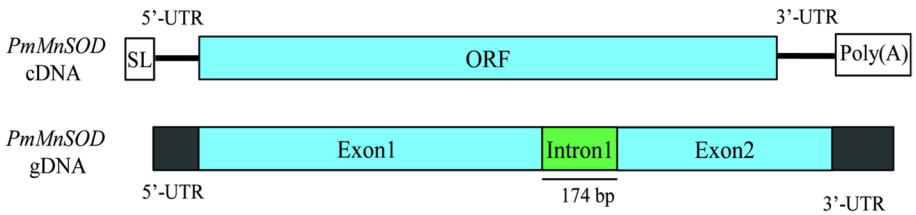


Fig. 3 Schematic structures of genomic DNA and cDNA of *PmCuZnSOD*

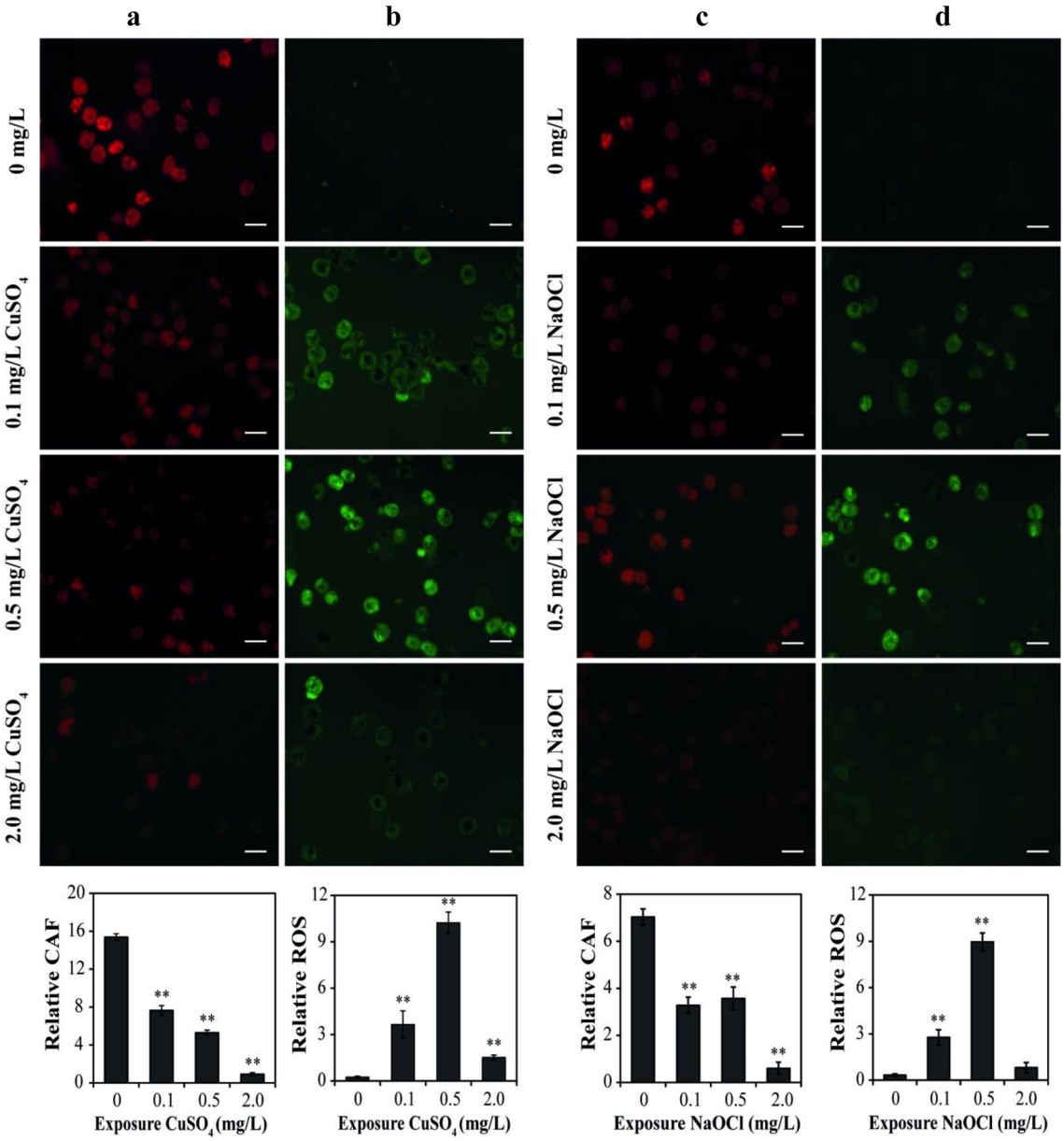


Fig. 5 The relative CAF level (**a**, **c**) and ROS production (**b**, **d**) of *P. minimum* after 24 h exposed to various $CuSO_4$ and NaOCl concentrations, respectively. Significant differences between the control and treated sample, as determined by one-way ANOVA, are highlighted $*p<0.05$; $**p<0.01$; $***p<0.001$. Scale bars represent: 20 µm

balance between their production and clearance, consequently undermining the cell. *PmMnSOD* expression was then induced and SOD activity increased accordingly. Apart from MnSOD, other SODs (i.e., CuZnSOD, FeSOD) and antioxidant enzymes, such as catalase (CAT) and glutathione peroxidase (GPx), are involved in counteracting

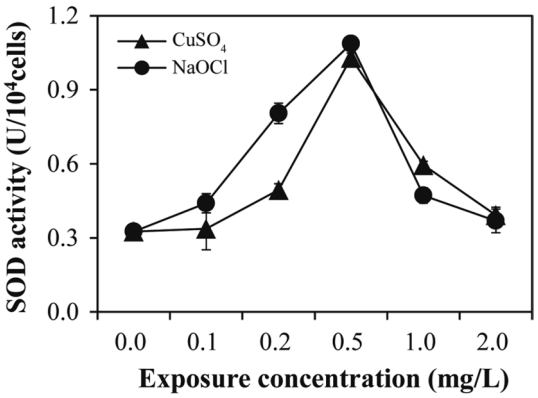


Fig. 6 Effects of $CuSO_4$ and NaOCl on the SOD activity of *P. minimum* after 24 h

oxidative stress within cells. Genetic research previously revealed the widespread presence of these antioxidant genes and suggested that they help microalgae to cope with challenges to their survival and development caused by environmental stressors [28, 63]. For instance, Guo and Ki [13] reported that the *KatG* gene of *P. minimum* functions in defence mechanisms associated with oxidative stress. This demonstrated that cells have evolved a protective strategy for eliminating oxidative stress caused by environmental stimuli.

In conclusion, this is the first study to characterize the gene structure, intracellular localization, and phylogenetic relationship of *MnSOD* in dinoflagellates, specifically *P. minimum*. Upon exposure to $CuSO_4$ and NaOCl, the dinoflagellate cells increased *PmMnSOD* expression and SOD activity to mitigate ROS over-production. Our findings will help in gaining a deeper understanding of *MnSOD* genes and their defensive roles in dinoflagellates.

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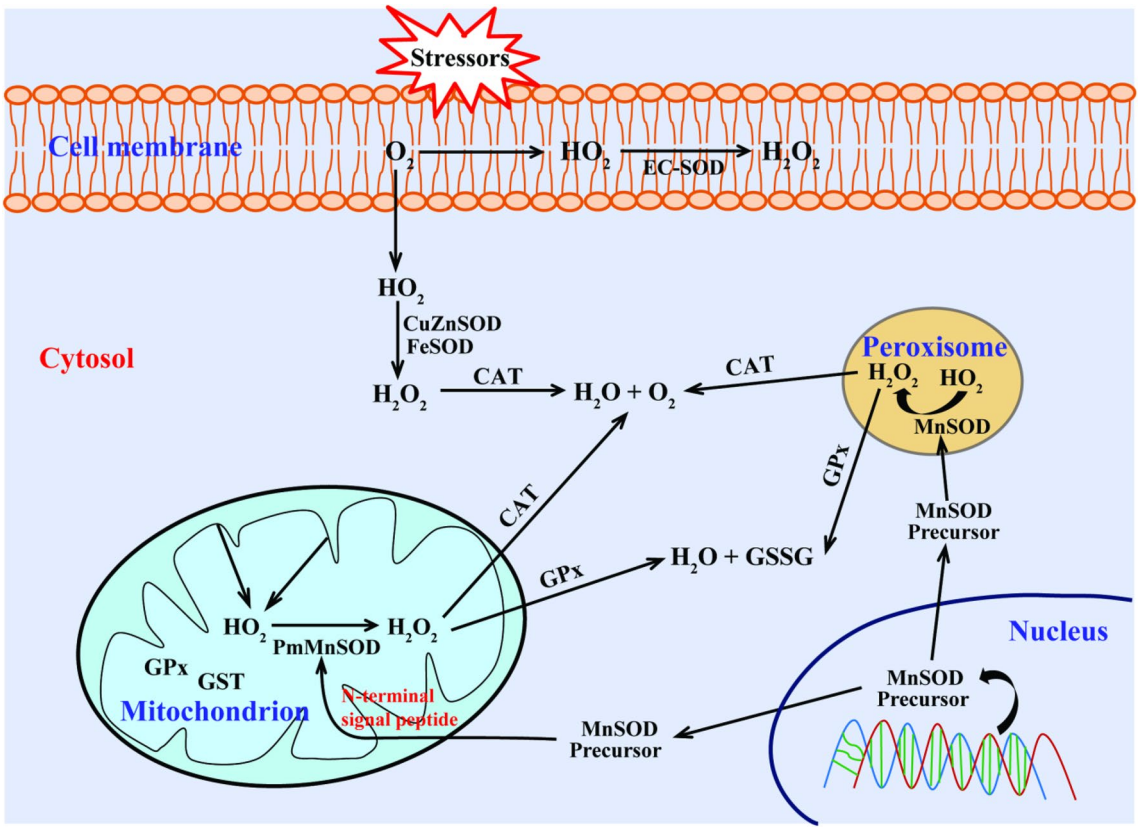

Fig. 7 A schematic representation of the SOD intracellular location and roles in *P. minimum*. The mode was adapted from Sheshadri and Kumar (2016). *CAT* catalase, *GPx* glutathione peroxidase, *GST* glutathione *S*-transferase, *GSSG* glutathione disulphide

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies conducted on human or animal subjects.

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