Molecular Immunology 93 (2018) 223–235

Contents lists available at ScienceDirect

Molecular Immunology

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

Identification and characterization of six peroxiredoxin transcripts from mud crab *Scylla paramamosain*: The first evidence of peroxiredoxin gene family in crustacean and their expression profiles under biotic and abiotic stresses

Dan-Dan Tu, Yi-Lian Zhou, Wen-Bin Gu, Qi-Hui Zhu, Bin-Peng Xu, Zhong-Kai Zhou, Ze-Peng Liu, Cong Wang, Yu-Yin Chen*, Miao-An Shu*

College of Animal Sciences, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Keywords:
Peroxiredoxin
Scylla paramamosain
Biotic stress
Abiotic stress
Molecular characterization
Peroxidase activity

ABSTRACTS

The peroxiredoxins (Prxs) define a novel and evolutionarily conserved superfamily of peroxidases able to protect cells from oxidative damage by catalyzing the reduction of a wide range of cellular peroxides. Prxs have been identified in prokaryotes as well as in eukaryotes, however, the composition and number of Prxs family members vary in different species. In this study, six *Prx*s were firstly identified from the mud crab *Scylla paramamosain* by RT-PCR and RACE methods. Six *Sp*Prxs can be subdivided into three classes: (a) three typical 2-Cys enzymes denominated as Prx1/2, 3, 4, (b) two atypical 2-Cys enzymes known as Prx5-1 and Prx5-2, and (c) a 1-Cys isoform named Prx6. The evolutionarily conserved signatures of peroxiredoxin catalytic center were identified in all six *Sp*Prxs. Phylogenetic analysis revealed that *Sp*Prx3, *Sp*Prx4, *Sp*Prx5 s and *Sp*Prx6 were clearly classified into Prx3-6 subclasses, respectively. Although *Sp*Prx1/2 could not be grouped into any known Prx subclasses, *Sp*Prx1/2 clustered together with other arthropods Prx1 or unclassified Prx and could be classified into the typical 2-Cys class. The comparative and evolutionary analysis of the *Prx* gene family in invertebrates and vertebrates were also conducted for the first time. Tissue-specific expression analysis revealed that these six *SpPrx*s were expressed in different transcription patterns while the highest expression levels were almost all in the hepatopancreas. Quantitative RT-PCR analysis exhibited that the gene expression profiles of six *SpPrx*s were distinct when crabs suffered biotic and abiotic stresses including the exposures of *Vibrio alginolyticus*, poly (I:C), cadmium and hypoosmotic salinity, suggesting that the *SpPrx*s might play different roles in response to various stresses. The recombinant proteins including the *Sp*Prx1/2, *Sp*Prx4, *Sp*Prx5-1 and *Sp*Prx6 were purified and the peroxidase activity assays indicated that all these proteins can reduce H_2O_2 in a typical DTT-dependent manner. To our knowledge, this is the first study about the comprehensive characterization of *Prx* gene family in *Scylla paramamosain* and even in crustaceans. These results would broaden the current knowledge of the whole *Prx* family as well as be helpful to understand and clarify the evolutionary pattern of *Prx* family in invertebrate and vertebrate taxa.

1. Introduction

Reactive oxygen species (ROS) are constantly generated in aerobic organisms during physiological metabolism process and innate immune defense reaction (Aguirre et al., 2005; Circu and Aw, 2010; Finkel, 1998; Halliwell and Cross, 1994). As normal metabolic intermediates, ROS play important roles in cell proliferation, differentiation, signaling transduction and immune functions (Bogdan et al., 2000; Ermak and Davies, 2002; Yu, 1994). However, excessive accumulation of ROS could cause serious oxidative damage to cell components including lipids, membranes, proteins and DNA, resulting in various diseases (Yu, 1994; Zhang et al., 2007, 2008). To avoid the cellular damage caused by ROS, all organisms are equipped with a wide range of antioxidant proteins to maintain the intracellular redox homeostasis, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxin (Prx) (Mittler et al., 2004).

Prxs are ancestral thiol-dependent selenium and heme-free peroxidases highly expressed in virtually all living species (Rhee et al., 2005;

* Corresponding authors.
E-mail addresses: chenyy@zju.edu.cn (Y.-Y. Chen), shuma@zju.edu.cn (M.-A. Shu).

https://doi.org/10.1016/j.molimm.2017.11.029
Received 24 July 2017; Received in revised form 23 November 2017; Accepted 28 November 2017

Wood et al., 2003), and have an entirely different role and catalytic mechanism to reduce the peroxides. During catalysis, the conserved N-terminal catalytic cysteine (Cys-SH) is oxidized to sulfenic acid (Cys-SOH), which then reacts with another cysteine residue (Cys-SH) to form a disulfide that is subsequently reduced by an appropriate electron donor like thioredoxin (Trx) (Hanschmann et al., 2013; Rhee and Woo, 2011). Although Prxs are evolutionarily conserved, species may possess different number of *Prx* genes. Mammalian cells express six different Prx isoforms: four 2-Cys Prx isoforms (Prx1-4), one atypical 2-Cys Prx isoform (Prx5), and one 1-Cys Prx isoform (Prx6), while five Prxs are present in *Saccharomyces cerevisiae* (Arockiaraj et al., 2012; Zheng et al., 2010). These isoforms vary in subcellular localization, with Prx1, Prx2, and Prx6 being localized mainly in cytosol (Rhee et al., 2005), Prx3 being restricted in the matrix of mitochondria, only Prx4 containing a signal peptide that serves for localization in the endoplasmic reticulum and secretion into the extracellular space, and Prx5 being in cytosol, mitochondria, and peroxisomes (Banmeyer et al., 2004; Knoops et al., 1999; Rhee et al., 2005).

Previous researches had demonstrated that Prxs can not only protect the organisms against various oxidative stresses, but also mediate the intracellular signal transduction (Kang et al., 2005; Rhee et al., 2005). Furthermore, the expression of *Prx* genes can be regulated when organisms faced with oxidative stress caused by hydrogen peroxide and pathogen infection, including bacterial, viral and parasite infections (Valero et al., 2015; Zhang and Lu, 2015). Moreover, changes in physical parameters such as temperature and salinity can also cause the difference of expression level of *Prx* genes (Tolomeo et al., 2016; Van Horn et al., 2010). Toxic metal exposure can also influence the expression of *Prx* genes (Poynton et al., 2007). All these results suggested that Prx family may play important roles in response to biotic and abiotic stresses.

The mud crab, *Scylla paramamosain*, mainly inhabits estuaries and coastal waters throughout the tropical to warm temperate zone in the South-east Asia regions (Imai et al., 2004). Notably, *S. paramamosain* is also one of the most important marine culture crabs in China (Yu et al., 2017) and its yearly aquaculture production had reached 141,000 tons in China (Fishery Bureau of Ministry of Agriculture of China, 2015). However, biotic stresses such as bacterial and virus infections are threatening the survival of mud crabs, causing high mortality and significant economic loss in commercial mud crab aquaculture (Escobedo-Bonilla et al., 2008; Lin et al., 2010; Yang et al., 2014). Moreover, the estuary mud crabs also suffer abiotic stresses including the exposures to heavy metal and fresh water which flux from land to ocean by estuary. Generally, all these biotic and abiotic stresses could result in significant increase and accumulation of ROS in crustacean (Chen and Chia, 1996; Duan et al., 2015; Junkunlo et al., 2016; Ma et al., 2013; Paital and Chainy, 2012; Piller et al., 1995; Robles et al., 2002; Thitamadee et al., 2014; Wang et al., 2013). Accordingly, it might be useful to fully investigate the relationships between the *Prx*s genes and biotic or abiotic stresses.

Up to date, *Prx* genes except *Prx3* had been cloned in several crustaceans. *Prx1/2* had been isolated in Chinese shrimp (*Fenneropenaeus chinensis*) (Zhang et al., 2007), kuruma shrimp (*Marsupenaeus japonicus*) (Bacano Maningas et al., 2008), flatback mud crab (*Eurypanopeus depressus*) (Van Horn et al., 2010), swimming crab (*Portunus trituberculatus*) (Chen et al., 2011), Indian white shrimp (*Fenneropenaeus indicus*) (Kiruthiga et al., 2012), oriental river pawn (*Macrobrachium nipponense*) (Sun et al., 2014) and black tiger shrimp (*Penaeus monodon*) (Bu et al., 2017). *Prx4* had been identified in black tiger shrimp (*Penaeus monodon*) (Qiu et al., 2010), kuruma shrimp (*Marsupenaeus japonicus*) (Chen et al., 2013), Chinese shrimp (*Fenneropenaeus chinensis*) (Zhang et al., 2014) and Pacific white shrimp (*Litopenaeus vannamei*) (Zeng et al., 2015). Moreover, *Prx5* had been amplified in freshwater prawn (*Macrobrachium rosenbergii*) (Arockiaraj et al., 2012), ridgetail white prawn (*Exopalaemon carinicauda*) (Duan et al., 2013), mud crab (*Scylla paramamosain*) (Tu et al., 2017) and red crayfish (*Procambarus clarkii*) (Wu et al., 2017), while *Prx6* had been cloned in Chinese mitten crab (*Eriocheir sinensis*) (Mu et al., 2009). However, little is known about the existence and stress response of the whole *Prx*s gene family under biotic and abiotic stresses in crustacean. In this study, six transcripts of the *Prx* family were identified in *Scylla paramamosain* and the comparison of the *Prx* gene family in invertebrates and vertebrates were conducted for the first time. The tissue expression profiles of *SpPrx*s, as well as their expression patterns under biotic and abiotic stresses including the exposures of *Vibrio alginolyticus*, virus-analog poly (I:C), cadmium and hypoosmotic salinity, were also investigated.

2. Materials and methods

2.1. Experimental animals

Healthy mud crabs (*Scylla paramamosain*, weighing 75 ± 10 g) were obtained from Sanmen Bay of Zhejiang Province (China) and maintained in PVC tanks supplied with a continuous flow of aerated artificial seawater (salinity at 18 ppt) at 28 ± 2 °C.

2.2. Total RNA extraction and cDNA synthesis

Target tissues were collected from different treatment groups, and immediately frozen in liquid nitrogen and stored at −80 °C for RNA isolation. Total RNA was extracted from tissues of mud crabs using RNAiso Plus (Takara) reagent following manufacturer's specifications. The purity and integrity were determined by analysis on agarose gel electrophoresis and measuring the absorbance at 260/280 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

All RNA samples treated with gDNA Eraser were subjected to syntheses cDNAs for real time PCR using PrimeScript™ RT reagent Kit (Takara). To synthesize first-strand cDNA for Rapid Amplification cDNA End (RACE) reactions, 1 μg RNA from hepatopancreas was reverse transcribed using reagents and a protocol provided in a SMARTer™ RACE cDNA Amplification Kit (Clontech). The first-strand cDNA synthesis was performed following the manufacture's instruction using 5′-CDS Primer A and SMARTer II A oligo (5′-RACE Ready cDNA) and 3′-CDS Primer A (3′-RACE-Ready cDNA). The first-strand cDNA was also synthesized using 3′ RACE Adaptor of 3′ Full RACE Core Set Ver. 2.0 (Takara) according to the manufacturer's protocol.

2.3. Isolation and sequencing of SpPrxs

The related gene-specific primers were designed based on the conserved sequences of *Prx*s genes in other species obtained from NCBI GenBank database. The full length of *Prx*s genes were cloned using the 3′- and 5′- RACE methods. The PCR products were separated on 1.0% (w/v) agarose gels, cloned into pMD19-T plasmid vectors (Takara), and the recombinant plasmids were transformed into DH5α chemically competent cell of *Escherichia coli* (Takara). The positive clones were screened by PCR and then sequenced. Primers were listed in Table 1.

2.4. Bioinformatic analysis of SpPrxs

Program BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was utilized to compare the obtained sequences to a sequence database. DNAStar software was used to find the open reading frames (ORF) and translate the DNA sequences into amino acids. The theoretical isoelectric point (pI) and molecular mass of the deduced protein were predicted by Compute pI/MW program (http://www.expasy.org/proteomics). The putative domains and mitochondrial targeting sequences were predicted by Pfam program (http://pfam.xfam.org/search#tabview=tab1) and MitoProt (https://ihg.gsf.de/ihg/mitoprot.html), respectively. Multiple sequence alignment was generated using the CLUSTALW program (http://www.genome.jp/tools/