Fish & Shellfish Immunology 37 (2014) 38–45

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Molecular characterization, immune response against white spot syndrome virus infection of peroxiredoxin 4 in *Fenneropenaeus chinensis* and its antioxidant activity

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form
26 December 2013
Accepted 29 December 2013
Available online 10 January 2014

Keywords:
Peroxiredoxin (Prx)
White spot syndrome virus (WSSV) infection
Recombinant protein
Antioxidant activity

ABSTRACT

Peroxiredoxins (Prx) are a family of antioxidant proteins and perform important functions in intracellular signal transduction. Here, we report a Prx gene from Chinese shrimp *Fenneropenaeus chinensis*. The full-length cDNA of *Fc*Prx gene contained an open reading frame of 735 bp encoding a polypeptide of 275 amino acids. The molecular mass of the deduced amino acid of *Fc*Prx is 27445.43 Da with an estimated pI of 5.71. Sequence comparison showed that the *Fc*Prx shares high identities with Prx IVs and it was named *Fc*Prx4. A real-Time PCR (qRT-PCR) assay was developed to assess the mRNA expression of *Fc*Prx4 in different tissues and temporal expression in hemocytes and hepatopancreas of *F. chinensis* challenged by white spot syndrome virus (WSSV). Transcripts of *Fc*Prx4 can be detected in all tissues examined. The expression of *Fc*Prx4 showed significant up-regulation in shrimp hemocytes and hepatopancreas after artificial infection with WSSV. A fusion protein containing *Fc*Prx4 was produced in vitro and was confirmed by Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) assay. And activity analysis indicated that the recombinant *Fc*Prx4 proteins can reduce H_2O_2 in the presence of dithiothreitol.

1. Introduction

Hemocytes play significant role in invertebrate innate defense reactions. When the organism is attacked by microorganisms or viruses, invertebrate hemocytes can remove pathogens by phagocytosis [1]. In the process of phagocytosis, the host's NADPH-oxidase gets activated and results in the production of a mass of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) [2]. Though the production of ROS is an important host defense mechanism for killing invading pathogens, the mass accumulation of these reactive molecules in hosts will cause serious cell damage and more pathogen replication [3,4]. Organisms have evolved protective enzymatic systems, including superoxide dismutase, catalase, and many kinds of peroxidases to reduce and regulate the level of ROS [5,6]. Among the peroxidases, peroxiredoxins (Prx), also named thioredoxin peroxidase (TPx), play a particularly central role in elimination of hydroperoxide with thioredoxin as an immediate hydrogen donor [7].

Prx is a selenium independent peroxidase protein, and it is a large family of antioxidant proteins ubiquitously found in all living organisms from prokaryotes to eukaryotes [8]. In the past decades, Prxs have received increasing attention for their distinctive characteristics in catalytic activity and functioning as chaperonins [9–11]. *Saccharomyces cerevisiae* cytosolic TPx I was the first peroxiredoxin isolated from a eukaryotic cell [9]. Subsequently, the presence of multiple isoforms of Prxs and their characterization were reported in mammalian cells. In mammals, Prxs are divided into six subtypes with Prx1–4 belonging to the typical 2-Cys type, Prx5 to the atypical 2-Cys type and Prx6 to the 1-Cys type [12].

Prxs have been shown to be participating in immune response following pathogen infection in many species including fly, yellow croaker and human [13–15]. Recently, different isoforms of Prx have been found in some species of crustacean such as *Fenneropenaeus chinensis* [16], *Marsupenaeus japonicus* [17], *Eriocheir sinensis* [18], *Eurypanopeus depressus* [19], *Penaeus monodon* [20], *Litopenaeus vannamei* [21], *Fenneropenaeus indicus* [22], and *Macrobrachium rosenbergii* [23]. The research results of the literature

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http://dx.doi.org/10.1016/j.fsi.2013.12.026

Table 1
Primers used in the experiments.

Primer name	Primer sequences
*Fc*Prx4-F1	5′-CCTCGTCTTCTTCTTCTACC-3′
*Fc*Prx4-R1	5′-TCATCTTGCGGAACTCCT-3′
*Fc*Prx4-RT-F	5′-CCGCAAGATGAACACAGA-3′
*Fc*Prx4-RT-R	5′-ACACCGTAGTCCTGAGAG-3′
*Fc*Prx4-EF	5′-GCTAGCATGGATCTGCGGGCGGTT-3′
*Fc*Prx4-ER	5′-GAATTCGATGACCAACCGAAAGAAC-3′
18S–F	5′-GCCTGAGAAACGGCTACCAPRXC-3′
18S-R	5′-GTAGTAGCGACGGGCGGTGTGT-3′
BDA-oligo	5′-AAGCAGTGGTATCAACGCAGAGTACGCGGG-3′
AOLP	5′-CCACGCGTCGACTAGTAC(T)16(A/C/G)-3′
NUP	5′-AAGCAGTGGTATCAACGCAGAGT-3′
AP	5′-GGCCACGCGTCGACTAGTAC-3′

The underlined characters show the sequences recognized by the restriction enzyme *Nhe* I and *EcoR* I, respectively.

above-mentioned also revealed the fact that Prxs were involved in the immune reactions against pathogen infection in crustaceans.

In the present study, we report the molecular cloning and characterization of a Prx4 homologue (*Fc*Prx4) from *F. chinensis*. The expression profiles of *Fc*Prx4 mRNA in hemocytes and hepatopancreas of shrimp after infection with white spot syndrome virus (WSSV) were studied, and the enzyme activity of recombinant *Fc*Prx4 protein was assayed.

2. Materials and methods

2.1. Shrimp and immune challenge

Chinese shrimp *F. chinensis*, about 16 g in body weight, were purchased from a local shrimp farm and reared in 8 m^3 fiber glass

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1   GTG GGC CGG TCT GTG GAT GAG CCC CTG CGT CTG GTT CAG GCC TTC CGG CTT GCG GTG GAA 60
61  GGA GCA GCA AGG AGA ATC CTA GAA GAA ATA GGG GGG AGT TGA GAG CAA GAT GCA GAT CAG 120
121 AGG GAG AAG TTG AGA GTA ATG GGA TCC TCC AGT CCC GTG CGA AAT CGA CGC CGG CCA TCT 180
181 CCA CCG CCA GCC CGG CCC GTC CAG CTG AAC CAA AAC ACA AGC TCA AAG CCC GAA TTT CCT 240
241 CCG CAA AAC GAC CCT CTT TTA AGC CCC TTC GCG ATG GAT CTG CGG GCG GTT TTG GCG ACA 300
1                                           M  D  L  R  A  V  L  A  T  9
301 GCG CTG TGC CTG AGT GTG GGC GTG ATG GGT GCG GCG CCG GAA GAG CAA TGC CAT ACG TTC 360
10  A  L  C  L  S  V  G  V  M  G  A  A  P  E  E  Q  C  H  T  F  29
361 GCG GGC GGT GCA GTT TAC CCA AAT ACA GAA GGC AGG GCT TCG GGA CAC AGC CTG CAG TGG 420
30  A  G  G  A  V  Y  P  N  T  E  G  R  A  S  G  H  S  L  Q  W  49
421 ACC AAA GCC ATG ATC AGC AGA CCG GCC CCA GTG TGG GAA GGC ACT GCG GTC ATC GAC GGA 480
50  T  K  A  M  I  S  R  P  A  P  V  W  E  G  T  A  V  I  D  G  69
481 GAG TTC CGT GAG CTC AAG CTT AAG GAT TAC CGC GGG AAA TAC CTC GTC TTC TTC TTC TAC 540
70  E  F  R  E  L  K  L  K  D  Y  R  G  K  Y  L  V  F  F  F  Y  89
541 CCC TTG GAC TTT ACA TTT GTC TGC CCA ACG GAG ATC CTG GCT TTC AAT GAC CGC GTT GAG 600
90  P  L  D  F  T  F  V  C  P  T  E  I  L  A  F  N  D  R  V  E  109
601 GAG TTC CGC AAG ATG AAC ACA GAG GTC GTG GCC TGT TCC ATA GAC TCG CAT TTT ACC CAT 660
110 E  F  R  K  M  N  T  E  V  V  A  C  S  I  D  S  H  F  T  H  129
661 CTT GCT TGG ACT AAC ACG CCA CGC AAG GAT GGT GGC CTG GGA AAG CTC AAG ATC CCA CTG  720
130 L  A  W  T  N  T  P  R  K  D  G  G  L  G  K  L  K  I  P  L  149
721 CTG TCT GAC ATC ACC CAC AAG ATC TCT CAG GAC TAC GGT GTC TAC CTA GAG GAC CAA GGC 780
150 L  S  D  I  T  H  K  I  S  Q  D  Y  G  V  Y  L  E  D  Q  G  169
781 ATT GCC CTC AGG GGC CTG TTC ATC ATT GAT GAC AAG GGA GTA CTG AGA CAG ATC ACC ATG 840
170 I  A  L  R  G  L  F  I  I  D  D  K  G  V  L  R  Q  I  T  M  189
841 AAC GAC CTC CCT GTG GGC CGG TCT GTG GAT GAG ACC CTG CGT CTG GTT CAG GCC TTC CAG 900
190 N  D  L  P  V  G  R  S  V  D  E  T  L  R  L  V  Q  A  F  Q  209
901 TTC ACA GAC CAG CAT GGG GAA GTC TGC CCA GCC GGA TGG AAG CCT GGG GAT GAC ACG ATC 960
210 F  T  D  Q  H  G  E  V  C  P  A  G  W  K  P  G  D  D  T  I  229
961 ATC CCC AAC CCA GAA GAG AAA CTC AAA TAC TTC AAG AAA GCC AAC CAG TAG GGC CAG TCC1020
230 I  P  N  P  E  E  K  L  K  Y  F  K  K  A  N  Q  *                    245
1021AGC TGT TGC ATG GGG TTG CAA AAC AGG CTT GCG GTG GAA GGA GCA GCA AAG AGA ATC CTA 1080
1081GAA GAA ATA GGG GGA AGT TGA GAG CAA GAT GAG GAA AGC AGA TCA GAG GGA GAA GTT GAG 1140
1141AGC AAT GGG TTC CTC CAG TCC CGT GCG AAA TCG AGT GGA TTT GGT GGC GGT GGT GAT GAT 1200
1201CGC TTC TTA AGA GTG TTC TGT AGA CGC ATT TTC GAG GAT GTA CAA AAC CCG GTG ATG GGA 1260
1261CTA TTG GAG TAT GTC ATG AGG AAA TTT GAT GCT AAA GTG ATT TGT TGT TGT TGT TTT TTT 1320
1321GGT TGT TTG TTT TTG ATG ATT TTG TTG ATA TAT CTG GGT TGA TGA TGA CTG GTG ATG TTT 1380
1381TTG CAT ACA GGT ATT TGA ATT TTG TTT AGA TGT AAG AAT AAA GCT TTA TTT TTT CAT AAA1440
1441TAT TTT AAT GTG TTC AAT AGC AAG ACA GTT ATA TTT ATT ACA TTC CTA GTG CAA TTA AAC 1500
1501ATG TCA AAA TCA GCA ATA AAG CCA GAC TTT AGA CTC ATA TTG ATT TTG TCA TAC ATT CCA 1560
1561ATT GTA AAT AAA AAA AAA AAA AAA 1584
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Fig. 1. The complete nucleotide and deduced amino acid sequence of *Fc*Prx4 from Chinese shrimp *F. chinensis*. The letters in boxes indicate the start codon (ATG) and the polyadenylation signal sequence (AATAAA and AATTAAA). The Prx signature motifs (FYPLDFTFVCPTEI and GEVCPA) are shaded.