Fish & Shellfish Immunology 34 (2013) 556–563

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

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

Miiuy croaker (*Miichthys miiuy*) Peroxiredoxin2: Molecular characterization, genomic structure and immune response against bacterial infection

Liping Ren, Tianjun Xu, Rixin Wang, Yuena Sun*

Laboratory for Marine Living Resources and Molecular Engineering, College of Marine Science, Zhejiang Ocean University, Zhoushan 316000, PR China

ARTICLE INFO

Article history:
Received 27 September 2012
Received in revised form
28 November 2012
Accepted 30 November 2012
Available online 16 December 2012

Keywords:
Miiuy croaker
Peroxiredoxin2 (Prx2)
Molecular cloning
Genomic structure
Gene expression

ABSTRACT

Peroxiredoxin2 (Prx2) protein is an important member in cellular antioxidant protein superfamily. Prx2 exists widely in prokaryotes and eukaryotes, it not only plays a part in eliminate reactive oxygen, but also takes effect in many other metabolic activities, such as stimulate epithelial cell proliferation, participate in the signal transduction in cells and so on. After molecular cloning we got that the complete cDNA sequence of Prx2 consists 882 bp, including a 5′-UTR of 46 bp, an open reading frame (ORF) of 591 bp, and a 3′-UTR of 245 bp. The complete gene of miiuy croaker Prx2 has 5 exons and 4 introns. The deduced 197 amino acid residues of miiuy croaker Prx2 consists a Val-Cys-Pro (VCP) motifs. In order to better elucidate the immune mechanisms of the Prx2 in the lower vertebrates, we conducted a research about the Prx2 gene of miiuy croaker and its expression pattern after bacterial infection. Real-time PCR (RT-PCR) results showed that expression of Prx2 was up-regulated in kidney, liver and spleen under infection with *Vibrio anguillarum*, and expressed level differently in ten different uninjected tissues. Our results suggested that Prx2 might be involved in immune defence in miiuy croaker.

1. Introduction

Peroxiredoxin (Prx) is a selenium independent peroxidase protein, it is great different in catalytic activity and protein sequence with other antioxidant. Prx proteins, initially characterized in yeast, constitute a family of antioxidant enzymes with no homology with conventional antioxidant proteins. They were first named thioredoxin peroxidases because they disassembled H_2O_2 to water using thioredoxin as an intermediate electron donor, however, later renamed for Prxs not requiring thioredoxin [1]. Prx proteins played important roles in some cellular functions. Such as involved in protein and lipid protection against oxidative injury [2], cell proliferation, differentiation and intracellular signaling pathways regulating apoptosis [3]. According to the amount of conservative homocysteine, this Prx superfamily had been divided into two main groups, namely 1-Cys Prx and 2-Cys Prx [4]. These two groups were further divided into six subtribes, namely Prx1, Prx2, Prx3, Prx4, Prx5 and Prx6. There was a highly conservative homocysteine locating in the 52 site of the former class. While the later's highly conservative homocysteine were located in the site of 51 and 172. Prx proteins could eliminate the metabolic peroxide or superoxide through the thioredoxin. That was essential for organisms' surviving. Even though other functions, such as participating in cell proliferation and differentiation, enhancing the activity of NK cells [5–7], protecting free radicals sensitive proteins, participating the metabolism of the hemoglobin, participate in signal cascade amplification of cell factors and so on.

Up to now, many researches had implied that Prx proteins were involved in the immune response. For example, the study of PrxV in bay Scallop *Argopecten irradians*, the expression analysis of PrxV indicated that PrxV was involved in the immune response of bay scallops [8]. As to the study of Prx6 from Chinese mitten crab *Eriocheir sinensis*, the results showed that Prx6 was involved in responses against bacterial infection, and also indicated its functional importance in the immune system of *E. sinensis* [9].

Prx2, the target we studied in this experiment is a member of peroxiredoxin family belonging to the Prxs group. It was showed that Prx2 protein had a positive reaction in human metabolism from current studies [10]. Prx2 expressed abundantly in the erythrocyte [11], and its absence in knock-out mice gives rise to hemolytic anemia. These studies demonstrated that Prx2 protein was greatly efficient at clearing away H_2O_2 inductively in the

* Corresponding author. College of Marine Science, Zhejiang Ocean University, No. 105 Wenhua Road, 316000 Zhoushan, Zhejiang province, PR China. Tel.: +86 580 2550826.
E-mail address: yuenasun@163.com (Y. Sun).

1050-4648/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsi.2012.11.042

erythrocyte. The cysteine at the active site on one subunit was oxidized to a sulfenic acid when Prx2 protein reacted with peroxide [12]. A second conserved cysteine at the C-terminal end of the resolving cysteine then reacted with the sulfenic acid to form a disulfide bridge. Reduction of the disulfide by thioredoxin regenerated Prx2 protein and completed the cycle. An interesting trait of mammalian 2-Cys Prxs was when peroxide was at a high level, the peroxidatic Cys changed overoxidized to the sulfinic (SO_2H) or sulfonic (SO_3H) acid form [13].

Miiuy croaker, *Miichthys miiuy* is one of the most important elements in the fishery market both in the economic value and nutritional value [14]. Miiuy croaker is mainly distributed from the western Japan to the East China Sea. In China especially in the coast cities, miiuy croaker is very popular not only for its great taste but also its high nutrition and medicinal value. Today, infectious diseases is an important factor that depresses miiuy croaker industry greatly, such as, *Vibrio anguillarum* was one of the conditional pathogens which did harm for the fish industry. Therefore, learning how to improve the disease resistance of miiuy croaker is essential for fishery production. To improve fish health, a better understanding of the teleost immune system is required. An important step toward deciphering immune mechanisms is the identification, characterization and function analysis of genes that are regulated in response to vaccination and pathogen exposure.

2. Materials and methods

2.1. Sample and experiments preparations

Healthy miiuy croaker were collected from Zhoushan Fisheries Research Institute (Zhejiang, China). The collected fish were fed twice daily and were kept in aerated water tanks with a flow-through seawater supply at ambient temperature and under a natural photoperiod for at least one week.

For the stimulation experiment, *V. anguillarum* was cultured in the 2216E media at 28 °C, and then 1 ml resuspended bacteria was injected miiuy croaker after centrifugation to approximately 3.0×10^7 CFU/ml in phosphate-buffered saline. The infected health fish were killed at 6 h, 12 h, 24 h, 36 h, 48 h, and 72 h respectively. Uninfected fish were still kept in separate tanks as control. To examine the expression of Prx2 in miiuy croaker, ten uninfected tissues samples (liver, spleen, kidney, intestines, heart, muscle, stomach, brain, fin, gill) and three infected tissues samples (liver, spleen, kidney) were collected from miiuy croaker. The samples were then immediately frozen in liquid nitrogen after dissection and then separately stored at −80 °C prior to RNA extraction.

2.2. DNA and RNA extraction, cDNA synthesis

Genomic DNA isolated from samples using the standard phenol–chloroform method [15]. The isolated DNA was assessed by agarose gel electrophoresis and finally stored at −20 °C for future use. For the expression of miiuy croaker Prx2 experiment, total RNA was extracted using Trizol reagent (Qiagen) according to the manufacturer's instructions from various tissues and the products were analyzed in a 1.5% agarose gel. The cDNA was synthesized using Quantsript RT kit (Qiagen) according to the instructions of manufacturer and the reaction carried out without the template was used as blank, cDNA was stored at −20 °C for future experiment.

2.3. Primer design, PCR amplification and cloning

To obtain the complete sequence of miiuy croaker Prx2, five pairs of primers (Prx2-intron1 F/R, Prx2-intron2F/R, Prx2-intron3F/R, Prx2-intron4F/R, Prx2-intron 5F/R) were designed based on complete mRNA sequence (Table 1). The position of the introns was sited according to the known sequence of vertebrate species form Genebank. In addition, two pairs of primers (Prx2-RT-F/R and

Table 1
Primers used in this study.

Primer name	Sequence (5′–3′)
Prx2-intron1F	CAACACTAAGATTGGCAAGTC
Prx2-intron1R	AGCGGGTAGAAGAAGAAGAT
Prx2-intron2F	AATATGGGCTGCGAGGTTAT
Prx2-intron2R	GGAGATGGACTTGGTAAGGT
Prx2-intron3F	GAGGCAGATCACCATCAATG
Prx2-intron3R	GTTAGGAAGTCAGCTAGGAGA
Prx2-intron4F	TTACCAAGTCCATCTCCAGAG
Prx2-intron4R	CCCACAGGCAAGTCATTGAT
Prx2-intron5F	CTGACAAATTCGGAGAAGTT
Prx2-intron5R	AAGAGAAGTGCTACGGTTAG
Prx2-RT-F	GGGGCTTCTCGGCTTTACGTGCAGA
Prx2-RT-R	TCTGCACGTAAAGCCGAGAAGCCCC
β-actin-RT-F	GTGATGAAGCCCAGAGCA
β-actin-RT-R	CGACCAGAGGCATACAGG

Table 2
Prxs sequences and relative information of other species for sequence analysis.

Species	Transcript IDs	Prxs class	Length (bp)	Protein (aa)
Gorilla	ENSGGOT00000005437	I	600	199
Human	ENST00000319248	I	1021	199
Cow	ENSBTAT00000004751	I	889	199
Hedgehog	ENSEEUT00000000378	I	513	171
Armadillo	ENSDNOT00000018487	I	513	171
Coelacanth	ENSLACT00000016328	I	846	127
Cod	ENSGMOT00000013016	I	585	194
Medaka	ENSORLT00000013205	I	582	171
Cod	ENSGMOT00000006388	II	654	218
Medaka	ENSORLT00000018208	II	835	205
Hedgehog	ENSEEUT00000008088	II	585	194
Armadillo	ENSDNOT00000005119	II	373	124
Coelacanth	ENSLACT00000000522	II	483	109
Gorilla	ENSGGOT00000034213	II	1007	198
Human	ENST00000301522	II	987	198
Cow	ENSBTAT00000015996	II	1013	199
Cod	ENSGMOT00000006622	III	660	219
Medaka	ENSORLT00000017891	III	799	251
Chicken	ENSGALT00000015262	III	1443	257
Armadillo	ENSDNOT00000001359	III	582	193
Coelacanth	ENSLACT00000016741	III	1565	254
Gorilla	ENSGGOT00000030124	III	1582	256
Human	ENST00000298510	III	1584	256
Cod	ENSGMOT00000002617	IV	663	221
Medaka	ENSORLT00000008268	IV	814	258
Coelacanth	ENSLACT00000002451	IV	971	269
Anole lizard	ENSACAT00000007498	IV	822	273
Chicken	ENSGALT00000026387	IV	1406	288
Hedgehog	ENSEEUT00000013407	IV	792	263
Gorilla	ENSGGOT00000032416	IV	935	271
Human	ENST00000379341	IV	1005	271
Cow	ENSBTAT00000008107	IV	972	274
Anole lizard	ENSACAT00000006412	V	651	216
Cod	ENSGMOT00000015001	V	477	158
Coelacanth	ENSLACT00000020514	V	1888	190
Armadillo	ENSDNOT00000004646	V	477	157
Cow	ENSBTAT00000011403	V	869	219
Gorilla	ENSGGOT00000001345	V	829	214
Human	ENST00000265462	V	893	214
Anole lizard	ENSACAT00000015555	VI	675	224
Cod	ENSGMOT00000018680	VI	672	224
Medaka	ENSORLT00000012564	VI	699	233
Coelacanth	ENSLACT00000018243	VI	2588	224
Armadillo	ENSDNOT00000004168	VI	675	224
Hedgehog	ENSEEUT00000004020	VI	675	224
Cow	ENSBTAT00000006383	VI	1667	224
Gorilla	ENSGGOT00000027489	VI	1691	227
Human	ENST00000340385	VI	1751	224