Fish & Shellfish Immunology 38 (2014) 340–347

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Short communication

Molecular cloning, tissue distribution and expression analysis of a manganese superoxide dismutase in blunt snout bream *Megalobrama amblycephala*

Shengming Sun [a], Jian Zhu [a,*], Xiaojun Jiang [b], Bing Li [a], Xianping Ge [a,b,*]

[a] Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, PR China
[b] Wuxi Fishery College Nanjing Agricultural University, Wuxi 214081, PR China

ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form
19 March 2014
Accepted 30 March 2014
Available online 13 April 2014

Keywords:
Megalobrama amblycephala
Mitochondrial manganese superoxide dismutase
Nitrite
Transcriptional analysis

ABSTRACT

The full-length mitochondrial manganese superoxide dismutase cDNA of blunt snout bream *Megalobrama amblycephala* (denoted as MamMnSOD) was identified in liver using homology cloning and rapid amplification of cDNA ends. The full-length cDNA of MamMnSOD consisted of 986 bp, with an open reading frame encoding 224 amino acids, a 58-bp 5′ untranslated region and a 256-bp 3′ untranslated region. The deduced amino acid sequences of MamMnSOD showed high sequence homology to mitochondrial MnSODs from crustaceans. Several motifs, including three mitochondrial MnSOD signatures, amino acid residues responsible for coordinating the manganese, and the putative active center, were almost completely conserved in the deduced amino acid sequences of MamMnSOD. The mRNA expression of MamMnSOD in the tissues of heart, liver, spleen, kidney, muscle, intestine, and gill was examined by quantitative real-time PCR; the highest expression was in the liver. Transcription of MamMnSOD was kinetically modulated in response to nitrite stress in liver and gill tissues. The purified recombinant MamMnSOD showed potent antioxidant activity. Polyclonal antibodies generated from the recombinant product of MamMnSOD were used to specifically identify the native protein in liver of *M. amblycephala*. Collectively, the findings of this study strongly suggested that MamMnSOD combats oxidative stress and cellular damage induced by nitrite, by detoxifying harmful reactive oxygen species in *M. amblycephala*.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The generation of reactive oxygen species (ROS) and intermediates is an unavoidable consequence of aerobic metabolism in most aerobic organisms and an excess of ROS can lead to oxidation, loss of cell function, damage of cell membranes, and ultimately apoptosis or necrosis [1–4]. Organisms have well-developed defense systems against ROS, involving both limiting the formation of ROS and instituting its removal [5]. Prolonged nitrite exposure resulted in the formation of excess ROS that caused oxidative stress, the formation and removal of ROS are in balance. However, the excess ROS produced in living organisms under stress conditions has the potential to damage key cellular components, including lipids, proteins, and DNA [6]. To balance the harmful and positive effects of ROS production, organisms have evolved antioxidant systems to maintain oxygen radicals at optimal concentrations [7,8].

Superoxide dismutases (SOD, EC 1.15.1.1) are a family of antioxidant enzymes that catalyze the dismutation of superoxide into hydrogen peroxide and oxygen [9]. They are classified into three distinct groups according to their metal cofactor: copper and zinc SODs (Cu- and Zn-SOD), manganese and iron SODs (Mn- and Fe-SOD), and nickel SOD (Ni-SOD) [10,11]. Mn-SOD, which contains a manganese ion, is found mostly in the mitochondrial matrix (mMn-SOD) [12], but some cytosolic Mn-SOD (cMn-SOD) has also been found in the mitochondrial matrix [13]. Given that at least 90% of generated ROS originate from mitochondria in most vertebrate species [14,15], we believe that Mn-SOD, a mitochondrial protein, plays an important role in host defense against oxidative stress in

* Corresponding authors. Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, No. 9 East Shanshui Road, Wuxi Jiangsu 214081, PR China. Tel.: +86 510 85557892.
E-mail addresses: sunsm@ffrc.cn (S. Sun), zhuj@ffrc.cn (J. Zhu), gexp@ffrc.cn (X. Ge).

http://dx.doi.org/10.1016/j.fsi.2014.03.036
1050-4648/© 2014 Elsevier Ltd. All rights reserved.

various species, including crustaceans. More importantly, it has also been demonstrated that this protein plays an important role in promoting cellular differentiation and tumorigenesis [16], in immune responses induced by bacteria, virus infection, hypoxia, and heavy-metal exposure [17–21].

At present, molecular cloning and functional expression of SODs represent an important focus of the fish research community. To date, the Mn-SOD of *Danio rerio* [22], *Hemibarbus mylodon* [23], *Hypophthalmichthys molitrix* [19], and *Takifugu obscurus* [20], among others, have been cloned. However, there are no reports on the SODs of blunt snout bream *Megalobrama amblycephala*, a species that accounts for an important proportion of Chinese aquaculture. The main objectives of the present study were to: (1) clone the full-length cDNA of mMn-SOD from *M. amblycephala* (denoted as MamMnSOD); (2) investigate the tissue expression profile of MamMnSOD; (3) examine the temporal expression profile of MamMnSOD transcript in the liver and gill after environmental stress exposure; (4) analyze the antioxidant activity of recombinant MamMnSOD; and (5) generate the polyclonal antibodies and detect the native protein in liver by Western blot analysis.

2. Materials and methods

2.1. Animals and materials

M. amblycephala juveniles were obtained from Yixing fish farm of Fresh-water Fisheries Research Center, Chinese Academy of Fishery Sciences. Fish were fed with a commercial diet during the acclimation period before the experiment. In the nitrite stress tests, two nitrite concentration treatment groups were used: 0.01 (normal freshwater as control) and 15 mg L^{-1} (adjusted by diluting 10 g L^{-1} $NaNO_2$). For each treatment, liver and gill tissues from three individuals were sampled at 0, 3, 6, 12, 24, and 48 h after nitrite exposure, and samples were snap-frozen in liquid nitrogen for RNA extraction. At the same time, tissue samples from the heart, liver, spleen, kidney, muscle, intestine, and gills were collected and immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. RNA extraction, reverse transcript, and partial MnSOD cDNA cloning

Total RNA was extracted using an Unizol reagent kit (Biostar, Shanghai, China) according to the manufacturer's protocol. The first-strand cDNA synthesis was performed by using superscript™ III RNAse H reverse transcriptase (Invitrogen, CA, USA) to transcribe poly $(A)^+$ RNA, with oligo-d (T) 18 as the primers. Reaction conditions complied with the manufacturer's instructions.

A large number of genes in the liver of *M. amblycephala* have been discovered by expressed sequence tag (EST) and annotation analysis [24]. One EST (isotig08118) in the library was homologous to the MnSOD of *H. molitrix* (ADM86391.1). These sequences were chosen for further amplification of the MnSOD gene from *M. amblycephala*. The primers (Table 1) designed from isotig08118 were used to amplify the partial cDNA MnSOD from the *M. amblycephala* liver. The PCR reactions were performed as follows: 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 30 s, and elongation at 72 °C for 3 min, followed by a 10 min extension at 72 °C and cooling to 4 °C. The PCR fragments were subjected to electrophoresis on a 1% agarose gel for length difference and cloned into the pMD-18T vector (Takara, Japan). After transformation into the competent cells of *Escherichia coli* DH5α, recombinant bacteria were identified by blue-white screening and confirmed by PCR. Three of the positive clones were sequenced in both directions and the resulting sequences were verified and subjected to cluster analysis in NCBI.

Table 1
Description of primers used in this study.

Primer	Primer sequence (5′–3′)
Mn-SOD-F1 (3′-RACE out primer)	CAGGGCTCAGGCTGGGGCTGGTTGGG
Mn-SOD-F2 (3′-RACE in primer)	GACCACATTACAACCACCAAACTCAG
Mn-SOD-R1 (3′-RACE out primer)	AAGGGAGACTTGGGTCGTCACATCAC
Mn-SOD-R2 (3′-RACE in primer)	CTGGAGGTCAGGTTTGTGTGTGCTTC
Mn-SOD-F (Real-time primer)	TGTTGGAGGCCATTAAGCGT
Mn-SOD-R (Real-time primer)	CGCAAGCAGCAATTCTCAGT
β-Actin F (Real-time primer)	TCGTCCACCGCAAATGCTTCTA
β-Actin R (Real-time primer)	CCGTCACCTTCACCGTTCCAGT

2.3. cDNA cloning of MnSOD in M. amblycephala

Total RNA was isolated from mixed tissues of prawns using RNAiso Plus Reagent (TaKaRa, Japan) according to the manufacturer's protocols. First-strand cDNA was synthesized using the Reverse Transcriptase M-MLV Kit (TaKaRa, Japan). The 3′- rapid amplification of cDNA ends (RACE) and 5′-RACE were performed using 3′-full RACE Core Set Ver. 2.0 Kit and 5′-full RACE Kit (TaKaRa, Japan) to obtain the cDNA 3′ and 5′ ends of five chitinases. All the primers used in the cloning are listed in Table 1. The PCR products were purified using a gel extraction kit (Sangon, China), and sequenced on an ABI3730 DNA Analyzer after insertion into the PMD-18T vector.

2.4. Sequence analysis of MamMnSOD

The MamMnSOD gene sequence was analyzed using the BLAST algorithm from the NCBI website (http://www.ncbi.nlm.nih.gov/blast), and the deduced amino acid sequence was analyzed using the Expert Protein Analysis System (http://www.expasy.org/). SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/) was employed to predict the signal sequence of MamMnSOD. Multiple alignment of MamMnSOD was performed using the ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/). Phylogenetic analysis of the putative amino acid sequences of MnSOD was carried out by the neighbor-joining method using ClustalW 2.0. The reliability of the estimated phylogenetic tree was evaluated by the bootstrap method with 1000 pseudo-replications.

2.5. Analysis of MamMnSOD expression in tissues

The expression of MamMnSOD mRNA in the heart, liver, spleen, kidney, muscle, intestine, and gills was separately detected by quantitative real-time PCR (qRT-PCR). Total RNA was extracted as described in Section 2.2. First-strand cDNA was synthesized using MMLV reverse transcriptase (TaKaRa, Dalian, China) with 5 μg of total RNA. Gene-specific primers (Table 1) were used to amplify the MamMnSOD transcript, and the PCR products were sequenced to verify the specificity of the PCR primers. The β-actin primers were used to amplify the β-actin fragments that were used as an internal control [25]. An SYBR Green qRT-PCR assay was conducted to determine MnSOD mRNA expression. The PCR temperature profile and reaction conditions were specified by the manufacturer of SYBR® PrimeScript™ qRT-PCR Kit (Takara, Japan) on an ABI step of the qRT-PCR system (Applied Biosystems, USA). Each sample was run in triplicate along with the internal control gene. To ensure that only one PCR product was amplified and detected, the dissociation curve analysis of amplification products was performed at the end of each PCR reaction. The comparative threshold cycle (C_t) method was used to analyze the expression level of MamMnSOD. The difference in C_t between the target tissue and the internal control (ΔC_t) for each sample was subtracted from the calibrated C_t to yield

Download English Version:

https://daneshyari.com/en/article/2431657

Download Persian Version:

https://daneshyari.com/article/2431657

Daneshyari.com