Available online at www.sciencedirect.com
ScienceDirect
Developmental and Comparative Immunology 31 (2007) 783–789
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Tissue-specific expression and molecular modeling of cytosolic manganese superoxide dismutases from the white shrimp *Litopenaeus vannamei*

Gracia A. Gómez-Anduro, Rogerio R. Sotelo-Mundo, Adriana Muhlia-Almazán, Gloria Yepiz-Plascencia*

Aquatic Molecular Biology Laboratory, Centro de Investigación en Alimentación y Desarrollo, P.O. Box 1735; Hermosillo Son; 83000, México

Received 26 April 2006; received in revised form 3 November 2006; accepted 13 November 2006
Available online 14 December 2006

Abstract

Manganese superoxide dismutases (MnSODs) are usually mitochondrial enzymes, although there are few examples of cytosolic MnSODs (cMnSOD). We have previously characterized a cMnSOD cDNA from *Litopenaeus vannamei* hemocytes, and to obtain new insights into the tissue specific expression and the protein structure, we characterized three more different cMnSOD transcripts (*cMnsod1*, *cMnsod2* and *cMnsod3*) and modeled the three-dimensional protein structure using human MnSOD as a template. The nucleotide sequences differ in seven positions. Four differences are silent; while three produce changes in amino acid sequence. *cMnsod1*, *cMnsod2* and *cMnsod3* are differentially expressed in nervous system, hepatopancreas and hemocytes. The structural protein model predicts *bona fide* MnSODs with proper coordination for the enzymatic activity.

Keywords: Antioxidant; Cytosolic MnSODs; Crustacean; Differential expression; Protein modeling

1. Introduction

Superoxide dismutase (EC 1.15.1.1) catalyzes the conversion of two molecules of superoxide anion to one molecule of hydrogen peroxide and one of oxygen [1]. SODs can have different metallic cofactors such as: copper–zinc SOD (CuZnSOD) [2], nickel SOD (NiSOD) [3], manganese–iron SOD (MnFeSOD) [4], iron–zinc SOD (FeZnSOD) [5], iron SOD (FeSOD) and manganese SOD (MnSOD). Crustaceans have the typical mitochondrial MnSOD (mMnSOD) and an additional cytoplasmic MnSOD (cMnSOD). The former is a homotetramer and the later a homodimer of ~25 kDa subunits [6,7], coded by different genes [8]. The cMnSOD was proposed to replace the normal cytosolic CuZnSOD in the blue crab *Callinectes sapidus* as an adaptation to cope with the need of high blood copper levels in hemocyanin, a Cu-based oxygen-carrier that is highly mobilized during molting in this crab and in other crustaceans [8].

Although there are complete cDNA sequences reported for some crustacean cMnSODs such as the blue crab *C. sapidus* (GenBank AF264030.1) [8], the

*Corresponding author. Tel.: +52 662 289 24 00; fax: +52 662 280 04 21.
E-mail address: gyepiz@cascabel.ciad.mx (G. Yepiz-Plascencia).

0145-305X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2006.11.007

freshwater prawn *Macrobrachium rosenbergii* [9], the shrimp *Penaeus monodon* (GenBank AAW50395), *Litopenaeus vannamei* (GenBank DQ005531) [10]; and partial sequences from *Farfantepenaeus aztecus*, (AY211085); *Palaemonetes pugio*, (AY211084) and *Marsupenaeus japonicus* (AY211084), the existence of cMnSOD isoforms is unknown. In this study, we report the characterization of three cMnSOD isoforms from the shrimp *L. vannamei* and describe their differences and tissue-specific expression. Structural modeling of the primary sequence predicts that they adopt the canonical MnSOD fold and have the SOD enzymatic activity, and the slight differences in the predicted amino acid sequence of the three cDNA sequences do not affect the tertiary and quaternary protein structure.

2. Materials and methods

2.1. cDNAs sequencing and specific primers design

The three different cDNA sequences were obtained using cMnSODF and reverse cMnSODR primers (Table 1), that were designed based on the previously determined cMnSOD cDNA from white shrimp (GenBank accession number DQ005531). A PCR was done using 0.5 μM of each primer, 21 μl of platinum PCR Supermix (Invitrogen) and the cDNA obtained from ~230 ng total RNA (see below). PCR amplification conditions were: 95 °C, 1 min; 95 °C, 30 s, 63 °C, 1 min, 68 °C, 3 min (one cycle); 95 °C, 30 s, 60 °C, 1 min, 68 °C, 3 min (34 cycles); and 72 °C, 10 min in a DNA Thermal Cycler (PTC-200 DNA Engine, MJ Research), and kept at 4 °C until used. Each of the three DNA fragments containing the complete coding sequence were cloned into the pCR 2.1 TOPO vector using TOP 10 *E. coli* cells (Invitrogen). All clones were thoroughly sequenced in both strands at the GATC facility (Genomic Analysis and Technology Core), The University of Arizona. The sequence determined from each single PCR cloned fragment was compared with the previously determined cDNA of the cMnSOD from white shrimp (GenBank accession number DQ005531). Based on the coding region from each cloned fragment, specific primers were designed to distinguish differences in one base (Table 1). PCR reactions were optimized using specific clones for every cDNA as template.

2.2. Molecular analysis of the cMnSOD cDNAs

cDNAs sequences and the deduced protein sequences were compared to non-redundant nucleotide and protein databases using the BLAST algorithm [11]. For initial protein modeling, the human MnSOD sequence (GenBank P04179) was selected as the most similar protein to shrimp cMnSOD, and aligned with the deduced amino acid sequences from *L. vannamei* cDNAs *cMnsod1*, *cMnsod2* and *cMnsod3*, using Clustal W [12]. The three-dimensional structure of shrimp cMnSOD was obtained by homology modeling using a human MnSOD structure (PDB 1VAR) [13]. The selection of this template was not trivial, since this structure is a variant of the human mitochondrial MnSOD that destabilizes the tetrameric interface [13]. Crustacean cMnSODs are postulated to be dimeric [6,8] and we considered that 1VAR was a better choice for the starting homology model. The modeling was started using the Swiss-Model server [14], and the input shrimp sequence did not include the cytosolic amino terminal residues 1–85, since they do not have significant similarity to the template model. The Refmac5 program [15] of the CCP4 crystallographic computational suite [16,17] was used to refine the

Table 1
Name, sequence and position of the primers in the MnSODs cDNA sequences

Primer name	Sequence (5′–3′)	cDNA* position (nt)
cMnSODF	ATGGCTGAGGCAAAGGAAGCTTAC	1–24
cMnSODR	CAATGACCTGCATTCTTACGAG	864–842
cMnSODF1	CTCATGCTTTGCCACCC	257–273
cMnSODF2	TAACAACCTAATTGCCGCTACA	360–381
cMnSODF3	TGCTCATGCTTTGCCACCT	255–273
cMnSODR1	CATGACGCTCATTCACGTTCT	835–815
cMnSODR2	CTCATAACGCTCATTCACGTTCT	837–815
cMnSODR3	CATAACGCTCATTCACGTTCC	835–815

*Gómez-Anduro et al. [10].