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A teleostan homolog of catalase from black rockfish (*Sebastes schlegelii*): Insights into functional roles in host antioxidant defense and expressional responses to septic conditions

Don Anushka Sandaruwan Elvitigala [a, b], Thanthrige Thiunuwan Priyathilaka [a, b], Ilson Whang [b], Bo-Hye Nam [c], Jehee Lee [a, b, *]

[a] Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea
[b] Fish Vaccine Research Center, Jeju National University, Jeju Special Self-Governing Province 690-756, Republic of Korea
[c] Biotechnology Research Division, National Fisheries Research and Development Institute, 408-1 Sirang-ri, Gijang-up, Gijang-gun, Busan 619-705, Republic of Korea

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ABSTRACT

Antioxidative defense renders a significant protection against environmental stress in organisms and maintains the correct redox balance in cells, thereby supporting proper immune function. Catalase is an indispensable antioxidant in organisms that detoxifies hydrogen peroxides produced in cellular environments. In this study, we sought to molecularly characterize a homolog of catalase (RfCat), identified from black rockfish (*Sebastes schlegelii*). *RfCat* consists of a 1581 bp coding region for a protein of 527 amino acids, with a predicted molecular weight of 60 kD. The protein sequence of RfCat harbored similar domain architecture to known catalases, containing a proximal active site signature and proximal heme ligand signature, and further sharing prominent homology with its teleostan counterparts. As affirmed by multiple sequence alignments, most of the functionally important residues were well conserved in RfCat. Furthermore, our phylogenetic analysis indicates its common vertebrate ancestral origin and a close evolutionary relationship with teleostan catalases. Recombinantly expressed RfCat demonstrated prominent peroxidase activity that varied with different substrate and protein concentrations, and protected against DNA damage. *RfCat* mRNA was ubiquitously expressed among different tissues examined, as detected by qPCR. In addition, *RfCat* mRNA expression was modulated in response to pathogenic stress elicited by *Streptococcus iniae* and poly I:C in blood and spleen tissues. Collectively, our findings indicate that RfCat may play an indispensable role in host response to oxidative stress and maintain a correct redox balance after a pathogen invasion.

1. Introduction

Reactive oxygen species (ROS) play an indispensable role in host anti-microbial defense, acting directly or as signaling molecules in host inflammatory or innate immune responses [1–4]. Nevertheless, excessive ROS generation can have detrimental effects, including host cell damage due to oxidation of biomolecules, DNA mutagenesis, activation of pro-cell death factors, and tumorigenesis [5–7]. Moreover, accumulation of ROS in cellular environments can compromise host immunity [8]. Therefore, a proper ROS balance must be maintained for survival. This balance is regulated by the cellular antioxidant system, consisting of ROS scavengers, such as the enzymes catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), thioredoxin, thioredoxin reductase (TrxR), and peroxiredoxins (Prx) [9] along with non-enzyme constituents, including glutathione, vitamin A, E, and C [10]. Among enzymatic components, catalase plays an indispensable role in detoxifying hydrogen peroxide to form the nontoxic end-products water and oxygen [9]. Generally, two types of catalases are found in organisms, classical Fe heme enzymes and catalase-peroxidases. The former belongs to a small group of manganese enzymes, whereas the latter contains a covalent triplet of distal side chains, which catalyze peroxidatic and catalytic reactions

* Corresponding author. Marine Molecular Genetics Lab, Department of Marine Life Sciences, College of Ocean Science, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju 690-756, Republic of Korea. Tel.: +82 64 754 3472; fax: +82 64 756 3493.
E-mail address: jehee@jejunu.ac.kr (J. Lee).

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following a different mechanism than the classical heme enzymes [11]. In addition to hydrogen peroxide, catalase can also breakdown several other substrates, including phenol, methanol, ethanol, and nitrites [12]. Interestingly, catalase is an efficient enzyme with a high turn-over rate; each second, one catalase molecule can convert millions of peroxide molecules into water and oxygen [13].

Catalase is ubiquitously expressed in prokaryotes and eukaryotes, consisting of four identical subunits of 50–60 kD [14,15]. Each monomeric subunit contains a single heme group and NADPH molecule on its surface [16,17]. This NADPH prevents the enzyme from oxidation by its own substrate. Much of the information on catalase, especially regarding its structure and the regulation of its expression, has been reported in mammals [18,19], plants [20], and bacteria [21]. Human catalase is a member of the peroxisomal glycoprotein family with four subunits, each harboring four conceptual domains, including β-barrel, N-terminal threading arm, wrapping loop, and C-terminal helixes [22].

There are some credible evidences which suggest that catalase can mediate host immune responses, besides its main ROS scavenging role in cells. For instance, *Caenorhabditis elegans* catalase was found to stimulate innate immune gene response [23], and catalase was shown to mediate a main host defense system required in host–microbe interactions in the gastrointestinal tract of fruit flies [24]. In addition, catalase activity and/or expression was shown to be altered by viral infections in crustaceans [25–28], and enhanced *catalase* transcription was detected in mollusks with bacterial infection [29,30]. Hence, gaining insight into different counterparts of catalase in different taxons is essential to understand its potential, but unrevealed roles in organisms.

Information on lower vertebrate catalase homologues, especially of teleost origin, is relatively scarce. Among fish catalases, both rock bream (*Oplegnathus fasciatus*) [31] and zebrafish (*Danio rerio*) [32,33] have been evaluated to date. According to these reports, teleostan catalases demonstrated a strong peroxidase activity at a broad spectrum of temperatures and pH conditions, and its expression can be modulated by pathogen invasion. In addition, another study showed that gene expression of catalase can be temporarily induced by starvation in rock breams [34].

Mariculture is considered as a productive way to increase marine fish and shellfish production. Therein, fish and shellfish are cultured at a high density, either in an enclosed section of ocean or in tanks filled with seawater. Therefore, these creatures are more susceptible to environmental stress factors, including pathogen infections, which can negatively affect their survival and growth. Moreover, oxidative stress in these species can suppress their immune response, and in turn make them more vulnerable to infections [35]. Therefore, gaining insight into molecular defense mechanisms against stress is a primary step for the development of sustainable mariculture, by identifying targets for potential therapies. Since catalase is an important antioxidant and a relevant immune molecule, we sought to identify and molecularly characterize its homolog in black rockfish (*Sebastes schlegelii*), which is an economically important and mariculturally farmed comestible, especially in the Asia Pacific region.

In the present study, a teleostan homolog of catalase (RfCat) was identified from black rockfish and characterized at the molecular level, providing evidence for molecular expression during pathogenic stress, along with its putative functional roles against oxidative defense.

2. Materials and methods

2.1. Black rockfish cDNA database

A database of black rockfish cDNA sequences was created by 454 GS-FLX™ sequencing technique [36]. In brief, the total RNA was extracted from blood, liver, head kidney, gill, intestine, and spleen tissues from three fish (~100 g) challenged with immune stimulants including *Edwardsiella tarda* (10^7 CFU/fish), *Streptococcus iniae* (10^7 CFU/fish), lipopolysaccharide (1.5 mg/fish), polyinosinic:polycytidylic acid (poly I:C, 1.5 mg/fish). The extracted RNA was then cleaned using an RNeasy Mini kit (Qiagen, USA) and assessed for quality and quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Canada), resulting in an RNA integration score (RIN) of 7.1. Subsequently, the GS-FLX™ 454 shotgun library was constructed, and a cDNA database was established using fragmented RNA (average size – 1147 bp) from the aforementioned RNA samples (Macrogen, Korea).

2.2. Identification and sequence characterization of RfCat

We identified the complete cDNA sequence of *RfCat* from the black rockfish cDNA database using the Basic Local Alignment Search Tool (BLAST) algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, the identified sequence was characterized using different bioinformatics tools. The full-length putative coding sequence of *RfCat* was identified, and the corresponding amino acid sequence was derived using DNAsist 2.2 software. Domain architecture corresponding to the derived primary structure of RfCat was predicted using ExPASy Prosite database (http://prosite.expasy.org). Moreover, some of the physicochemical properties of the derived protein sequence were determined by the ExPASy ProtParam tool (http://web.expasy.org/protparam). Comparison of the RfCat protein sequence with its homologues was carried out through pairwise and multiple sequence alignments, executed by Matgat software [37] and ClustalW2 (http://www.Ebi.ac.uk/Tools/clustalw2) program, respectively. The phylogenetic reconstruction of RfCat was generated according to the neighbor-joining method using Molecular Evolutionary Genetics Analysis (version 4.0) software (MEGA 4.0) [38], validated by 1000 bootstrap replications. In addition, the tertiary structure of RfCat was modeled by the I-TASSER online server [39,40] and visualized using PyMOL 1.7 program (http://www.pymol.org).

2.3. Overexpression and purification of recombinant RfCat (rRfCat)

rRfCat was expressed as a fusion protein with maltose binding protein (MBP) as previously described, but with some modifications [41]. Briefly, the coding sequence of *RfCat* was amplified using the sequence-specific primer pair, RfCat-F and RfCat-R, which contained restriction enzyme sites for *Eco*RI and *Sal*I, respectively (Table 1). PCR was performed in a TaKaRa thermal cycler (TaKaRa, Otsu, Shiga, Japan) in a total volume of 50 μL, containing 5 U TaKaRa ExTaq polymerase, 5 μL of 10× TaKaRa ExTaq buffer, 8 μL 2.5 mM dNTPs, 80 ng of template, and 40 pmol of each primer (Table 1). The PCR was performed under the following conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. The PCR product (~1.6 kbp) was resolved on a 1% agarose gel, excised, and purified using the Expin™ Gel SV kit (GeneAll, Korea). Digested pMAL-c2X vector (150 ng) and the PCR product (108 ng) were ligated using Mighty Mix (5.0 μL; TaKaRa) at 4 °C overnight. The ligated pMAL-c2X/RfCat product was transformed into DH5α cells and sequenced. After sequence conformation, the recombinant expression plasmid was transformed into *Escherichia coli* BL21 (DE3) competent cells. Expression of the rRfCat fusion protein was induced using isopropyl-β-D-galactopyranoside (IPTG, 0.5 mM). *E. coli* BL21 cells were grown in 500 mL Luria broth (LB) supplemented with ampicillin (100 μg/mL) and glucose (0.2%) at 20 °C for 9 h. Induced *E. coli* BL21 (DE3) cells were then cooled on ice for 30 min and harvested by centrifugation at 3500 rpm for 30 min at