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Molecular characterization of a catalase from *Hydra vulgaris*

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ABSTRACT

Catalase, an antioxidant and hydroperoxidase enzyme protects the cellular environment from harmful effects of hydrogen peroxide by facilitating its degradation to oxygen and water. Molecular information on a cnidarian catalase and/or peroxidase is, however, limited. In this work an apparent full length cDNA sequence coding for a catalase (HvCatalase) was isolated from *Hydra vulgaris* using 3′- and 5′- (RLM) RACE approaches. The 1859 bp *HvCatalase* cDNA included an open reading frame of 1518 bp encoding a putative protein of 505 amino acids with a predicted molecular mass of 57.44 kDa. The deduced amino acid sequence of HvCatalase contained several highly conserved motifs including the heme-ligand signature sequence RLFSYGDTH and the active site signature FXRERIPERVVHAKGXGA. A comparative analysis showed the presence of conserved catalytic amino acids [His(71), Asn(145), and Tyr(354)] in HvCatalase as well. Homology modeling indicated the presence of the conserved features of mammalian catalase fold. Hydrae exposed to thermal, starvation, metal and oxidative stress responded by regulating its *catalase* mRNA transcription. These results indicated that the *HvCatalase* gene is involved in the cellular stress response and (anti)oxidative processes triggered by stressor and contaminant exposure.

1. Introduction

Catalases (= Catalatic hydroperoxidases, CHPs) are ubiquitous enzymes and these proteins are placed into four main groups: (1) the classic heme-containing monofunctional catalases for which hydrogen peroxide is both electron donor and acceptor, (2) the heme-containing bifunctional catalatic peroxidases (CPXs) in which the catalatic activity is much higher than the peroxidatic activity, (3) the nonheme-containing catalases (Allgood and Perry, 1986), and (4) a miscellaneous group containing proteins with minor catalatic but no peroxidatic activities (Jones and Masters, 1978; Nadler et al., 1986). Most CHPs exist as tetramers of 60–65 kDa subunits (Nadler et al., 1986).

More than 300 CHPs sequences are now available, divided among monofunctional catalases (>225), bifunctional catalase-peroxidases (>50) and manganese-containing catalases (>25) (Chelikani et al., 2004). Frequently, organisms use different isozymes, which are expressed simultaneously or under developmental-stage- and environment-specific conditions (Schrempf et al., 1999) to decompose hydrogen peroxides to ground-state O_2. Catalases directly dismutate hydrogen peroxide to water and dioxygen by two-electron transfer redox reactions where as peroxidases remove the H_2O_2 by using it to oxidize another substrate (Schubert and Wilmer, 1991).

In an ultrastructural localization study (Hand, 1976) it is shown that catalase activity (diaminobenzidine reaction product) is present in small round or elongated bodies resembling microperoxisomes in the epitheliomuscular, digestive and gland cells of hydra. In the same study it is also demonstrated that microperoxisome-like bodies reactive for L-alpha-hydroxy acid oxidase is present in the epidermal cnidoblasts; however, catalase could not be demonstrated in them. This study (Hand, 1976) provided the first cytochemical evidence for the presence of an H_2O_2-producing oxidase in microperoxisomes. Peroxidase like activity has also been observed in the ectodermal foot mucous cells (Hoffmeister-Ullerich et al., 2002) and in lithium treated hydra (Jantzen et al., 1998). Hydroperoxides are also observed to play the role of second messengers in peroxidase activity. However peroxidase or catalase activity is not specifically attributed to any gene products in hydra, though few redox (and stress regulatory) proteins are reported in hydra (Brennecke et al., 1998; Gellner et al., 1992; van Dam et al., 2010). Our group have cloned and characterized two superoxide dismutases, manganese superoxide dismutase (HvMnSOD) and extracellular superoxide dismutase (HvEC-SOD) (Dash et al., 2007), and two phospholipid hydroperoxide glutathione peroxidases,

Abbreviations: H., Hydra; Hv, *Hydra vulgaris*; cDNA, DNA complementary to RNA; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; RACE, rapid amplification of cDNA ends; ORF, open reading frame; UTR, untranslated region; nt, nucleotide(s); aa, amino acid(s); bp, base pair(s); kDa, kilodalton(s); dNTP, deoxyribonucleoside triphosphate; EDTA, ethylenediaminetetraacetic acid; ppm, parts per million; Cu, copper; Zn, zinc; Cd, cadmium; Cr, chromium; As, arsenic; Se, selenium; Na, sodium; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; EST, expressed sequence tag; catalase, catalase protein; *catalase*, gene, cDNA or mRNA encoding catalase; *HvCatalase*, gene, cDNA or mRNA encoding HvCatalase protein; NADPH, nicotinamide adeninedinucleotide phosphate reduced form; CAT, catalase.

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mitochondrial (HvGPx41) and nuclear (HvGPx42) (Dash et al., 2006), from *Hydra vulgaris*.

Hydra, a fresh water cnidarian, is used as an ideal environmental toxicological model to study the acute and chronic toxicity effects of several environmental toxicants. Our laboratory (Lum et al., 2003; Mayura et al., 1991; Taylor et al., 2009) and other have used the changes in external gross morphology and anatomy, and physiology are useful as markers of toxicity or toxicity end points in the hydra bioassays (Ferreira et al., 2011; Holdway et al., 2001; Johnson et al., 1982; Karntanut and Pascoe, 2000; Pascoe et al., 2002; Pollino and Holdway, 1999; Trenfield et al., 2011; van Dam et al., 2010; Vernouillet et al., 2010). At molecular level, it may be postulated that, the detection of stress and/or redox sensitive messages in hydra can constitute an early-warning marker for the presence of potentially deleterious agents in water. Because *H. vulgaris* is sensitive to a variety of compounds, the detection stress protein messages such as catalase messages could be applied as a prescreening tool in determining the relative toxicity of many toxicants, and new compounds that are yet to be screened for toxicity.

In this work, a cDNA encoding a monofunctional catalase was identified and isolated from *H. vulgaris*. The expression of hydra monofunctional *catalase* (*HvCatalase*) mRNA is assayed with respect to both environmental contaminant challenge (i.e., arsenic, cadmium, zinc and copper) and stress (both oxidative and non-oxidative) in order to explore its possibility for use as biomarker of stress and toxicity.

2. Materials and methods

2.1. Hydra culture

Hydra vulgaris (formerly known as *Hydra attenuata*) were originally obtained from E. Marshall Johnson, Jefferson Medical College (Philadelphia, PA, USA). *H. vulgaris* were maintained in shallow glass dishes at 18 °C in a medium containing 1 mM $CaCl_2 \cdot 2H_2O$, 0.012 mM EDTA, and 0.458 mM TES (N-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid, sodium salt) buffer (pH 7.0). Daily, hydrae were fed with brine shrimp (*Artemia nauplii)* hatched in a solution of 1% sodium chloride and treated with iodine (40 μg ml^{-1}). Hydrae were maintained free from bacterial and fungal contamination and were not fed for 24 h before initiating the experiments. Deionized water was used throughout this portion of the study (Mayura et al., 1991).

2.2. RNA isolation and cleanup

Total RNA was extracted from hydra by application of 2 ml of TRIzoL® reagent (Invitrogen, USA) to approximately 20 mg of fresh tissue, using the manufacturer's instructions. The RNA was quantified by ultraviolet absorbance at 260 nm. Integrity of the total RNA was confirmed by 1% formaldehyde agarose gel electrophoresis. The RNA isolated was cleaned up from contaminating DNA using RNeasy Mini Kit (Invitrogen, USA) following the manufacturer's instruction.

2.3. Identification of partial fragments of H. magnipapillata catalase cDNA

An EST was found from the hydra, *H. magnipapillata*, under the accession number gi|60408694. The expressed sequence tag coded for a catalase similar to the N-terminal end of *Sus scrofa* catalase. This EST sequence was used to design primers F2 (5′-ATGGTGTTGGATCGTAATCCTG-3′) and R3 (5′-CTTGAGGGCCATTAAAGCTG-3′) to clone a fragment of catalase from *H. vulgaris*.

2.4. Cloning and identification of partial fragments of H. vulgaris catalase cDNA

All oligonucleotides except as mentioned were procured from IDT Inc. (IA, USA). All polymerase chain reaction (RACE-PCR and RT-PCR) experiments were performed using *Taq* DNA polymerase (Invitrogen, USA) and a thermal cycler (MJ Research, USA).

RNA (5 μg) was reverse-transcribed to cDNA at 37 °C for 60 min using the oligo(dT) bifunctional primer N (5′-AACTGGAAGAATTCGCGGCCGCAGGAAd$(T)_{18}$-3′) and the AMV RT supplied in the cDNA synthesis kit (Amersham Biosciences, USA). The first-strand cDNA was amplified using the primer pairs: F2 and R3 for cloning and identifying partial fragments of *H. vulgaris catalase* cDNA. The PCR was performed for 30 cycles, consisting of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min and a final extension at 72 °C for 10 min. The resultant PCR products were subcloned into the pCR®II–TOPO® vector using a TA cloning kit (Invitrogen, USA). Multiple independent clones were sequenced using automated methods (DNA Technologies Lab, Department of Veterinary Pathobiology, Texas A&M University) on an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, USA) using a Big-Dye sequencing kit (Applied Biosystems, USA) and M13 primers. The identity of the clones was evaluated by matching the sequences to the nucleotide/protein sequences available at the GenBank. The cloned sequence constituted residues 1019 to 1247 in the catalase nucleotide sequence as shown in Fig. 1.

2.5. 3′-RACE of the HvCatalase cDNA

In order to clone the 3′-end of the *HvCatalase* cDNA, the first-strand cDNA prepared above was amplified using the oligo(dT) bifunctional primer N and a gene-specific primer F2 (complementary to nucleotide (nt) 1019 to 1040, Fig. 1) for 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 3 min. The first-round PCR products were reamplified using the primer F3 (5′-CGGGTGTTGAGACATCTCCT-3′) (complementary to nucleotide (nt) 1096 to 1115, Fig. 1) and oligo(dT) bifunctional primer N using the same temperature parameters. The PCR products were subcloned and sequenced as described above. The identity of the clones was evaluated by matching the sequences to the nucleotide/protein sequences available at the GenBank.

2.6. 5′-RACE of the HvCatalase cDNA

FirstChoice® RLM-RACE (RNA Ligase Mediated Rapid Amplification of cDNA Ends) (Ambion Inc., USA) was employed to clone the 5′-end of the *HvCatalase* cDNA. In brief total RNA (10 μg) was treated with Calf Intestine Alkaline Phosphatase (CIP) to remove free 5′-phosphates from molecules such as ribosomal RNA, fragmented mRNA, tRNA, and contaminating genomic DNA. The cap structure found on intact 5′ ends of mRNA is not affected by CIP. The RNA was then treated with Tobacco Acid Pyrophosphatase (TAP) to remove the cap structure from full-length mRNA, leaving a 5′-monophosphate. A 45 base RNA adapter oligonucleotide (5′-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA-3′) was ligated to the RNA population using T4 RNA ligase. The adapter cannot ligate to dephosphorylated RNA because these molecules lack the 5′-phosphate necessary for ligation. During the ligation reaction, the majority of the full length decapped mRNA acquires the adapter sequence as its 5′ end. A random-primed reverse transcription reaction using MMLV reverse transcriptase and nested PCR then amplified the 5′ end of the catalase transcript. First round of PCR was performed using 5′ RACE outer primer (O1) (5′-GCTGATGGCGATGAATGAACACTG-3′) and a 5′ RACE gene specific outer primer (R3). Second round of PCR used 5′ RACE inner primer (O2) (5′-CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG-3′) and 5′ RACE gene specific inner primer (R2) (5′-AGGAGATGTCTCAACACCCG-3′).

2.7. GenBank accession number

The nucleotide sequence of the HvCatalase mRNA is available in the GenBank databases under the accession number JN580276.