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Short communication

Sequence analysis, characterization and tissue distribution of channel catfish (*Ictalurus punctatus* Rafinesque, 1818) myeloperoxidase cDNA

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ABSTRACT

Myeloperoxidase (EC 1.11.1.7), a heme-containing lysosomal glycoprotein, is found predominantly in azurophilic granules of neutrophils. This enzyme upon activation catalyzes hydrogen peroxide in the presence of various halide ions to form hypohalous acids. Subsequently, these reagents are able to kill the invading microorganisms. In this study, we report the identification, characterization and expression analysis of the channel catfish myeloperoxidase transcript. The full-length nucleotide sequence of channel catfish myeloperoxidase cDNA had 3157 nucleotides, including an open reading frame, which appears to encode a putative peptide of 771 amino acid residues with a calculated molecular mass of 87.14 kDa. By comparison with the human counterpart, the channel catfish myeloperoxidase peptide can be divided into domains and has conservative features, including peroxidase catalytic sites, covalent linkage sites for the heme group and all cysteine residues. The channel catfish myeloperoxidase transcript was detected by RT-PCR in anterior kidneys, where the major leukocyte population is neutrophil precursors. Reagent development and the role of this enzyme in *Edwardsiella ictaluri* infection are under investigation.

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1. Introduction

Peroxidases, distributed ubiquitously in both prokaryotes and eukaryotes, catalyze hydrogen peroxide into water and various organic and inorganic substrates [1]. According to the recently published peroxidase databases [2,3], there are more than 6000 peroxidase sequences from over 940 organisms. The peroxidases can be classified into two large groups: heme-containing and non-heme-containing peroxidases [1–3]. The latter contains alkylhydro-, glutathione-, halo-, NADH-peroxidases, peroxiredoxins and manganese catalases. The former includes catalases, dyp-type peroxidases, di-heme cytochrome C peroxidases, haloperoxidases, and animal and non-animal peroxidases. The animal peroxidases are further classified and included "mammalian" and "non-mammalian vertebrate" peroxidases [2,3]. After we and others identified channel catfish peroxiredoxins (non-heme-containing peroxidases) [4,5], we found another peroxidase—myeloperoxidase—EST was up-regulated in the early stage of *Edwardsiella ictaluri* infection in catfish ovary cell line (CCO cells) [Yeh and Klesius, unpublished data].

Myeloperoxidase (EC 1.11.1.7), a heme-containing lysosomal glycoprotein, is found predominantly in azurophilic granules of neutrophils, and in much lower amounts in monocytes and some tissue macrophages [6–11]. Upon activation, neutrophils produce hydrogen peroxide and release myeloperoxidase from the granules that the latter catalyzes the former in the presence of chloride ions to form hypochlorous acid [7,12–15]. Subsequently, this reagent is able to kill the invading microorganisms [6,16]. However, myeloperoxidase has also been detected in microglia and macrophages surrounding pathological lesions of multiple sclerosis and Alzheimer's disease [17,18]. Recently, the studies of this enzyme have been focused on the roles in chronic inflammation [see references [11,19,20] for reviews].

In teleost fish, the myeloperoxidase activity has been detected in channel catfish [21], black bullhead (*Ameiurus melas* Rafinesque, 1820) [22], Indian major carps (including *Cirrhinus mrigala* Hamilton, 1822, *Catla catla* Hamilton, 1822 and *Labeo rohita* Hamilton, 1822) [23], zebrafish (*Danio rerio* Hamilton-Buchanan, 1822) [24] and goldfish (*Carassius auratus* Linnaeus, 1758) [25]. The fish myeloperoxidase gene transcript has been cloned in zebrafish [26,27], and the protein has been purified from turbot (*Psetta maxima* Linnaeus, 1758) anterior kidney neutrophils [28]. However, the roles of this enzyme in fish infected with various microorganisms have not been characterized. In this study, the channel catfish myeloperoxidase transcript was completely sequenced and characterized, and the expression profile in various tissues was determined.

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2. Materials and methods

2.1. Fish

Channel catfish (NWAC103 strain, weighed 25–30 g) were used in this study. Prior to aseptical tissue excision, the catfish were euthanized by immersion in 300 mg/ml of tricaine methanesulfonate according to the Guidelines for the Use of Fishes in Research [29]. Spleen, anterior kidney, liver, intestine, skin and gill were collected and immersed in 1 ml of TRI reagent (Molecular Research Center, Inc., Cincinnati, OH). The Institutional Animal Care and Use Committee of the USDA ARS Aquatic Animal Health Research Unit in Auburn, AL approved the experiment.

2.2. RNA isolation and rapid amplification of cDNA ends (RACE)

Total RNA from channel catfish tissues was isolated by using a Tri reagent (Molecular Research Center, Inc.) according to the manufacturer's instruction. The quality and quantity of total RNA were determined by using RNA 1200 chips on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). A GeneRacer kit (Invitrogen, Carlsbad, CA) was used to obtain full-length 5′- and 3′-end of cDNA according to the manufacturer's instruction. Total RNA (5 μg) from three catfish anterior kidneys were used for this RACE construction.

2.3. Myeloperoxidase gene amplification

Both specific full-length 5′- and 3′-RACE of the myeloperoxidase gene transcript were PCR amplified. The PCR reaction mixtures (50 μl per reaction) contained the following reagents (in final concentrations): 1× Prime STAR PCR buffer (TaKaRa, Madison, WI), 200 μM dNTP mix (TaKaRa), 300 μM each of gene specific primer and GeneRacer primer, 1.25 U Prime STAR HS DNA polymerase (TaKaRa) and 1 μl of cDNA template (equivalent to 250 ng RNA input). The amplification was performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA) according to the protocol described in the GeneRacer manual. The primers for PCR amplification are listed in the Table 1. The amplified PCR products were purified by agarose gel electrophoresis, and ligated into the pSC vector, followed by transformation of the vectors into the Solo Pack® competent *Escherichia coli* cells (Agilent Technologies) according to the manufacturer's instruction. At least six colonies per PCR product were randomly selected for DNA sequencing.

2.4. DNA sequencing and bioinformatics

The DNA sequencing reactions were carried out at the USDA ARS Genomics and Bioinformatics Research Unit in Stoneville, MS with an ABI 3730 × 1 Genetic Analyzer (Applied Biosystems, Foster City, CA). Chromatograms were edited, trimmed and analyzed also at the USDA ARS Genomics and Bioinformatics Research Unit in Stoneville, MS. The amino acid sequence of channel catfish myeloperoxidase was deduced from nucleotide sequence by using Transeq [30], aligned with other myeloperoxidase amino acid sequences deposited in GenBank using ClustalW2 [31]. ExPASy server [32] was used to calculate the myeloperoxidase peptide molecular mass and pI, and to analyze the myeloperoxidase *N*-glycosylation sites. The signal peptide site in the amino acid sequence was detected with SignalP 3.0 software [33]. Phylogenetic relationships of myeloperoxidase amino acid sequences from various species were analyzed with MEGA 4.0 software [34] based on the ClustalW2 alignment results.

Table 1
Oligonucleotides used for PCR amplification in this study.

Primer	Sequence	Direction
GeneRacer 5′Primer (Invitrogen)	5′-GCTGTCAACGATACGCTACGTAACG-3′	Forward
GeneRacer 3′Primer (Invitrogen)	5′-CGACTGGAGCACGAGGACACTGA-3′	Reverse
MPX487–F	5′- CAAACCCTACCGCCTCCGTGCAGAAC–3′	Forward
MPX189-F	5′-TGCGGCCTGTCTGCACCTAGGAATGT–3′	Forward
MPX386-F	5′-GATCCGCCAAGGAGACAGGCTCTGGT–3′	Forward
MPX908–R	5′-CTGGGAATTTGGATGGGGAAGCATGG–3′	Reverse
MPX195-R	5′-GGCCGCAAAACCTCCACCATTCGTTA–3′	Reverse
MPX51-R	5′-GCCGTTCAGCTTAGCCGTCCTGCATT–3′	Reverse
β-Actin-F	5′-GACTTCGAGCAGGAGATGGG-3′	Forward
β-Actin-R	5′-AACCTCTCATTGCCAATGGTG-3′	Reverse

2.5. RT-PCR

Two-step RT-PCR assays were used to profile myeloperoxidase gene transcripts in various tissues as described previously [35–38]. β-Actin was used as an internal control. The amplified products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. Images were recorded by a KODAK Gel Logic 440 Imaging System (version 4.0.3) (Eastman Kodak, Rochester, NY), and processed with ImageJ software (version 1.41) [39].

3. Results and discussion

The full-length cDNA sequence of channel catfish myeloperoxidase consists of 3157 nucleotides, including a 90-nucleotide 5′-untranslated region (UTR), an open reading frame and a 741-nucleotide 3′-UTR (GenBank accession no. GQ429001). The 3′-UTR has a mRNA instability sequence (attta) and a polyadenylation signal sequence (tataaa) 168 and 86 nucleotides upstream of a 36-nucleotide polyadenylation tail, respectively. The human myeloperoxidase gene also uses the tataaa sequence as the polyadenylation signal [40]. The open reading frame appears to encode a 771 amino acid peptide with a calculated molecular mass of 87.14 kDa and a pI of 7.75 at pH 7.0. The peptide has five potential *N*-glycosylation sites at Asn^{111}, Asn^{181}, Asn^{379}, Asn^{426} and Asn^{614} (numbering after channel catfish), which may have a critical role in peroxidase activity. Castro et al. [28] reported that the turbot myeloperoxidase loses its peroxidase activity significantly, after this enzyme is treated with peptide-*N*-glycosidase F to remove *N*-linked carbohydrates. This deglycosylation modification may change the tertiary structure and therefore affect peroxidase activity of this enzyme [28,41,42].

The BLASTx searches of channel catfish myeloperoxidase against the NCBI *nr* database identified many related peroxidase orthologues from other species (*E* value $>3 \times 10^{-160}$) (Figs. 1 and 2). When we compared these myelopeorxidases, we found that the length of myeloperoxidases varies from 718 (mouse) to 783 amino acids (horse), and the degree of conservation ranges from 43% (vs. horse) to 65% (vs. zebrafish). Furthermore, when aligned with the human counterpart, the channel catfish myeloperoxidase can be synthesized from a single gene as a prepropeptide that is further proteolytically cleaved into (1) a signal peptide (cleavage at Val^{15}-$Ser^{16}Ala^{17}$–$Gln^{18}Thr^{19}$), (2) a 121-amino-acid propeptide, (3) a light chain and (4) a heavy chain [40,43–45]. Thus, the mature channel catfish myeloperoxidase may consist of a pair of heavy and light chain dimers with the insertion of the heme group [43,44]. Several important characteristics for myeloperoxidase functions are conserved in channel catfish [1,3,41]. First, catalytic residues at Gln^{232} (numbering after channel catfish) and His^{236} in the light chain and Arg^{395}, His^{433} and Asn^{588} in the heavy chain of myeloperoxidase are conserved among mammals and teleost fish (Fig. 1). Second, amino acid residues at Asp^{235} and Glu^{398} involved in the