Fish & Shellfish Immunology 30 (2011) 992–997

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Short communication

Molecular responses of ceruloplasmin to *Edwardsiella ictaluri* infection and iron overload in channel catfish (*Ictalurus punctatus*)

Hong Liu [a,b], Eric Peatman [a], Wenqi Wang [a], Jason Abernathy [a], Shikai Liu [a], Huseyin Kucuktas [a], Jeffery Terhune [a], De-Hai Xu [c], Phillip Klesius [c], Zhanjiang Liu [a,*]

[a] *The Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures and Program of Cell and Molecular Biosciences, Aquatic Genomics Unit, Auburn University, Auburn, AL 36849, USA*
[b] *College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China*
[c] *Aquatic Animal Health Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 990 Wire Road, Auburn, AL 36832, USA*

ARTICLE INFO

Article history:
Received 10 November 2010
Received in revised form
23 December 2010
Accepted 30 December 2010
Available online 8 January 2011

Keywords:
Teleost
Catfish
Immune
Edwardsiella ictaluri
Iron

ABSTRACT

Ceruloplasmin is a serum ferroxidase that carries more than 90% of the copper in plasma and has documented roles in iron homeostasis as well as antioxidative functions. In our previous studies, it has been shown that the ceruloplasmin gene is strongly up-regulated in catfish during challenge with *Edwardsiella ictaluri*. However, little is known about the function of this gene in teleost fish. The objective of this study, therefore, was to characterize the ceruloplasmin gene from channel catfish, determine its genomic organization, profile its patterns of tissue expression, and establish its potential for physiological antioxidant responses in catfish after bacterial infection with *E. ictaluri* and iron treatment. The genomic organization suggested that the catfish ceruloplasmin gene had 20 exons and 19 introns, encoding 1074 amino acids. Exon sizes of the catfish ceruloplasmin gene were close to or identical with mammalian and zebrafish homologs. Further phylogenetic analyses suggested that the gene was highly conserved through evolution. The catfish ceruloplasmin gene was mapped to both the catfish physical map and linkage map. The catfish ceruloplasmin gene was mainly expressed in liver with limited expression in other tissues, and it was significantly up-regulated in the liver after bacterial infection alone or after co-injection with bacteria and iron-dextran, while expression was not significantly induced with iron-dextran treatment alone.

1. Introduction

Iron is an essential trace element to almost all organisms with functions including oxygen transport, electron transport, DNA synthesis etc. However, in excess, free iron can be used to generate reactive oxygen species (ROS) through the Fenton reaction and then can significantly increase levels of oxidative stress and be extremely toxic to organisms [1,2]. Thus, it is very important to maintain an appropriate balance of iron in the body. The liver plays a central role in iron metabolism serving as the major storage site for iron and the principal site of synthesis of many iron regulatory proteins [3].

Ceruloplasmin is best known as a major copper-binding protein found in the plasma of vertebrate species and mainly synthesized by the liver [4]. After ceruloplasmin was originally isolated from plasma by Holmberg and Laurell in 1948 [5], this protein quickly became the subject of many investigations concerning its function, molecular structure and the physical properties of the copper ions bound to it [4,6–11]. The ceruloplasmin gene was identified on the human 3q25 chromosome with a molecular weight of ~132 kDa [8]. The molecule is composed of six compact domains, with large loop insertions, and is characterized by the presence of three types of spectroscopically distinct copper sites [9]. There are six copper atoms, three forming a trinuclear cluster at the interface of domains 1 and 6 and the other three forming mononuclear sites in domains 2, 4, and 6 [9]. The ceruloplasmin protein contains 90–95% of copper in the plasma, but rather than strictly functioning in copper metabolism, its main function appears to be a modulator of iron efflux via oxidation [4,6,7]. Ceruloplasmin has long been known to possess ferroxidase activity, the ability to oxidize ferrous to ferric iron. This activity is required for its function in iron homeostasis and is thought to reduce oxidative stress by inhibition of the Fenton reaction which uses Fe^2 to generate ROS [4,10]. Ceruloplasmin has also been demonstrated to function as an acute phase protein activated by the host immune system during stress conditions [4,11].

* Corresponding author. Tel.: +1 334 844 8727; fax: +1 334 844 9208.
E-mail address: zliu@acesag.auburn.edu (Z. Liu).

1050-4648/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2010.12.033

Studies of the ceruloplasmin gene in teleost fish to-date have been limited, compared with intensive studies of the gene in mammals. A ceruloplasmin gene was characterized in icefish and it was reported that increased ceruloplasmin expression served as a compensatory mechanism to prevent accumulation of ferrous iron in hemoglobin-less fish [12]. Channel catfish (*Ictalurus punctatus*) is the most important aquaculture species in the United States, accounting for more than 60% of all U.S. aquaculture production [13]. A number of genes involved in iron metabolism have been characterized in catfish recently [14–17]. In previous microarray studies of catfish transcriptomic responses to disease [18,19], gene features representing catfish ceruloplasmin were strongly up-regulated at three days following infection. To better characterize and analyze the catfish ceruloplasmin gene in relation to antioxidative and immune responses, here we generated the full genomic sequence of the ceruloplasmin gene, determined its genomic organization, localized it on the catfish physical and linkage maps, captured patterns of tissue expression and analyzed ceruloplasmin transcriptional responses to bacterial infection and iron overload.

2. Materials and methods

2.1. Identification of ESTs, BAC library screening and genomic sequencing of the catfish ceruloplasmin gene

To identify partial cDNAs encoding the channel catfish ceruloplasmin gene, BLAST searches were conducted using expressed sequence tags (ESTs) from previous sequencing efforts [20–24]. All channel catfish ESTs were assembled into contiguous sequences (contigs) using the sequence assembly program CAP3 program [25]. Two contigs each harboring partial cDNA of ceruloplasmin were identified by BLAST analyses, and were further used to design primers for BAC genomic sequencing.

The CHORI-212 BAC library [26] was screened as previously described [14–17] to identify positive BAC clones for the channel catfish ceruloplasmin gene. Briefly, screening for ceruloplasmin positive BAC clones was conducted using catfish gene-specific probes designed from cDNA sequences and generated by PCR using forward (5′-CATTGGAGGGAAATACAAGAAG-3′) and reverse (5′-ACTGTACTCCATGAGGCTGAAT-3′) primers. The PCR amplified segments of the ceruloplasmin cDNA were gel-purified and labeled with ^{32}P-dCTP using a Random Primed DNA Labeling kit (Roche Applied Science, Indianapolis, IN). After removing unincorporated nucleotides with Sephadex G50 spin columns, the labeled probes were denatured and added into hybridization tubes containing the BAC filters that had been under pre-hybridization at 63 °C for 2 h. The filters were hybridized at 63 °C for 16 h in 30 mL hybridization solution. The filters were then washed and exposed to X-ray film at −80 °C for 20 h. Positive clones were identified from the CHORI-212 BAC library, picked and cultured in 2× YT medium. BAC DNA was then isolated and sequencing reactions were performed using the BAC DNA with the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 3130XL automated DNA sequencer (Applied Biosystems) as previously described [27].

2.2. Southern blot analysis

Southern blot analysis was conducted as previously described [14–17] to determine the genomic copy number of channel catfish ceruloplasmin gene. In brief, genomic DNA was isolated from three individual adult channel catfish and 10 μg DNA was digested with the restriction endonucleases *Eco* RI, *Hin*d III or *Pst* I (New England Biolabs, Beverly, MA). The digested DNA samples were electrophoresed on a 0.7% agarose gel. The gel was submerged in 0.25 N HCl for 15 min, then in denaturation and neutralization buffer for 30 min, respectively. The DNA was transferred to positively-charged nylon membranes (Millipore, Bedford, MA) by capillary transfer and was fixed to the membrane using a UV cross-linker (Stratagene, La Jolla, CA) using the auto-crosslink setting. The membrane was hybridized with cDNA probes amplified using forward (5′-CACGAACTGCTTGAC CCGAG-3′) and reverse (5′-CCAGAATGCCCAGGTGTTCC-3′) primers, following the procedures described in 2.1.

2.3. Phylogenetic analysis

Amino acid sequences of ceruloplasmin genes from various vertebrates were retrieved from GenBank and Ensembl databases for multiple sequence alignment using ClustalW. Accession numbers for retrieved sequences were shown next to their names in the figure. Based on multiple sequence alignment, a phylogenetic tree was constructed using the neighbor-joining method within the Molecular Evolutionary Genetics Analysis 4 (MEGA 4) package [28]. Data was analyzed using Poisson correction, and gaps were removed by complete deletion. The topological stability of the trees was evaluated by 10,000 bootstrap replications.

2.4. Bacterial challenge and iron-dextran treatment

Bacterial challenge and iron-dextran treatment were conducted as previously described [14,15]. Briefly, channel catfish with an average body weight of 6.1 g and average body length of 9.5 cm, were kept at 27 °C in a flow-through system with heated municipal water. A total of 960 fish of four groups were treated by injection: (1) control group (phosphate-buffered saline, PBS (pH 7.4) injected); (2) *Edwardsiella ictaluri* challenged group; (3) iron-dextran treated group; and (4) iron-dextran treated and *E. ictaluri* challenged group.

A single colony of *E. ictaluri* grown on brain heart infusion (BHI) agar plate was isolated and cultured in BHI broth at 28 °C overnight. The bacterial culture was diluted with PBS, and 1×10^5 CFU of bacteria in 100 μL PBS were injected intraperitoneally. For the iron-dextran group, 100 μL of the iron-dextran solution (Sigma, St. Louis, MO) diluted in sterile PBS to a final concentration of 12.5 mg/mL was injected intraperitoneally. The fish in the combination group were injected with both bacteria and iron-dextran. Injections were carried out under anesthesia using tricaine methanesulfonate (MS 222) at 100 mg/L.

2.5. Tissue sampling and RNA extraction

Before dissection, fish were euthanized by MS 222 exposure at a concentration of 300 mg/L. To determine ceruloplasmin gene expression in various healthy catfish tissues, 11 tissue samples including brain, gill, heart, head kidney, trunk kidney, intestine, liver, muscle, skin, spleen, and stomach from control channel catfish were isolated, pooled, and immediately immersed in RNA*later* (Invitrogen, Carlsbad, CA). Similarly, the liver tissue from 45 fish (3 pools of 15 fish each) at 4 h, 24 h, 3 d and 7 d post-treatment in each group of the bacterial challenge/iron-dextran experiment were pooled and stored in RNA*later* (Invitrogen) for RNA extraction. Correspondingly, uninfected control samples were taken at each time interval. All samples were stored at −80 °C until RNA extraction. Pooled tissue samples were homogenized under liquid nitrogen using a mortar and pestle, and then total RNA was extracted using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA) according to the supplied protocol.

2.6. Quantitative real-time RT-PCR analysis

Quantitative real-time RT-PCR was carried out using a Fast Start RNA Master SYBR Green I Reagents Kit (Roche Applied Science,

Indianapolis, IN, USA) on a LightCycler 1.0 (Roche Applied Science), following manufacturer's instructions with modification [18,19]. The concentration of the total RNA was quantified by spectrophotometry using an Ultrospec 1100 pro (Amersham Biosciences, Fairfield, CT) and 100 ng total RNA was used in one reaction. Catfish ceruloplasmin specific forward (5′-CAGTCAGAGATCATGCTCCAAC-3′) and reverse (5′-AGATCAGCAGACCTGTCCTTTGG-3′) primers were used. The 18S rRNA gene (forward primer: 5′-AGAAACGGCTACCACATCC-3′ and reverse primer: 5′-GATACGCTCATTCCGATTACAG-3′) was used as a reference gene for comparative normalization of expression levels.

To assess the relative expression of ceruloplasmin in various catfish tissues, the spleen RNA sample was arbitrarily chosen as the calibrator (1×) and the relative abundance of ceruloplasmin was calculated for the remaining 10 tissues by ratio with the calibrator. Meanwhile, the fold induction of the channel catfish ceruloplasmin gene after different treatments was quantified in the liver. Cycle threshold (Ct) values generated by q-RT-PCR were converted to fold differences by the relative quantification method using the Relative Expression Software Tool version 2009 (REST 2009) [29]. Expression differences between control and treatment groups were assessed for statistical significance using a pairwise fixed reallocation randomization test within the REST software. The fold-change of the channel catfish ceruloplasmin gene after different treatments was made into a graphical representation; differential regulation was considered significant at $p < 0.05$.

3. Results and discussion

3.1. Sequence analysis of the channel catfish ceruloplasmin gene

Screening of a channel catfish CHORI-212 BAC library [26] led to the identification of two BAC clones (36_M7 and 58_P3) positive for the ceruloplasmin gene. The clone (58_P3) was used for sequencing of the channel catfish ceruloplasmin gene. A genomic sequence of 13,469 bp of ceruloplasmin gene was obtained and the nucleotide sequence of channel catfish ceruloplasmin gene has been deposited to GenBank with the accession number of GU936972.

Alignment of the genomic sequence with cDNA sequences indicated that similar to the zebrafish ceruloplasmin gene, the catfish ceruloplasmin gene had 20 exons and 19 introns. The sizes of ceruloplasmin exons appeared to be similar or identical among all vertebrate ceruloplasmin genes with the exception of exon 9, which was found in catfish and zebrafish sequences but not in those of mammalian species (Fig. 1). The human ceruloplasmin gene appears to have different isoforms. In hepatocytes, the human ceruloplasmin gene is biosynthesized as two transcripts of 3.7 kb and 4.2 kb, which arise from use of an alternative polyadenylation site within the 3′ UTR [8,30]. Several studies have demonstrated a GPI (glycophosphatidylinositol)-linked ceruloplasmin isoform is generated by alternative splicing of exon 19 and 20 in astrocytes and Sertoli cells [31–33]. Another human ceruloplasmin mRNA arising from alternative splicing of exon 18 and predicted to result in a protein with 4 additional amino acids has also been detected in multiple extrahepatic cell types [34]. Although no alternative isoforms of catfish ceruloplasmin were detected in this work, it is possible that other isoforms could be expressed in tissues or conditions other than those analyzed here.

The consensus full-length ceruloplasmin cDNA contained an ORF of 3222 bp encoding a putative protein of 1074 amino acids. Multiple sequence alignments using deduced amino acid sequences of ceruloplasmin genes from several organisms indicated that this gene is conserved throughout vertebrate evolution. Multiple sequence analyses revealed that of the 20 copper-binding residues within the ceruloplasmin gene, 18 amino acids are invariant in catfish, zebrafish and human, while amino acids at the other 2 sites varied in catfish and zebrafish (Fig. S1). It was reported in the icefish ceruloplasmin gene, that 4 of the 20 amino acids were changed compared with the human ceruloplasmin and 3 were different from that of zebrafish [12]. The catfish ceruloplasmin showed 62–78% identity with ceruloplasmin protein sequences of other teleost fish, and 54–55% identity with mammalian ceruloplasmin (Table 1).

3.2. Phylogenetic analysis of the channel catfish ceruloplasmin gene

In order to analyze the evolutionary relationships of ceruloplasmin genes among catfish and other vertebrate species, phylogenetic analysis was conducted based on amino acid sequences retrieved from GenBank and Ensembl databases. The ceruloplasmin homologues of the mammalian, chicken and teleost species were supported by strong bootstrap values. As shown in Fig. 2, the ceruloplasmin gene from catfish was clustered with other teleosts including zebrafish, tetraodon, medaka and ocellated icefish. As expected, the catfish ceruloplasmin gene was most closely related to zebrafish ceruloplasmin, with high bootstrap support. The mammalian ceruloplasmin genes clustered together and grouped with the chicken ceruloplasmin and formed a separate clade from the teleost ceruloplasmin genes. Two ceruloplasmin proteins, likely the result of alternative splicing, were identified in mouse.

3.3. Determination of copy number and genomic mapping of the channel catfish ceruloplasmin

Using Southern blot analysis to determine the genomic copy number, the ceruloplasmin gene was found to exist as single copy in the channel catfish genome. As shown in Fig. 3, two bands were

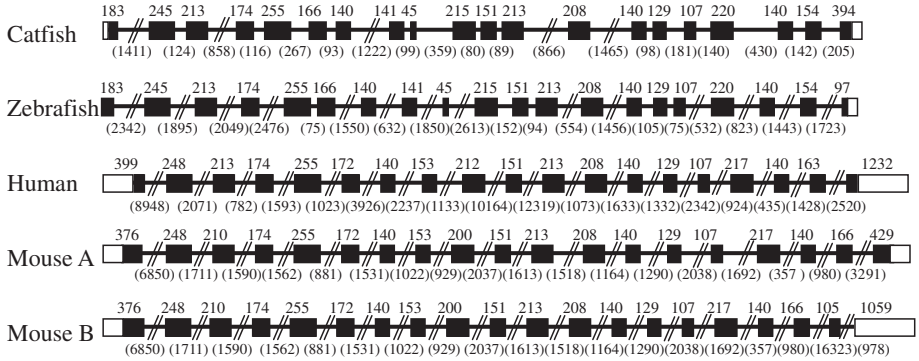

Fig. 1. Schematic diagram of ceruloplasmin gene structure. Exons are represented by black boxes, whereas the white boxes indicate non-coding regions. Values on the top of boxes are the length of the region in base pairs. The length of introns is represented in parentheses below each gene structure. Double slashes indicate non-proportional representation of the introns.

Table 1
Pairwise similarities of selected ceruloplasmin proteins.

Catfish	Zebrafish	Medaka	Tetraodon	Ocellated icefish	Human	Rat	Mouse A	Mouse B	Cattle	Chicken	
	78	63	65	62	55	54	54	55	54	56	Catfish
	65	68	64	54	54	53	54	54	57	Zebrafish
		78	78	50	50	49	49	50	52	Medaka
			79	51	51	51	51	50	52	Tetraodon
				47	48	47	47	47	50	Ocellated icefish
					82	83	83	86	60	Human
						90	90	78	59	Rat
							99	79	58	Mouse A
								79	59	Mouse B
									60	Cattle
										Chicken

observed with restriction enzyme digestion for ceruloplasmin using *Eco* R1, and only one band was observed using *Hin*d III and *Pst* I. An examination of restriction sites within the genomic sequence indicated the presence of one *Eco* R1 site within the genomic sequence. The restriction patterns highly suggested the presence of a single copy of the ceruloplasmin gene in the catfish genome.

In addition to the copy number analysis, we have determined the location of the two BAC clones (36_M7 and 58_P3) containing the ceruloplasmin gene. Through an examination of the existing physical map of the catfish genome, both BAC clones fell within a single contig, contig 482, confirming that the catfish ceruloplasmin gene exists as a single copy gene [35]. Further analysis revealed that the BAC clones positive for the ceruloplasmin gene had sequences available from both 5′ and 3′ ends of the clones [36] and the clones (36_M7) harbored a microsatellite sequence within the BAC-end sequence. This clone was subsequently mapped to the linkage map of the catfish genome linkage group LG06 [37].

3.4. Tissue expression and expression after bacterial infection and/or administration of iron-dextran of the channel catfish ceruloplasmin gene

Quantitative RT-PCR was used to determine relative tissue distribution of ceruloplasmin gene expression in 11 catfish tissues including brain, gill, head kidney, trunk kidney, heart, intestine, liver, muscle, skin, spleen and stomach. The catfish ceruloplasmin gene was mainly expressed in liver, with limited expression observed in the spleen, and very low expression observed in the other tissues tested including brain, gill, intestine, muscle, skin and stomach (Fig. 4a).

Ceruloplasmin gene expression in the liver of channel catfish after *E. ictaluri* challenge and/or iron-dextran treatment was also detected using qRT-PCR. The catfish ceruloplasmin gene was significantly up-regulated in the liver at 24 h and 3 d after injection of *E. ictaluri*. The expression of channel catfish ceruloplasmin was not significantly changed by treatment with iron-dextran alone. In groups of fish injected with both *E. ictaluri* and iron-dextran, the ceruloplasmin gene was significantly up-regulated in the liver at 24 h post-administration (Fig. 4b).

Previous studies using microarrays showed that the most highly up-regulated group of functionally related catfish genes following *E. ictaluri* infection was composed of genes involved in iron homeostasis, including intelectin, haptoglobin, hemopexin (*Wap65*), ceruloplasmin, transferrin and ferritin [18,19]. Some of these results have been confirmed and extended through further studies on several such genes as intelectin [16], *Wap65* [17] transferrin [14] and ferritin [15]. In the present study, the channel catfish ceruloplasmin was found to be induced in the liver after bacterial infection only and treatment with both bacteria and iron, confirming previous observations [18,19]. The ceruloplasmin gene is an acute phase reactant and serum concentration of ceruloplasmin has been shown to increase during inflammation and infection [11]. Fe^{+2} must be oxidized to Fe^{+3} by ceruloplasmin or hephaestin [38,39] before it can bind to its blood transport protein, transferrin, for distribution to cells of the bone marrow and elsewhere. Ceruloplasmin appears to act as a positive acute phase protein in catfish together with transferrin in order to increase iron storage to make it unavailable for bacterial growth. The function of ceruloplasmin in relation to health, as studied in humans, is yet to be defined. In spite of its antioxidant properties, it has also been noted to generate reactive oxygen species (ROS) such as superoxide and hydrogen

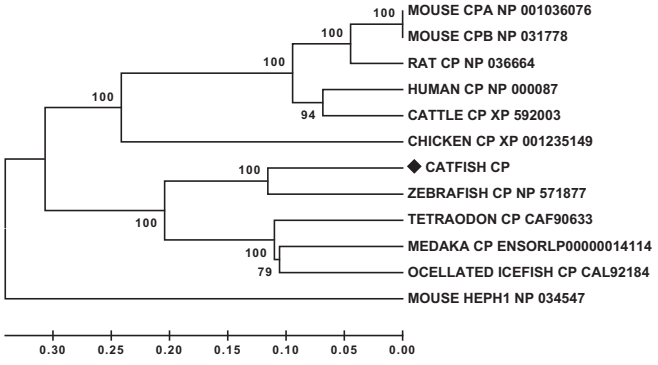

Fig. 2. Phylogenetic analysis of catfish with vertebrate ceruloplasmin genes. The phylogenetic tree was constructed using the neighbor-joining method, based on a ClustalW multiple sequence alignment of amino acid sequences. The topological stability of the tree was evaluated by bootstrapping; percentage values are indicated by numbers at the nodes.

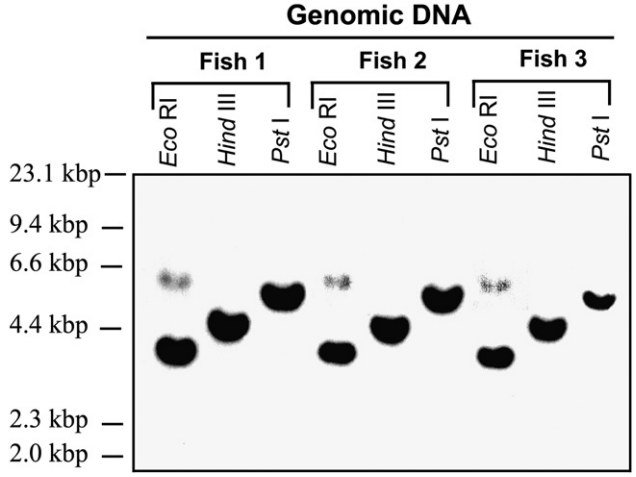

Fig. 3. Southern blot analysis of the catfish ceruloplasmin gene using genomic DNA of three channel catfish individuals. DNA markers (Kb) are indicated on the left margin.

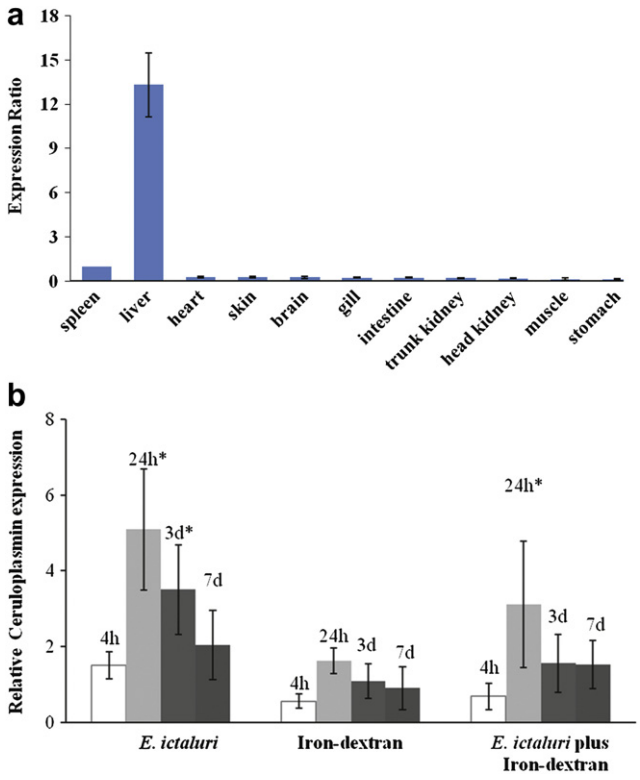

Fig. 4. (a) Relative expression of ceruloplasmin in channel catfish determined by qRT-PCR. The *Y*-axis represents normalized relative expression values of ceruloplasmin. Tissue RNA samples are labeled along the *X*-axis. Expression levels in all tissues are presented relative to that in the spleen tissue (1X). (b) Fold induction of channel catfish ceruloplasmin gene after *E. ictaluri* and/or iron-dextran treatment in the liver. Relative calreticulin expression was expressed as fold change over control samples taken at the same time interval as normalized to change in expression in the 18S control. The bars indicate mean expression of 3 tested pools (15 fish each) ±SE. Asterisks indicate statistical significance at the level of $P < 0.05$ relative to appropriate control.

peroxide, presumably through its coppers to promote vasculaopathic effects in humans [10].

In the healthy individual, copper is tightly bound to ceruloplasmin and its transfer to cells is carefully regulated. In the case of iron overload, reactive oxygen species disrupt copper binding to ceruloplasmin, thereby impairing its normal protective function while liberating copper which in turn may promote oxidative pathology and cause disease [10]. However, catfish ceruloplasmin expression was not significantly regulated by the iron-dextran treatment alone in comparison with the control, suggesting that the influx of iron did not negatively regulate the expression of the ceruloplasmin gene as might be expected. Similar results were also found in recent studies of transferrin in sea bass [40] and catfish [14], suggesting that basal levels of the transferrin gene may be sufficient to deal with the excess iron introduced in the system. Further studies are needed to better understand the crucial interplay between bacterial stimulation, antioxidant responses, and iron level-dependent signaling in the expression and function of teleost iron homeostasis genes.

Acknowledgements

This project was supported by a grant from USDA AFRI Animal Genome Basic Genome Reagents and Tools Program (USDA/NRICGP award# 2009-35205-05101), and in part by a Specific Cooperative Agreement with USDA ARS Aquatic Animal Health Laboratory, Auburn, AL under Contract Number 58-6420-5-030. The authors would like to thank Fei Chen, Jianguo Lu, Lilian Wong, Ludmilla Kaltenboeck, Parichart Ninwichian, Shaolin Wang, Yanliang Jiang and Yoona Lee for their help with collecting tissue samples.

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.fsi.2010.12.033.

References

[1] Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem 2005;12:1161–208.
[2] Crichton RR, Wilmet S, Legssyer R, Ward RJ. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J Inorg Biochem 2002;91:9–18.
[3] Anderson GJ, Frazer DM. Hepatic iron metabolism. Semin Liver Dis 2005;25 (4):420–32.
[4] Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. Annu Rev Nutr 2002;22:439–58.
[5] Holmberg CG, Laurell CB. Investigations in serum copper.II. Isolation of the copper containing protein and a description of some of its properties. Acta Chem Scand 1948;2:550–6.
[6] Musci G, Polticelli F, Calabrese L. Structure/function relationships in ceruloplasmin. Adv Exp Med Biol 1999;448:175–82.
[7] Sargent PJ, Farnaud S, Evans RW. Structure/function overview of proteins involved in iron storage and transport. Curr Med Chem 2005;12:2683–93.
[8] Yang F, Naylor S, Lum J, Cutshaw S, Jl M, Naberhaus K, et al. Characterization, mapping and expression of the human ceruloplasmin gene. Proc Natl Acad Sci USA; 1986:83.
[9] Zaitseva I, Zaitsev V, Card G, Moshkov K, Bax B, Ralph A, et al. The X-ray structure of human serum ceruloplasmin at 3.1 Å: nature of the copper centres. J Biol Inorg Chem 1996;1:15–23.
[10] Shukla N, Maher J, Masters J, Angelini GD, Jeremy JY. Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? Atherosclerosis 2006;187:238–50.
[11] Gitlin JD. Transcriptional regulation of ceruloplasmin gene expression during inflammation. J Biol Chem 1988;263:6281–7.
[12] Scudiero R, Trinchella F, Riggio M, Parisi E. Structure and expression of genes involved in transport and storage of iron in red-blooded and hemoglobin-less antarctic notothenioids. Gene 2007;397:1–11.
[13] USDA. Catfish processing report. National Agriculture Statistics Service; 2006.
[14] Liu H, Takano T, Abernathy J, Wang S, Sha Z, Jiang Y, et al. Structure and expression of transferrin gene of channel catfish, *Ictalurus punctatus*. Fish Shellfish Immunol 2010;28:159–66.
[15] Liu H, Takano T, Peatman E, Abernathy J, Wang S, Sha Z, et al. Molecular characterization and gene expression of the channel catfish ferritin H subunit after bacterial infection and iron treatment. J Exp Zool A Ecol Genet Physiol 2010;313A(6):359–68.
[16] Takano T, Sha Z, Peatman E, Terhune J, Liu H, Kucuktas H, et al. The two channel catfish intelectin genes exhibit highly differential patterns of tissue expression and regulation after infection with *Edwardsiella ictaluri*. Dev Comp Immunol 2008;32:693–705.
[17] Sha Z, Xu P, Takano T, Liu H, Terhune J, Liu Z. The warm temperature acclimation protein Wap65 as an immune response gene: its duplicates are differentially regulated by temperature and bacterial infections. Mol Immunol 2008;45:1458–69.
[18] Peatman E, Baoprasertkul P, Terhune J, Xu P, Nandi S, Kucuktas H, et al. Expression analysis of the acute phase response in channel catfish (*Ictalurus punctatus*) after infection with a Gram-negative bacterium. Dev Comp Immunol 2007;31:1183–96.
[19] Peatman E, Terhune J, Baoprasertkul P, Xu P, Nandi S, Wang S, et al. Microarray analysis of gene expression in the blue catfish liver reveals early activation of the MHC class I pathway after infection with *Edwardsiella ictaluri*. Mol Immunol 2008;45:553–66.
[20] Cao D, Kocabas A, Ju Z, Karsi A, Li P, Patterson A, et al. Transcriptome of channel catfish (*Ictalurus punctatus*): initial analysis of genes and expression profiles of the head kidney. Anim Genet 2001;32:169–88.
[21] Ju Z, Karsi A, Kocabas A, Patterson A, Li P, Cao D, et al. Transcriptome analysis of channel catfish (*Ictalurus punctatus*): genes and expression profile from the brain. Gene 2000;261:373–82.
[22] Karsi A, Cao D, Li P, Patterson A, Kocabas A, Feng J, et al. Transcriptome analysis of channel catfish (*Ictalurus punctatus*): initial analysis of gene expression and microsatellite-containing cDNAs in the skin. Gene 2002;285:157–68.
[23] Li P, Peatman E, Wang S, Feng J, He C, Baoprasertkul P, et al. Towards the ictalurid catfish transcriptome: generation and analysis of 31,215 catfish ESTs. BMC Genomics 2007;8:177.
[24] Wang S, Peatman E, Abernathy J, Waldbieser G, Lindquist E, Richardson P, et al. Assembly of 500,000 inter-specific catfish expressed sequence tags and large scale gene-associated marker development for whole genome association studies. Genome Biol; 11:R8.

[25] Huang X, Madan A. CAP3: a DNA sequence assembly program. Genome Res 1999;9:868–77.
[26] Wang S, Xu P, Thorsen J, Zhu B, de Jong PJ, Waldbieser G, et al. Characterization of a BAC library from channel catfish *Ictalurus punctatus*: indications of high levels of chromosomal reshuffling among teleost genomes. Mar Biotechnol (NY) 2007;9:701–11.
[27] Xu P, Wang S, Liu L, Peatman E, Somridhivej B, Thimmapuram J, et al. Channel catfish BAC-end sequences for marker development and assessment of syntenic conservation with other fish species. Anim Genet 2006;37:321–6.
[28] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596–9.
[29] Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;30:36.
[30] Koschinsky M, Funk W, VanOost B, MacGillivray R. Complete cNDNA sequence of human preceruloplasmin. Proc Natl Acad Sci USA 1986;83:5086–90.
[31] Patel BN, David S. A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. J Biol Chem 1997;272:20185–90.
[32] Patel BN, Dunn RJ, David S. Alternative RNA splicing generates a glycosylphosphatidylinositol-anchored form of ceruloplasmin in mammalian brain. J Biol Chem 2000;275:4305–10.
[33] Salzer JL, Lovejoy L, Linder MC, Rosen C. Ran-2, a glial lineage marker, is a GPI-anchored form of ceruloplasmin. J Neurosci Res 1998;54:147–57.
[34] Yang FM, Friedrichs WE, Cupples RL, Bonifacio MJ, Sanford JA, Horton WA, et al. Human ceruloplasmin. Tissue-specific expression of transcripts produced by alternative splicing. J Biol Chem 1990;265:10780–5.
[35] Xu P, Wang S, Liu L, Thorsen J, Kucuktas H, Liu Z. A BAC-based physical map of the channel catfish genome. Genomics 2007;90:380–8.
[36] Liu H, Jiang Y, Wang S, Ninwichian P, Somridhivej B, Xu P, et al. Comparative analysis of catfish BAC end sequences with the zebrafish genome. BMC Genomics 2009;10:592.
[37] Lu J, Peatman E, Yang Q, Wang S, Hu Z, Reecy J, et al. The catfish genome database cBARBEL: an informatic platform for genome biology of ictalurid catfish. Nucleic Acids Res; 2010. doi:10.1093/nar/gkq765.
[38] De Domenico I, Ward DM, di Patti MC, Jeong SY, David S, Musci G, et al. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J 2007;26:2823–31.
[39] Chen H, Attieh ZK, Su T, Syed BA, Gao H, Alaeddine RM, et al. Hephaestin is a ferroxidase that maintains partial activity in sex-linked anemia mice. Blood 2004;103:3933–9.
[40] Neves JV, Wilson JM, Rodrigues PNS. Transferrin and ferritin response to bacterial infection: the role of the liver and brain in fish. Dev Comp Immunol 2009;33:848–57.