Purification and characterization of glutathione peroxidase 1 in the red muscle of Pacific bluefin tuna *Thunnus orientalis*

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Abstract Glutathione peroxidase GPx1 was purified from the red muscle of Pacific bluefin tuna *Thunnus orientalis* and its enzymatic properties were characterized. The enzyme was optimally active at pH 7.4 with a specific activity for hydrogen peroxide and a K_m of 6.7 μ M. cDNA was also isolated and it contained a predicted open reading frame (ORF) encoding a 188 amino acid protein. The phylogenetic tree shows that fish including Pacific bluefin tuna, pufferfish, and zebrafish, but not mammals, possess two genetically distinct types of GPx1, i.e., GPx1a and GPx1b. The purified enzyme was classified as a fish GPx-1b enzyme on the basis of the phylogenetic tree of the GPx1 family.

Keywords Pacific bluefin tuna · Fish · Glutathione peroxidase · Red muscle · Selenium · Selenoprotein

Introduction

Glutathione peroxidase (GPx, EC1.11.1.9) reduces hydroperoxides, including hydrogen peroxides, in the presence of reduced glutathione as a means of protecting organisms from oxidative damage. Several GPx isozymes have been identified in animal cells and these have been classified into different groups according to their cellular location and substrate specificity. In humans, eight types of GPx have been identified from GPx1 to GPx8 [1]. GPx1, whose preferred substrate is hydrogen peroxide [1], is the most

abundant isozyme and is localized in the cytoplasm of animal tissues. GPx1 is a tetramer of identical subunits. Each subunit contains a selenocysteine residue in the active center that facilitates the two-electron reduction of peroxide. Glutathione acts as the electron donor to regenerate the reduced form of the selenocysteine residue.

Selenium is an essential micronutrient for human health [1–3]. Food sources include fish and other seafood, red meat, eggs, chicken, and liver [3]. Selenium is incorporated in selenocysteine residues during protein synthesis to produce antioxidant enzymes such as GPx [4–17], thioredoxin reductase [18], and selenoprotein P [19].

Recently, a novel selenium-containing compound, selenoneine, was identified as the major form of organic selenium in the muscle and other tissues of tuna [20–22]. This compound has a unique selenoketone structure and a strong antioxidant capacity. The dietary intake of selenoneine through the consumption of fish may be important in free radical detoxification mechanisms. In addition, GPx1 has been identified as a major selenoprotein in tuna muscle [20–22]. Our recent study showed that selenoneine is incorporated into human and fish cells by the organic cation/carnitine transporter OCTN1 where it mediates methylmercury detoxification (Yamashita et al., submitted). Thus, these two selenium compounds, selenoneine and GPx1, may be involved in multiple nutritional pathways and may serve as selenium antioxidants in tuna tissues. Preliminary studies have indicated that selenoneine induces the expression of GPx1 in cultured fish and mammalian cells, suggesting that selenoneine might be used as a selenium source in selenoprotein synthesis. It has been suggested that a selenium-switch controls mRNA stability and helps determine the transcript abundance of GPx1 [1]. In addition, selenium regulation has been identified in selenoprotein translation mechanisms.

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Therefore, there may be a close relationship between selenium redox status and selenoprotein synthesis. It is not known, however, whether the selenoneine that is abundant in fish cells and tissues is used in selenoprotein synthesis. Protein and mRNA levels for several selenoproteins, such as GPx1, are downregulated dramatically by selenium deficiency [23]. GPx1 activities in rat tissues increase with increasing dietary selenium and reach defined plateaus at the selenium requirement [23]. Thus, GPx1 may be a sensitive biomarker for selenium deficiency and redox status in fish.

This report focuses on the molecular characterization and expression of GPx1 in fish. GPx1 was purified and cloned from Pacific bluefin tuna muscle tissue and its biochemical properties were characterized.

Materials and methods

Materials

Pacific bluefin tuna *Thunnus orientalis* (body weight approximately 40 kg) was obtained from an aquaculture farm in the Kyushu Islands of Japan and tissues were stored at -80°C until use. Reduced glutathione, hydrogen peroxide, cumene hydroperoxide, and *t*-butyl hydroperoxide were purchased from Wako Pure Chemical (Osaka, Japan). NADPH and glutathione reductase were purchased from Oriental Yeast (Osaka, Japan). A protein calibration kit was purchased from Roche Molecular Biochemicals (Mannheim, Germany).

Enzyme assay

GPx activity was examined by monitoring the oxidation of NADPH in the presence of GSH reductase, which catalyzes the reduction of oxidized GSH formed by GPx [4]. The activity was measured at 37°C in a solution containing 10 mM sodium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM sodium azide, 2.5 mM reduced glutathione, 0.25 U/ml glutathione reductase, and 0.16 mM NADPH. Solution fluorescence was monitored with a spectrofluorophotometer (Hitachi 850, Hitachi) at 460 nm with an excitation wavelength of 360 nm. Kinetic parameters K_m and $V_{\max}$ were obtained using hydrogen peroxide, cumene hydroperoxide, and *t*-butyl hydroperoxide as substrates at pH 7.0. Data were analyzed with nonlinear regression of the Michaelis–Menten equation with Prism ver. 5 (GraphPad Software, La Jolla, CA, USA). Protein concentration was determined with Bio-Rad Protein Assay (Bio-Rad Japan, Tokyo, Japan) using bovine serum albumin as a standard. One enzyme unit of GPx activity is defined as 1 μmol of NADPH oxidized per minute at 37°C .

Purification of GPx1 from tuna red muscle

Red muscle of Pacific bluefin tuna (600 g) was cut and homogenized with a Polytron homogenizer (Kinematica, Switzerland) in three volumes of 20 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA-2Na and 1 mM GSH. The homogenate was centrifuged at $7000\times g$ for 30 min and the supernatant was passed through a DEAE-Sephadex A-50 (Sigma-Aldrich Japan) column (60 mm $\times$ 200 mm) that had been equilibrated in the same buffer described above. Fractions were concentrated with polyethylene glycol-20000 (Wako Pure Chemical Industries, Osaka), dialyzed against the buffer used to equilibrate a DEAE-Sephadex A-50 column, and passed through a second DEAE Sephadex-A50 column (60 mm $\times$ 300 mm). The column was washed with the buffer and the enzymes were eluted with a linear gradient of NaCl (0–0.2 M). The active fraction was concentrated to 5 ml with an Amicon stirred cell 8050 equipped with a PM-50 membrane (Millipore, Billerica, MA, USA), run through a gel filtration chromatography Hiprep Sephacryl S-200 column (16 mm $\times$ 600 mm, Pharmacia, Sweden), and eluted with 10 mM Tris–HCl buffer (pH 6.8) containing 100 mM NaCl, 1 mM EDTA-2Na, and 1 mM GSH. Then the active fraction was concentrated with an Amicon stirred cell 8050, equilibrated with 20 mM HEPES buffer (pH 6.8) containing 1 mM EDTA-2Na and 1 mM GSH, purified with a Mono Q column (5 mm $\times$ 20 mm, Pharmacia), washed with buffer, and eluted with a linear gradient of NaCl (0–0.5 M).

Isolation of cDNA clones

The red muscle was lysed in TriZol reagent (Invitrogen, Carlsbad, CA, USA) and the total RNA was harvested by ethanol precipitation according to the manufacturer's instructions. Poly(A) RNA was purified with oligo(dT)-resin (Oligotex; Takara Bio, Ohtsu, Japan). Double-stranded cDNA was synthesized using oligo(dT) primers and a cDNA library was prepared using a Rapid Amplification of cDNA Ends kit (Clontech Laboratories, Mountain View, CA, USA). The cDNA fragments encoding GPx1 were amplified with the following PCR primers: *gpx-1*, upstream 5'- TTT TAC GAS CTG TCC GCA AAR CTM YTG TCA GGA GA -3' corresponding to the amino acid sequence FYDLSAKLLSGD of zebrafish GPx-1a, downstream 5'- TCT TTT ACA TAT TTA TCA RAA WCY CCC CT -3' corresponding to the 3' noncoding sequence of zebrafish GPx-1a. These were subcloned into the pGEM-T Easy vector (Promega, USA). Two distinct cDNA clones encoding GPx1 were isolated. We synthesized specific PCR primers corresponding to both cDNA clones, namely TunaGpx1A-1a (5'- CTC TCC AAC GGC CAT GAA GTT TAC TTG ACC -3') and TunaGpx1B-1a (5'- CGG TCG

GAC AAC GTT TTG GAT TAG TTT GCC -3'), and amplified 5' sequences for GPx1a and GPx1b cDNA.

Sequence analysis

Nucleotide sequences were determined with a DNA sequencer (ABI model3100; Perkin Elmer, Waltham, MA, USA) and a BigDye terminator cycle sequencing kit (Perkin Elmer).

Mass spectrometry

Mass measurements were made after peak smoothing and internal calibration (Quattro II, Micromass, UK). After carboxymethylation of the purified enzyme and digestion with trypsin, the peptide fragments were identified on the basis of the deduced amino acid sequence of the cDNA using electron spray ionization mass spectrometry (ESI-MS) according to previously described methods [24].

Western blotting

Rabbit polyclonal antibodies were raised against the synthetic peptide HQENCKNDEI [amino acid (aa) 71–80] corresponding to the conserved amino acid sequence between rainbow trout GPx1 (AF281338) and Pacific bluefin tuna GPx1s. The obtained antiserum was affinity-purified using a protein G Sepharose column (GE Healthcare Japan, Tokyo, Japan). Sample tissues were homogenized and centrifuged at $10000\times g$ for 10 min at 4°C. The extracted proteins from the tissues were mixed with 1/4 volume of $5\times$ sample buffer [0.2 M Tris-HCl, pH 6.8, 10% sodium dodecyl sulfate, 10 mM 2-mercaptoethanol, 20% glycerol, 0.05% bromophenol blue, and protease inhibitor cocktail (Roche Diagnostics, Tokyo, Japan)], and heated at 100°C for 1 min. Proteins were separated in a 12.5% gel by SDS-PAGE and electroblotted onto a polyvinylidene difluoride membrane (GE Healthcare Japan). Protein bands were visualized using an ECLTM Western blotting detection kit (GE Healthcare Japan) and Hybond-ECL film (GE Healthcare Japan) according to the manufacturer's protocol.

Results

Purification

GPx was purified 565-fold by four stages of gel chromatography on DEAE-Sephadex A-50, Sephacryl S-200, and MonoQ ion exchange columns with an overall yield of 0.8% (Fig. 1; Table 1). Homogeneity was confirmed by polyacrylamide gel electrophoresis and the molecular mass was estimated to be 22 kDa. Using gel filtration on

Sephacryl S-200, we estimated the molecular mass of the complete enzyme to be 90 kDa, indicating that the native enzyme is tetramer.

Enzyme activities

The activity of the purified enzyme was characterized. The activity against hydrogen peroxide was optimal at pH 7.4 at

Fig. 1 Purification of GPx1 from the red muscle of Pacific bluefin tuna. **a** Sephacryl S-200 chromatography. Bar indicates fractions collected for the further purification step. Arrows indicate elution of marker proteins, such as ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), serum albumin (68 kDa), and ovalbumin (45 kDa). **b** monoQ ion exchange chromatography. **c** SDS-PAGE (12.5% gel) was performed after reducing the sample. The gel was stained with Coomassie Brilliant Blue R-250

37°C. K_m and V_{max} values were 6.8 μM and 1.25 s^{-1} for hydrogen peroxide, 16.0 μM and 0.38 s^{-1} for cumene hydroperoxide, 7.0 μM and 0.71 s^{-1} for *t*-butyl hydroperoxide. The K_m of the enzyme for glutathione was 1.51 mM.

cDNA cloning and expression

We isolated two distinct types of cDNA clones encoding GPx1. (Fig. 2). The selenocysteine residue in the enzymes corresponded to a TGA codon in the coding region of the cDNAs, similar to mammalian GPx1 genes and other selenoproteins. Both cDNA clones that encoded glutathione

peroxidase possessed significant homologies with the zebrafish GPx1a and GPx1b [25], and were named Pacific bluefin tuna GPx1a and GPx1b, respectively. The bluefin tuna GPx1a cDNA contained a predicted open reading frame (ORF) encoding a 189 amino acid protein (predicted molecular weight, 21728), whereas GPx1b cDNA contained a predicted ORF encoding a 188 amino acid protein (predicted molecular weight, 21451) (Fig. 3). They showed conservation of the selenocysteine residue at the active center that was encoded in a TGA codon.

GPx1a shares 88, 78, 73, 73, and 70% sequence homology with tilapia GPx1, zebrafish GPx1a [25], zebrafish GPx1b [25], southern bluefin tuna GPx1 [26], and bovine GPx1 [15], respectively (Fig. 3). GPx1b shares 97 and 76% sequence homology with southern bluefin tuna GPx1 [26] and zebrafish GPx1b [25], respectively. The phylogenetic tree of GPx1 in vertebrates shows that fish including Pacific bluefin tuna, zebrafish and *Takifugu*, but not mammals, possess two genetically distinct types of GPx1, known as GPx1a and GPx1b (Fig. 4).

The purified protein was S-carboxymethylated, and digested with trypsin. ESI-MS analysis of the resulting peptide mixture detected an ion corresponding to the amino acid sequence VVLIVNVASLUGTTTR, [M + 2H] m/z 597.54 (observed), 596.785 (calculated), which contains an S-carboxymethylated selenocysteine residue of bluefin tuna GPx1b. Thus, the purified enzyme was due to GPx1b, and selenocysteine-37 was identified at the active center of the enzyme (Fig. 3). These findings indicate that the purified enzyme consists of four identical subunits of GPx1b.

Thus, fish including Pacific bluefin tuna, *Takifugu*, and zebrafish, but not mammals, possess two genetically distinct types of GPx1, i.e., GPx1a and GPx1b. Thus, mammals

Table 1 Purification of glutathione peroxidase from the red muscle of bluefin tuna

	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg)	Purification (fold)	Yield (%)
1. Crude extract	18700	56.0	0.006	1	100
2. DEAE Sephadex A-50	1266	19.5	0.031	5	35
3. DEAE Sephadex A-50	245	10.5	0.043	7	9
4. Sephacryl S-200	79	5.3	0.067	11	4.7
5. DEAE Sephadex A-50	20	5.3	0.067	44	4.7
6. Sephacryl S-200	2.3	2.2	0.94	156	1.9
7. Mono Q	0.25	0.9	3.39	565	0.8

Fig. 2 Nucleotide sequence and deduced amino acid sequence of Pacific bluefin tuna GPx1. A putative TGA codon encoding a selenocysteine residue is *underlined*. Selenocysteine insertion sequence (SECIS) in the 3'-noncoding region is *boxed*

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1  TTGAGAAAGGGAAATAGGTGGCACAATA 28
ATGGCTAAAAAATTTACGACTTCGAGGCCAACTCTTAACGGGAGAAACGTTTGATTTCTCCACACTGCAGGGCAAAGTTGTCTCTCAT 118
M A K K I Y D F E A K L L T G E T F D F S T L Q G K V V L I
GTGAATGTGGCGTCTCTCTGAGGTACGACCACCGAGGATTACACCCAGATGAACGAGCTCCACGAGCGGTACCCGGCAAGGGGCTCGTG 208
V N V A S L I G T T T R D Y T Q M N E L H E R Y A G K G L V
ATTCTGGGAGTGCCCTGCAATCAGTTTCGGCCATCAGGAGAACTGCAAGAACGAAGAAATCCTCTTGCTCTGAAGTATGTCCTCGTCTGGA 298
I L G V P C N Q F G H Q E N C K N E E I L L S L K Y V R P G
AACGGCTTTGAGCAAAGTTTCAGCTCCTGGAGAAAGTGGATGTGAACGGGAAGAATACCCATCCCTGTTTCAGTTCTCTGAAGGAAAGT 388
N G F E P K F Q L L E K V D V N G K N T H P L F T F L K E S
CTCCCATCCCCAGTGATGAGCAACACACTTTTGTGAACGACCCAAAGTTGATCATCTGGAGCCAGCTGCAGGAACGACGTGGCCCTGG 478
L P S P S D E Q H T F L N D P K L I I W S P V C R N D V A W
AACTTTGAGAAGTTCTTAATCGGGTCAGACGGAGTGCCTTCAAGCGTTTACAGCAGGAGGTTCTCACCAGTGACATCGACGGAGACATC 568
N F E K F L I G S D G V P F K R Y S R R F L T S D I D G D I
CAGAAGCTCCTCAACCAGGCAAATAATCCAAAACGTTGTCCGACCGTCATACAGCTTCCACAGCACTACGCCAGTGTGTGTGCGTGTCC 658
Q K L L N Q A N *
ATGAATACTCCTCAGCTTCAGTAGATGGTGTGTGACACTTTTCAACACACCTGTGCTCTCTTGACTCGATGACTGTGTTCTGTTCTTT 748
TACTAAATGAAGGCATCCTCTGAAACCAAGCTAGTTGTGAGGGGGGTATCTGAATGAAGTAAAGGCTTAACGTTTCAGCTCCATTGAG 838
TGCATGTAAAGCACAGCTGTGGTTGATGTGTTAAGTGGACCA 881

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Fig. 3 Deduced amino acid sequences of Pacific bluefin tuna GPx1a, GPx1b, and bovine GPx1. A putative selenocysteine residue in the active center is boxed

bluefin tuna GPx1a	MAVRRFYDLTVKLLSG-ETFSLSALKGKVLIENVASL	GGTTTRDYT
bluefin tuna GPx1b	MAKKIYDFEAKLLTG-ETFDFTLQGGKVVLIENVASL	GGTTTRDYT
bovine GPx1	MCAAQRSAAALAAAPRTVYAFSARPLAGGEPFNLSRLGKVLLIENVASL	GGTTVRDYT
bluefin tuna GPx1a	QMNELHSRYSKGLVILGVPCNQFGHQENCKNDEILRSLKYVRPGNGFEPNFQLEKVDV	
bluefin tuna GPx1b	QMNELHERYAGKGLVILGVPCNQFGHQENCKNEEILLSLKYVRPGNGFEPKFQLEKVDV	
bovine GPx1	QMNDLQRRLLGPRGLVVLGFPCNQFGHQENAKNEEILNCLKYVRPGGGFEPNFMLFEKCEV	
bluefin tuna GPx1a	NGKDAHPLFVYLKERLPSPCDDAMALMNDPKFIIWSPVSRNDVSWNFEKFLVSPDGEPYK	
bluefin tuna GPx1b	NGKNTHPLFTFLKESLPSPSDEQHTFLNDPKLIWSPVCRNDVAWNFEKFLIGSDGVVPFK	
bovine GPx1	NGEKAHPLFAFLREVLPSPDDATALMTDPKFIWSPVCRNDVSWNFEKFLVSPDGVPVR	
bluefin tuna GPx1a	RYSRNFLTIDIEADIKELLKRVK	
bluefin tuna GPx1b	RYSRNFLTSDIDGDIQKLLNQAN	
bovine GPx1	RYSRNFLTIDIEPDITLLSQGASA

Fig. 4 Phylogenetic relationships among vertebrate GPx1 enzymes. The tree was constructed with a protein maximum likelihood program, PHYML 3.0 [30]. Bootstrap confidence values for the sequence groupings are indicated in the tree ($n = 100$). The Pacific

bluefin tuna GPx1a and GPx1b, identified in this study, are *underlined*. Sequences database accession numbers in DDBJ/EMBL/GenBank are indicated in *parentheses*. The *scale* indicates the evolutionary distance of one amino acid substitution per site

possess a single copy of *GPx1*, whereas fish possess two distinct types of *GPx1*.

Gene synteny analysis showed that zebrafish GPx1b (*zgp1b* on zebrafish chromosome 6) was orthologous to human GPx1 (human chromosome 3, p21.31). In contrast,

the gene synteny of zebrafish GPx1a (*zgp1a* on zebrafish chromosome 11) was not comparable to those of human chromosomes. Therefore, the fish *GPx1a* cluster members, including Pacific bluefin tuna *GPx1a*, zebrafish *GPx1a*, and *Takifugu GPx1a*, may be derived from

duplication of the ancestral *GPx1* gene during vertebrate evolution.

The selenocysteine insertion sequence (SECIS) is an RNA element of approximately 60 nucleotides that adopts a stem-loop structure and directs the cell to translate UGA codons as selenocysteines. These sequences were present in the 3′-noncoding region of the isolated GPx1 cDNA clones, indicating that the genes encoding selenoproteins include one or more selenocysteine residues (Fig. 2).

Specific polyclonal antibody was raised against the fish GPx1 and the expression of GPx1 in several tissues of bluefin tuna was examined by Western blot analysis (Fig. 5). The antibody cross-reacted with the 22-kDa protein band in red muscle (Fig. 5a, lane 1). The staining disappeared completely when the antibody was pre-incubated with the purified GPx1 (Fig. 5a, lane 2), indicating that the antibody is specific to bluefin tuna GPx1. GPx1 expression was detected in red muscle, heart, spleen, and hepatopancreas, but not in white muscle.

Fig. 5 Expression of GPx1 in various tissues of Pacific bluefin tuna by Western blotting. **a** Water-soluble proteins (10 μ g/lane) in the red muscle were separated in a 12.5% SDS-PAGE gel. GPx1 expression was detected by Western blotting using an anti-GPx1 antibody (lane 1). The arrow indicates a 22-kDa protein. The staining disappeared completely when the antibody (20 ng of IgG) was pre-incubated with the purified tuna GPx1 (0.5 μ g) to remove the cross-reactive antibodies (lane 2). **b** Water-soluble proteins (17 μ g/lane) in the white muscle, red muscle, heart, spleen, and hepatopancreas were separated in a 12.5% SDS-PAGE gel. GPx1 was detected by immunostaining with anti-GPx1 antibody, and enolase was detected as an internal control by Coomassie Brilliant Blue R-250 staining

Discussion

GPx1 was purified from the red muscle of Pacific bluefin tuna. The enzyme has a molecular weight of 90000 and consists of four identical 22-kDa subunits. The enzyme exhibited a broad substrate specificity against hydrogen peroxide with an optimal activity at pH 7.4 as well as organic hydroperoxides like cumene hydroperoxide and *t*-butyl hydroperoxide. K_m and V_{max} values were 6.8 μ M and 1.25 s^{-1} for hydrogen peroxide. The K_m value for hydrogen peroxide was lower than that of other known GPx1s: 83.3 μ M for human liver GPx1 [27], 12.4 μ M for southern bluefin tuna liver GPx1 [16], and 10 μ M for rainbow trout liver GPx1 [17]. We also found that K_m values of Pacific bluefin tuna GPx1 for cumene hydroperoxide and *t*-butyl hydroperoxide were 16.0 and 7.0 μ M, respectively. These values were lower than those for human GPx1 (27.8 μ M for cumene hydroperoxide and 33.3 μ M for *t*-butyl hydroperoxide) [27], southern bluefin tuna GPx1 (90 μ M for cumene hydroperoxide and 90 μ M for *t*-butyl hydroperoxide) [16], and rainbow trout GPx1 (48 μ M for cumene hydroperoxide and 3000 μ M for *t*-butyl hydroperoxide) [17]. These properties indicate that the purified enzyme is GPx1.

To examine its amino acid sequence and expression in vivo, cDNA clones encoding GPx1 were isolated. Two genetically distinct types of GPx1 have been successfully isolated, GPx1a and GPx1b. The GPx1 enzyme purified from the red muscle of tuna in the present study was identified as GPx1b on the basis of its amino acid sequence determined using mass spectrometry of trypsin digests.

In addition, SECIS is required for the incorporation of selenocysteine in selenoprotein synthesis. The nucleotide sequence of SECIS of GPx1 was well conserved in Pacific bluefin tuna.

Phylogenetic analysis of GPx1 among the known vertebrate GPx1 amino acid sequences (Fig. 4) indicated that mammals possess only a single copy of *GPx1*, whereas fish possess two distinct types of *GPx1*. Mapping of the zebrafish hox clusters showed that zebrafish has two copies of many human chromosome segments [28]. Postlethwait et al. [29] compared zebrafish genome organization with that of humans and found that large chromosome segments have been conserved for the 430 million years since the divergence of human and zebrafish lineages. At least four copies of some paralogous chromosome segments exist in zebrafish, and they generally correspond to orthologous chromosome segments in mammals, suggesting that whole genome duplication events occurred prior to the divergence of fish and mammal lineages. Thus, the fish *GPx1a* group may arise from the fish *GPx1b* group through an ancient chromosomal duplication event. Further study is required to characterize the transcriptional and translational regulation

mechanisms of these two GPx1 isozymes in fish in response to selenium exposure.

GPx1 is a major selenoprotein, and selenoneine is a low molecular weight compound that acts as a strong free radical scavenger in the red muscle of bluefin tuna [20–22]. These two organic selenium species may be important members of the redox cycle in a variety of physiological and nutritional processes in fish muscle [20, 21].

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