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Proteomic study of the effects of complex environmental stresses in the livers of goldfish (*Carassius auratus*) that inhabit Gaobeidian Lake in Beijing, China

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Abstract Recent advances in proteomics have provided an excellent opportunity to understand biological adaptation under complex environmental stress at the protein level. Gaobeidian Lake, located in Beijing, China, is characterized by complex environmental stresses by serving as both the effluent of a wastewater treatment plant and a coolant of a nearby thermal power plant. Liver is the primary organ of energy metabolism and xenobiotic detoxification. To further our understanding of how organisms that live in Gaobeidian Lake acclimatize themselves to these complex environmental stresses, hepatic protein expression patterns were examined in goldfish *Carassius auratus* that inhabit the lake. Huairou Reservoir, a drinking water source, was used as a reference site. Twenty four protein spots, which were differently expressed in the two sites, were further digested with trypsin and analyzed by matrix-assisted laser desorption/ionization (MALDI) tandem time of flight mass spectrometry (TOF/TOF). The expression of several energy metabolism and oxidative stress proteins, such as glutathione peroxidase (GPx), ferritin H3, and liver basic fatty acid-binding protein (Lb-FABP) were found to be altered in this stressful environment. In addition to the up-regulation of GPx translation, both the mRNA levels and enzymatic activity of GPx protein were elevated in goldfish living in Gaobeidian Lake. The expression of both peroxisome proliferator activated receptor (PPAR), one of the most important metabolism and stress regulation genes as well as cytochrome P450 1A1 (CYP1A1), a detoxification gene, was also detected by real-time PCR at the two sites. Increased expression levels of both PPAR-beta and CYP1A1 ($P < 0.1$) were observed in Gaobeidian Lake. Our study provides an integrative view of the expression levels of hepatic proteins and genes in goldfish under complex environmental stress that live in Gaobeidian Lake. Our results showed that anthropogenic environmental stresses in Gaobeidian Lake activated the regulation gene of lipid metabolism PPAR, elevated the lipid metabolism levels, and activated the anti-oxidative adaptation mechanism of organisms in the lake.

Keywords Goldfish · Environmental stress · 2D-PAGE · MALDI TOF/TOF · Metabolism · Anti-oxidative response

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Introduction

Gaobeidian Lake has a catchment area of approximately 0.15 km^2, and its water source is primarily the effluent of the Gaobeidian Wastewater Treatment Plant. Some physico-chemical data of water quality from Gaobeidian Lake were as follows: dissolved oxygen (DO) (3.1 ± 0.6 mg/l), suspended solid (SS) (16.0 ± 3.4 mg/l), total phosphorus (TP) (2.3 ± 0.7 mg/l), total nitrogen (TN) (27.8 ± 4.4 mg/l), chemical oxygen demand (CODcr) (46.3 ± 6.3 mg/l), and pH (7.7 ± 0.1). Water in this lake is also used as a coolant for the nearby Beijing Guohua Thermal Power Plant; the water is then returned to the lake at a higher temperature than the original temperature. Water temperatures in the lake are between 12°C and 41°C, which correspond to seasonal changes and are approximately 5–10°C higher than ambient

temperature. Organisms that live in the lake must acclimatize themselves to this complex environment and certainly environmental stressors can lead to important changes in their physiological status and reconstruction of their genotypes. In our previous study, we detected the expression profiles of heat shock proteins in goldfish inhabiting Gaobeidian Lake, and found significant up-regulation of several stress proteins, such as HSP30 in the lake (Wang et al. 2007a).

Proteins catalyze essentially every biological function. Therefore, they are the ultimate determinants of the phenotypes of cells, tissues, and organisms. High resolution, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) separates hundreds or thousands of proteins, whereas mass spectrometry (MS), in conjunction with database searching, provides a powerful means to identify these proteins (Lin et al. 2003). Therefore, proteomic methods provide a very elegant approach for understanding the homeostatic control and global physiological changes in organisms under specific conditions (Pandey and Mann 2000; Kultz and Somero 1996; Martin et al. 2003). By combining 2D-PAGE and MS, our present study investigated the protein expression of goldfish (*Carassius auratus*) that inhabit Gaobeidian Lake, as well as the transcription of several anti-oxidative and detoxification genes, as well as the nuclear receptor gene, PPAR, which is involved in lipid metabolism. Huairou Reservoir, a drinking water source to the north of Beijing, was used as a reference site. Gaobeidian Lake is a complex environment, which includes many stressors. This study attempted to analyze the influence of multiple environmental stresses on hepatic protein expression patterns and identify the genes responsible for stress tolerance.

Materials and methods

Experimental animals

Adult male goldfish (body weight: 50.4 ± 4.5 g, body length: 11.9 ± 0.6 cm) were obtained from both Gaobeidian Lake and Huairou Reservoir on November 23, 2005. The temperatures for the day of collection at the two sites were 20°C and 4°C, respectively. Fish were killed by a sharp blow to the head followed by severing the spinal cord. Hepatic tissues were dissected rapidly on ice, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis.

Sample preparation for proteomic analysis

Frozen hepatic tissue samples were pulverized to fine powder under liquid nitrogen using a mortar and pestle and homogenized using a hand-held homogenizer. The lysis buffer consisted of 40 mM Tris buffer (pH 7.5) containing 7 M urea, 2 M thiourea, 1% dithiothreitol (DTT), 4% 3-[3-(cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), and 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation (10 min at 12,000*g*, 4°C), the supernatant containing the soluble protein fraction was recovered and the protein concentration was determined using the Bradford assay (Bradford 1976).

Two-dimensional gel electrophoresis

Protein samples from 10 male fish per site were randomly pooled into three groups ($n = 3, 3, 4$). The soluble protein (150 μg) was dissolved in rehydration solution [8 M urea, 2% CHAPS, 0.5% immobilized pH gradient (IPG) buffer, and a trace of bromophenol blue] in a volume of 450 μl. Immobiline DryStrips (Amersham Pharmacia, 18 cm, linear pH gradient from pH 4–7) were allowed to rehydrate (12 h, 30 V) in the protein solution under low viscosity oil in strip holders. Then, isoelectric focusing (IEF) was performed at 100 V for 2 h, 200 V for 2 h, 500 V for 1 h, 1,000 V for 1 h, 4,000 V for 2 h, and 8,000 V for 6 h on a Multiphor II system (Amersham Pharmacia). The temperature was maintained at 20°C. After completion of the IEF program, the strips were equilibrated for 15 min in a IPG equilibration buffer [50 mM Tris–HCl solution (pH 8.8), 6 M urea, 30% glycerol, 2% sodium dodecylsulfate (SDS), and a trace of bromophenol blue] plus 1% DTT, followed by 10 min in IPG equilibration buffer plus 1% iodoacetamide. After equilibration, the IPG strips were placed on top of a 12% polyacrylamide gel and the proteins were then separated according to their molecular weights (Mr) using an electrophoresis system (Ettan Dalt, Amersham Pharmacia). Electrophoresis was carried out at 10 mA/gel for 15 min, followed by a 6-h run at 200 V until the bromophenol blue front reached the bottom of the gels. The gels were then stained with either Coomassie Brilliant Blue G-250 or silver nitrate. Three 2D electrophoresis gels were performed for each group. Unless stated otherwise, the gels shown were representative of the gels performed.

Spot detection and in-gel digestion

Images of the gels were scanned with an ImageScanner (Amersham Biosciences) and analyzed with ImageMaster software (Amersham Biosciences). Protein spots, which were differently expressed in the two sites and clearly separated in gels, were excised manually from Coomassie Brilliant Blue G-250-stained gels. Each spot was pooled from three to six gels, washed twice with 200 mM

ammonium bicarbonate in 50% acetonitrile/water (20 min at 30°C), and then dehydrated by use of acetonitrile and spun dry. Gels were subjected to digestion in 25 mM ammonium bicarbonate buffer containing 10 ng/μl modified trypsin (Promega, USA) at 37°C for 16 h.

MALDI-TOF and TOF/TOF analysis

Matrix-assisted laser desorption ionization-MS (MALDI-MS) and tandem mass spectrometry (MALDI-MS/MS) analyses were performed using the 4800 Proteomics Analyzer (Applied Biosystems, CA) with MALDI time of flight/time of flight (TOF/TOF) ion optics. We applied 1 μl of the digest mixture, mixed with 1 μl of 100 mM alpha-cyano-4-hydroxy-cinnamic acid (CHCA) in 50% acetonitrile and 0.1% trifluoracetate (TFA), onto a MALDI target plate. Data were acquired in positive MS reflector mode with a scan range from 900 Da to 4,000 Da, and five monoisotopic precursors (S/N > 200) were selected for MS/MS analysis. Due to low sample amounts, some of the spots were not analyzed by MS/MS. Peptide mass fingerprints (PMFs) and MS/MS spectra obtained were used for protein identification in an NCBI non-redundant database using the Mascot search engine (www.matrixscience.com). All mass values were considered monoisotopic and the mass tolerance was set at 75 ppm. One missed cleavage site was allowed for trypsin digestion; cysteine carbamidomethylation was assumed as a fixed modification and methionine was assumed to be partially oxidized. Results with C.I.% (Confidence Interval %) values greater than 95% were considered a positive identification.

Total RNA extraction, reverse transcription, and real-time quantitative PCR

Total RNA from hepatic samples in the two sites was isolated using the Trizol reagent (Invitrogen, USA) and treated with RNase-free DNase I (Qiagen, USA) to remove any remaining genomic DNA according to the manufacturer's instructions. RNA quality and quantity were assessed using agarose gel electrophoresis and spectrophotometric absorbency at 260/280 nm. One-microliter aliquots of total isolated RNA were used to synthesize first-strand cDNA using M-MuLV reverse transcriptase (New England Biolabs, UK) and an oligo $(dT)_{15}$ primer (Promega, USA). To confirm the alteration in lipid metabolism, and detect the anti-oxidant and detoxification response in the Gaobeidian Lake, quantitative PCR was performed on the transcriptional levels of liver basic fatty acid-binding protein (Lb-FABP), glutathione peroxidase (GPx), peroxisome proliferator activated receptor (PPAR) subtypes (PPAR-alpha, -beta, and -gamma), and cytochrome P450 1A1 (CPY1A1) from the two groups. Quantitative PCR was performed using a QuantiTect SYBR Green PCR kit (Qiagen, USA) and an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA) in a 50-μl total reaction volume including 25 μl of 2×QuantiTect SYBR Green PCR master mix, 2 μl cDNA template, and 0.2 μM each of the target-specific primers. Beta-actin served as an internal RNA control. Thermal cycling conditions were as follows: 95°C for 15 min and 45 cycles of 94°C for 20 sec, 58°C for 30 sec and 72°C for 30 sec. Fold differences in the levels of gene expression were calculated using the 2-DeltaDelta CT method (Livak and Schmittgen 2001). PCR primers for GPx, PPAR-alpha, -beta, and -gamma were developed based on goldfish sequences in GenBank (accession numbers DQ983598, AY198322, AY894894, AY894893, respectively). PCR primers for Lb-FABP and CYP1A1 for goldfish were developed based on the zebrafish (*Danio rerio*) (GenBank accession number AF254642), and the carp (*Cyprinus carpio*) sequences (GenBank accession number AB048939), respectively.

Homogenate and GPx assay

Homogenization of goldfish hepatic tissues was performed in ice-cold 50 mM potassium-phosphate buffer containing 0.5 mM EDTA (pH 7.0) and centrifuged for 10 min at 12,000*g* and 4°C. The supernatant was collected and used for the GPx assay by the method of Rotruck et al. (1973), which is based on the reaction between the glutathione remaining after the action of GPx and 5,5′-dithiobis-(2-nitrobenzoic acid) to yield a compound that absorbs light at 412 nm. One unit of GPx activity was defined as 1 μmol/l GSH consumption after 1 min at 37°C (U/mg protein/min).

Statistical analysis

All values are expressed as the mean ± SD. A statistical comparison was accomplished using a two-tailed *t* test. The level of significance was set at $P < 0.1$.

Results and discussion

Hepatic protein expression patterns in goldfish

An effective separation of goldfish hepatic proteins was obtained by 2D-PAGE, applying a linear pH (pH 4–7) gradient in the first dimension and 12% PAGE in the second dimension. Optimal resolution was obtained by loading samples containing 150 μg protein (Fig. 1).

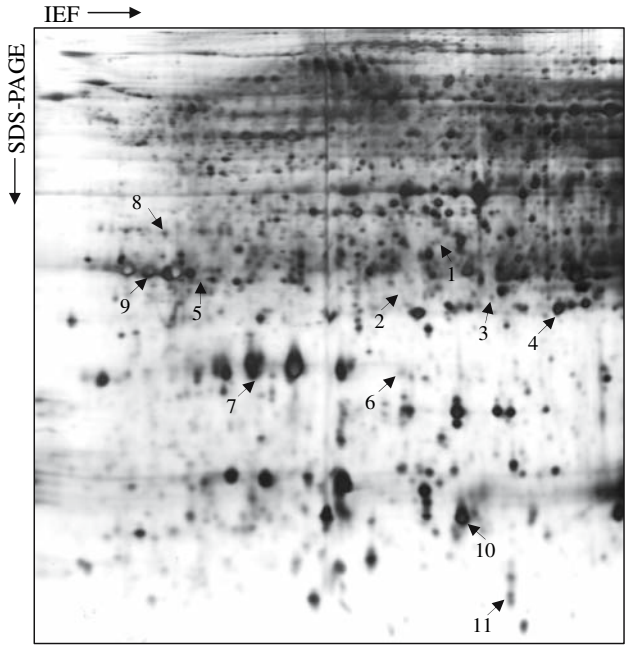

Fig. 1 Protein expression patterns of goldfish hepatic tissue from Gaobeidian Lake. Proteins (150 μg/sample) were separated by charge using Immobiline DryStrips (linear pH gradient from pH 4–7) and by Mr on a 12% polyacrylamide gel, and detected by silver staining. Spot numbers refer to the numbers given in Table 1

Attempts to increase the amount of loaded material resulted in poorly resolved gels (data not shown). Twenty-four spots in the gels were selected for digestion with trypsin. The masses of the resulting peptides (and ions) were measured by MALDI TOF/TOF MS, and matched to peptide (and ion) masses that were generated theoretically from proteins in the database (Table 1). Notwithstanding the fact that the PMFs and ion spectra approach is useful for identifying proteins in 2D gel spots, the mass spectrometric identification of proteins strongly relies on the presence of sequence information in the database. In our study, more than half of the 24 proteins selected for identification by spectrometry did not yield an unambiguous protein identification; most of the matches in the current study were homologous counterparts in organisms, such as zebrafish, in which genomic sequence information has been well-developed. The lack of unambiguous protein identification is most likely attributable to the absence of both genomic and proteomic goldfish sequences in the database. To date, there are only about 1,500 sequences in the goldfish nucleotide database at the NCBI. On the other hand, due to the absence of goldfish sequence information, it is difficult to determine the exact protein identity in a given sample. In our results, several protein spots were identified by MS/MS to be the same, such as ferritin H3 (spots 6 and 7) and Lb-FABP (spots 10 and 11). Given the fact that FABP is a member of a multigene family (Glatz and van der Vusse 1996), and several FABP variants are present in catfish (*Rhamdia sapo*), shark (*Halaetunus bivius*), and elephant fish (*Callorhynchus callorhynchus*) (Di Pietro et al. 1997; Cordoba et al. 1998, 1999), a rational explanation to the presence of two spots is that they likely represent paralogs of FABPs.

Table 1 List of spots/proteins identified by MS+MS/MS analysis from goldfish liver after 2D electrophoresis

Spot	Protein name	Organism	GenBank protein (gi)	Protein score	Protein score C.I.%	Total ion score	Total ion C.I.%	Expression change
1	Hypothetical protein LOC393297	*Danio rerio*	41056185	89	99.992	70	99.997	–
2	Betaine homocysteine methyltransferase	*Danio rerio*	56121765	105	100	92	100	–
3	Proteasome (prosome, macropain) subunit, beta type, 2	*Danio rerio*	50540284	85	99.98	47	99.356	–
4	Glutathione peroxidase	*Danio rerio*	29648593	85	99.98	63	99.966	+
5	Unnamed protein product	*Tetraodon nigroviridis*	47215453	66	98.228			–
6	Ferritin H3	*Oryzias latipes*	4585816	126	100	109	100	–
7	Ferritin H3	*Oryzias latipes*	4585816	134	100	112	100	+
8	14-3-3E1 protein	*Oncorhynchus mykiss*	34452069	139	100	83	100	–
9	Hypothetical protein LOC322453	*Danio rerio*	41054557	77	99.869	72	100	+
10	Liver basic fatty acid-binding protein (Lb-FABP)	*Rhamdia sapo*	3122083	98	99.999	61	99.958	–
11	Liver basic fatty acid-binding protein (Lb-FABP)	*Rhamdia sapo*	3122083	67	98.746	52	99.772	+

Spot numbers are as shown in Fig. 1; "+" indicates the spot was elevated from Gaobeidian Lake while "−" indicates the spot was attenuated from the lake

Functions and expression patterns of differentially expressed proteins exposed to environmental stress in Gaobeidian Lake

An important objective of this study is to elucidate how environmental stresses in Gaobeidian Lake alter the protein expression patterns of goldfish that inhabit the lake. Detailed studies on altered molecules will be helpful in understanding the molecular mechanisms of stress tolerance and the complex interactions between the rigorous environment and the aquatic species that live there. The altered protein expression patterns from the two sites identified by mass spectrometry included energy metabolism proteins (Lb-FABP and betaine homocysteine methyltransferase), anti-oxidative damage proteins (GPx and ferritin H3), 14-3-3E1, and the proteasome subunit, beta type 2. Their detailed patterns on silver-stained 2D gels from the two sites are shown in Fig. 2a–h. Two hypothetical proteins (LOC393297 and LOC322453), which were predicted from genomic sequences, and an unnamed protein product (GenBank gi 47215453) were also identified by MS in our study. Lb-FABP and GPx were selected for further evaluation of their transcription levels at the two sites by real-time PCR. Their altered transcription levels were consistent with the changes in their protein expression (Fig. 3a, b).

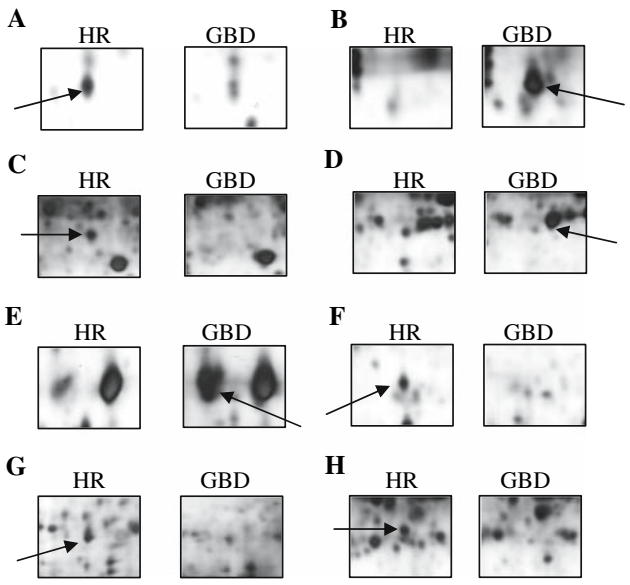

Fig. 2 Detailed patterns of protein expression (arrows) at the two sites. The representative silver stained images are shown: spot 10: Lb-FABP (**a**); spot 11: Lb-FABP (**b**); spot 2: betaine homocysteine methyltransferase (**c**); spot 4: GPx (**d**); spot 7: ferritin H3 (**e**); spot 6: ferritin H3 (**f**); spot 8: 14–3-3E1 (**g**); spot 3: proteasome subunit beta type 2 (**h**). There was differential protein expression in livers of goldfish from Gaobeidian Lake (GBD) compared with those from the Huairou Reservoir (HR)

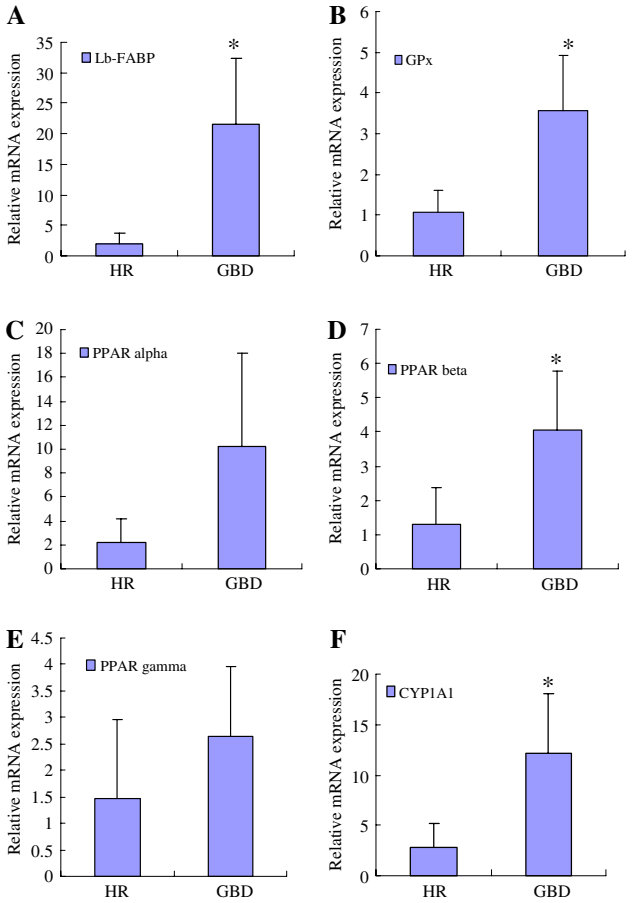

Fig. 3 Relative mRNA expression of Lb-FABP, GPx, PPAR-alpha, -beta, -gamma, and CYP1A1 at the two sites. Real-time PCR was performed on cDNA from goldfish hepatic tissues from Gaobeidian Lake (GBD) and the reference site, Huairou Reservoir (HR); results were normalized for beta-actin levels. Each value represents the mean ± SD. The * denote statistical significance ($P < 0.1$). (**a**) Lb-FABP: liver basic fatty acid-binding protein ($P = 0.036$); (**b**) GPx: glutathione peroxidase ($P = 0.042$); (**c**) PPAR-alpha: peroxisome proliferator activated receptor alpha ($P = 0.164$); (**d**) PPAR-beta ($P = 0.079$); (**e**) PPAR-gamma ($P = 0.369$); (**f**) CYP1A1: cytochrome P450 1A1 ($P = 0.069$)

Interpretation of the information contained in the comparative proteome with respect to biological function is challenging. In our study, two spots (spots 10 and 11) were identified by mass spectrometry as Lb-FABPs. Spot 11 was induced in the stressful environment in Gaobeidian Lake. We also found that the transcription of other genes involved in both lipid and fatty acid metabolism, such as apolipoprotein-AI (Apo-AI), Apo-CI precursor, and a 14-kDa apolipoprotein was elevated in Gaobeidian goldfish (Wang et al. 2007b). Lipids are an important fuel. The regulation of lipid metabolism in response to stress is an important element of the enantiostatic mechanism in animals (McClelland 2004). The elevation of these lipid binding and transport proteins in goldfish may be a kind of adaptation reaction and plays an important role in stress tolerance in Gaobeidian Lake.

The expression of PPAR subtypes

Changes in lipid metabolism can be regulated at the level of membrane transporters, at cytosolic FABP, and at fatty acid entry points into the mitochondria. The induction of many of these genes in response to stress occurs via peroxisome proliferator response elements (PPREs) at their promoter regions that bind PPAR (McClelland 2004).

PPAR is one of the most important nuclear receptor genes involved in the regulation of metabolism. Three subtypes of PPAR (-alpha, -beta, and -gamma) have been identified in mammals and they play key roles in lipid metabolism and energy homeostasis (Berger et al. 2005; Desvergne et al. 2004; Willson et al. 2000; Dreyer et al. 1992; Kliewer et al. 1994). Upon ligand binding, all of these subtypes can form heterodimers with the retinoid X receptor and then this complex regulates target gene expression, such as L-FABP through PPRE binding (Kliewer et al. 1992a, b). The three subclasses of PPARs also exist in fish (Ibabe et al. 2002; Mimeault et al. 2006). Like their mammalian homologs, fish PPARs bind to a variety of natural PPREs present in the promoters of mammalian or piscine genes (Leaver et al. 2005).

In mammals, the sequence that is upstream of the coding regions of L-FABP and Apo-AI was known to contain PPRE and the expression of these proteins was under the control of PPARs (Issemann et al. 1992; Vu-Dac et al. 1994). Although all PPAR subtypes are critical regulators of lipid homeostasis, they had been identified with distinct functions (Desvergne and Wahli 1999). To detect whether the PPAR subtypes were altered under environmental stresses in goldfish that inhabit the lake, real-time quantitative PCR experiments were performed using goldfish hepatic cDNA from the two sites. As shown in Fig. 3d, PPAR-beta transcripts from animals in Gaobeidian Lake showed higher expression levels ($P < 0.1$) than those at the reference site. Though both PPAR-alpha and -gamma altered in the same direction as PPAR-beta, the changes were not significant (Fig. 3c, e). Previous studies have shown that PPAR-beta, which is involved in the transcriptional control of the key genes of fatty acid oxidation, and activation of PPAR-beta with synthetic agonists both increase the expression of genes related to lipid oxidation (Tanaka et al. 2003; Muoio et al. 2002). Our results also emphasize the important role that PPAR-beta likely plays in the regulation of lipid metabolism in goldfish under conditions of environmental stress. The following events can be speculated: stressors in the environment result in the activation of PPAR-beta, one of the principal transcription regulators of lipid metabolism. The activated PPAR-beta then binds to the PPRE and activates its target gene expression, such as L-FABP and Apo-AI, and finally leads to the alteration of lipid metabolism status in goldfish inhabiting Gaobeidian Lake.

The transcriptional expression of GPx and CYP1A1, and GPx activity

One of our remarkable findings was the alteration of anti-oxidative genes (GPx and ferritin H3) at the two sites by 2D electrophoresis. The antioxidant defense system ensures that exposure to oxidants, including some xenobiotics, does not result in oxidative damage, including lipid peroxidation, DNA damage, and protein degradation (Choi et al. 2007).

GPx belongs to a family of cytosolic anti-oxidant enzymes and serves as the primary defense against oxidative stress by neutralizing free radicals (Kelly et al. 1998). GPx was identified by 2D electrophoresis to be up-regulated under stressful conditions in Gaobeidian Lake. An approximate 3.6-fold increase ($P < 0.05$) was detected for GPx transcripts by real-time PCR in the lake (Fig. 3b), and the GPx assay between the two groups showed similar results with 2D electrophoresis and real-time PCR (Fig. 4).

Ferritin is one of the major proteins of iron metabolism. It is ubiquitously expressed in all living organisms, from microorganisms to mammals (Harrison and Arosio 1996; Theil 1987; Andrews et al. 1992). Ferritin is also a member of the stress and inflammation response proteins and plays an important role in protection against oxidative damage. Its expression is modulated by a variety of conditions associated with oxidative stress (Arosio and Levi 2002). Previous experiments have shown that pre-exposure to acute iron loads can protect cells against oxidative damage caused by H_2O_2 and various types of oxidative insult (Balla et al. 1992; Lin and Girotti 1997), whereas artificial down-regulation of ferritin content using antisense oligonucleotides reduces resistance to oxidative stresses (Lin and Girotti 1998). Ferritin mRNA can also be induced in cells of cold-treated rainbow trout (Yamashita et al. 1996). In

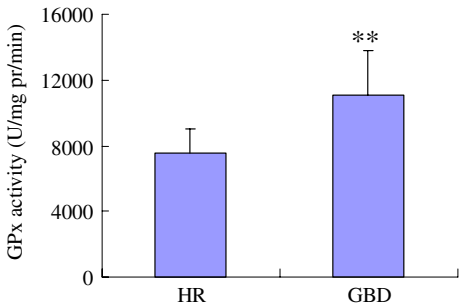

Fig. 4 GPx assay in goldfish from the two sites (GBD: Gaobeidian Lake; HR: Huairou Reservoir). Each value represents the mean ± SD. The "**" denotes $P < 0.01$

our study, two protein spots (spots 6 and 7), with a common molecular weight, were identified to be ferritin H3. The expression of protein spot 6 was attenuated in Gaobeidian Lake compared with the reference site, whereas spot 7 was significantly up-regulated in Gaobeidian Lake. These spots may represent two isoforms or paralogs of ferritin that were expressed in goldfish hepatic tissue. They were derived from alternative splicing or gene duplication, which play important roles in the generation of proteomic diversity in metazoan organisms (Blencowe 2006). Alternatively, they may represent post-translationally modified proteins, although this hypothesis cannot be verified by SDS-PAGE.

Our results for GPx and ferritin indicate that adaptive anti-oxidative responses in goldfish that inhabit Gaobeidian Lake can be triggered and may exert positive functions against oxidative damage. We also selected CYP1A1, one of the most common CYP450 family members, for analysis of its transcription levels at the two sites using real-time PCR. Its increased expression ($P = 0.069$) was observed in Gaobeidian Lake (Fig. 3f). Cytochrome P450s play key roles in elimination and detoxification of foreign compounds (Nelson et al. 1996, 2004; Nebert and Russell 2002). We did not detected other cytochrome P450s and, therefore, in terms of the activities of phase-I enzymes in the environmental stress, further work may be necessary.

In summary, using a 2D electrophoresis and MS strategy, we addressed the protein expression patterns of goldfish that inhabit Gaobeidian Lake. Some spots were further analyzed using mass spectrometry. Comparative analysis indicated that genes involved in lipid metabolism were altered and that anti-oxidative adaptation responses were activated in organisms under anthropogenic stress in Gaobeidian Lake. In three subtypes of PPAR, which is involved in the regulation of metabolism, it was PPAR-beta that was induced under conditions of complex stress. This result emphasized the probability that PPAR-beta plays an important role in the regulation of lipid metabolism in goldfish that inhabit stressful environments.

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