Quantitative Profiling of mRNA Expression of Glutathione *S*-transferase Superfamily Genes in Various Tissues of Bighead Carp (*Aristichthys nobilis*)

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ABSTRACT: The expression of glutathione S-transferase (GST) is a crucial factor in determining the sensitivity of cells and organs in response to a variety of toxicants. In this study, we cloned the core nucleotide of alpha, kappa, mu, mGST, pi, rho, and theta-like GST genes from bighead carp (*Aristichthys nobilis*). Their derived amino acid sequences were clustered with other vertebrate GSTs in a phylogenetic tree, and the bighead carp GST sequences have the highest similarity with those from common carp and zebrafish. We quantified the constitutive mRNA transcription of GST isoforms in eight different tissues (liver, kidney, spleen, intestine, muscle, heart, brain, and gill). The information obtained from the present study could be distilled into a few generalized principles: multiple GST isoenzymes were ubiquitously expressed in all tissues; majority of GSTs had high constitutive expression in intestine, liver, and kidney. These findings are in agreement with the roles of these tissues in xenobiotic metabolism. At the same time, some unique findings were detected in the current experiment: (1) higher expression of most GSTs was observed in spleen; (2) the expression of GST pi was highest in almost all the studied tissues except muscle; the other two isoforms, GST alpha and rho, were also highly expressed in liver, kidney, intestine, spleen, heart, and brain of bighead carp. All these results strongly imply an important role of these GST isoforms in detoxification of ingested xenobiotics. © 2010 Wiley Periodicals, Inc. J Biochem

INTRODUCTION

In recent decades, an increasing number of contaminants are actually present in aquatic ecosystems. Aquatic organisms suffer from increasing environmental stress, via exposure to a variety of xenobiotics, such as cynobactirial toxins, organochlorines, pesticides, phthalates, alkylphenolic compounds [1–4]. Exposure to these chemicals can affect behavior, reproduction, and physiology of fish and may also result in mutations or in death of organisms and their progeny. Moreover, a large portion of these contaminants can be transferred through the food chain, making them a potential threat to entire ecosystems and even human beings. The expression of glutathione S-transferase (GST; EC 2.5.1.18), which functions to protect cellular macromolecules from attack by reactive electrophiles, is a crucial factor in determining the sensitivity of cells and organs in response to a variety of toxicants.

The GSTs are a family of phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds [5,6]. Substrates for GSTs include many environmental pollutants, pesticides, antibiotics, antineoplastics, and carcinogenic products of phase I metabolism. Biochemical functions of GST enzymes include catalyzing the addition of GSH to electrophilic xenobiotics, facilitating the transfer of reducing equivalents to toxic products generated during oxidative stress, and chemical sequestration [7,8].

At least two ubiquitously distributed distantly related groups of GSTs are classified according to their location within the cell: microsomal and cytosolic. A third group of GSTs, the kappa class, are located in mammalian mitochondria and peroxisomes [9,10] and are structurally distinct from the microsomal and cytosolic GSTs [11]. Cytosolic (alpha, mu, omega, pi, sigma, theta, and zeta) and mitochondrial (kappa) GST(s) share some structural similarities but bear no structural resemblance to the microsomal GST (mGST) enzymes. Mammalian and most fish cytosolic GSTs are all dimeric with subunits of 199–244 amino acids in length; seven classes of cytosolic GST(s) designated alpha, mu, omega, pi, sigma, theta, and zeta in mammalian species and alpha, mu, pi, and theta in fish species are recognized [12,13]. The mammalian mitochondrial class kappa GST(s) are dimeric with subunits of 226 amino acids. Mouse, rat, human, and fish possess only a single kappa GST [5,13,14]. While in fish, a special GST isoform, which has no homologue molecules in mammals, was found in fish [15]. This special GST was named as rho class in red seabream *Pagrus major*, although its homologue molecule was found previously in the plaice *Pleuronectes platessa* and the largemouth bass *Micropterus salmoides* [6,16,17].

Profiles of GST isoenzymes constitutively expressed within tissues and changes subsequent to chemical exposure point to mechanisms regarding cellular responses to changes in environment. Profiling metabolic enzymes within tissues permits better predictions of sites of toxicity, metabolic intermediates, and responses to exposures to specific environmental pollutants. In humans, constitutive expression of the multiple isoforms of GST(s) is tissue specific, suggesting that some isoforms may have specialized functions [18]. In the mice model, the constitutive mRNA expression of 19 different GST enzymes was investigated in 14 different tissues and most GST isoforms are most highly expressed in the gastrointestinal (GI) tract and liver, which strongly suggests an important role of many GST isoforms in detoxification of ingested xenobiotics [12].

Fish GSTs play a critical role in xenobiotics detoxification and antioxidant defense. Measurement of enzyme activities or expression of GST transcripts in fish has been used as biomarker of exposure to xenobiotics [19,20,21]. Fu and Xie. [13] examined the mRNA abundance of nine GST isoforms in liver of common carp. Kim et al. [22] compared the expression of several GSTs (GST-alpha, GST-mu, GST-theta, and mGST) in different tissues of pufferfish. However, the majority of studies examining GST expression and activity have been performed using rat, mouse, and human GST(s) and tissue distribution and constitutive expression of all fish GST isoforms has not been fully characterized. The phytoplanktivorous fishes are of economic importance to humans because of their importance as food fish and their potential for biological management of cyanobacterial blooms [23,24]. Bighead carp (*Aristichthys nobilis*), one of the most important freshwater phytoplanktivorous fish, comprises not only much of the production of Chinese aquaculture [25,26] but also a substantial proportion (e.g., 6% in 1989) of the total world catch in inland waters. Therefore, the aims of the present study were to clone all known fish GSTs from bighead carp and determine tissue-specific expression of all these GST isoforms.

MATERIALS AND METHODS

Fish

Bighead carp (*Aristichthys nobilis*), a phytoplanktivorous fish, is a dominant fresh-water species in China. Male bighead carp (1 year of age with mean weight of 265 ± 25 g) were obtained from a fish farm and acclimatized in aerated fresh water tanks for 15 days before being used in the study. Water temperature was controlled at $25 \pm 1°C$, and dissolved oxygen was 6.8 ± 0.7 mg L^{-1}. Feeding of food pellets at a rate of 1% of the body weight per day was terminated 2 days before initiation of our experiment. A total of 10 different fish were killed, after cervical dislocation; the following tissues were taken: liver, kidney, spleen, muscle, intestine, brain, heart, and gill ($n = 10$). All tissues were snap-frozen in liquid nitrogen until use.

Total RNA Extraction and Reverse Transcription

Total RNA was isolated using Trizol reagent (Invitrogen, USA) and quantified by determination at OD_{260}, and the integrity of RNA was determined by gel electrophoresis. The purified total RNA (2 μg) was then reverse transcribed.

Reverse transcription was performed with oligo(dt)18 primer using first strand cDNA synthesis kit (Toyobo, Japan). The mRNA sequences of mammalian GSTs were used to search in zebrafish and pufferfish genome, and database for homologous genes, and degenerate primers were designed from conserved regions to clone partial GST cDNA sequences of bighead carp by PCR. PCR was carried out with the following conditions: 94°C/3 min; 30 cycles of 94°C/30 s, 55°C/30 s, 72°C/1 min; and 72°C/5 min. PCR products were cloned and sequenced.

Bioinformatics

The sequence identity of bighead carp GST genes with their homologues in other animals was analyzed by using the DNAstar software. Multiple sequence

TABLE 1. GST Sequences Used for Phylogenetic Tree Construction

Species	Protein	Accession No.	Species	Protein	Accession No.
Homo sapiens	Hs Alpha1	CAI13812	Tetraodon nigroviridis	Tn Mu	CAG07510
	Hs Alpha2	AAH02895		Tn Theta	CAG09655
	Hs Alpha3	NP_000838		Tn mGST1	CAF97117
	Hs Alpha4	AAH15523		Tn mGST2	CAG04538
	Hs Kappa1	AAH01231	Mus musculus	Mm Alpha1	AAH61134
	Hs Mu1	AAV38750		Mm Alpha2	AAH30173
	Hs Mu2	AAI05067		Mm Alpha3	AAH09805
	Hs Mu3	NP_000840		Mm Alpha4	AAH12639
	Hs Mu4	AAI08730		Mm Kappa1	NP_083831
	Hs Mu5	AAH58881		Mm mu1	NP_034488
	Hs Pi	AAV38753		Mm mu2	NP_032209
	Hs Theta1	NP_000844		Mm mu3	NP_034489
	Hs Theta2	AAG02373		Mm Mu4	AAH30444
	Hs omega1	NP_004823		Mm Mu6	AAH31818
	Hs omega2	NP_899062		Mm Pi	AAH61109
	Hs zeta 1	AAC33591		Mm Theta1	AAH12254
	Hs MGST	NP_064696		Mm Theta2	Q61133
Danio rerio	Dr alpha	AAH60914		Mm Theta3	AAH03903
	Dr Kappa1	XP_698521		Mm omega1	NP_034492
	Dr Mu	NP_997841		Mm omega2	NP_080895
	Dr Pi	AAH83467		Mm MGST	NP_064330
	Dr Theta	NP_956878			
	Dr Theta3	XP_692427			
	Dr Rho	CAK10882	Cyprinus carpio	Cc Alpha	ABD67507
	Dr mGST1	AAH74022		Cc Kappa	ABD67508
	Dr mGST3	XP_695658		Cc Mu	ABD67509
	Tn Alpha	CAG09409		Cc Pi	ABD67510
	Tn Kappa1	CAF97858		Cc Rho	ABD67511
				Cc Theta	ABD67512
				Cc mGST1	ABD67513

alignments were performed using the CLUSTALX program. The phylogenetic tree was constructed based on the results of alignments using the Mega 3.0 program. A bootstrap analysis was performed using 1000 replicates to test the relative support for particular clades. The GenBank accession numbers of sequences used are listed in Table 1.

Quantitative Real-Time PCR (Q-PCR) to Determine the Levels of Gene Expression

All the primers used in the Q-PCR are listed in Table 2. The specification of each pair of primers was confirmed by randomly sequencing six clones, and further confirmed by the melting curve analysis using Q-PCR. The amplification efficiency of each pair of primers was tested by constructing corresponding plasmid, and only primers with similar amplification efficiency were used in this experiment. The plasmid concentration of each GST isoform was measured at OD_{260}, and the corresponding copy numbers were calculated based on the formula that 1 μg of 1000 bp DNA equivalent to 9.1×10^{11} molecules.

To examine organ distribution of GST isoforms in different organs, Q-PCR was conducted by amplifying 1.0 μL of cDNA from the each sample, with the SYBR Green qPCR kit (Finnzymes, Finland) on a Chromo4 real-time detection system (MJ Research, USA). The

TABLE 2. Real Time PCR Primers Used in This Experiment

Target Gene	Primer Sequence (5′–3′)		Size (bp)
	Forward	Reverse	
Alpha	CTTCTGGAGGTCACTCTGATGCTGC	TGGCTCAACACCTCCTTCACAGTTT	188
Kappa	GTGCTGGTGGAGAGGGTGTCAAGAG	AAGTGAGGCAGGCTGGGTAATGTCCT	80
Mu	GCTTGCTCAACCAATCCGTCTG	GGGAGCGTCACCACAAGAATAGAAC	82
Pi	ATCTGTCCAACCTGCTCAAACCATT	AGGTCAAACAGGTTGTAGTCCGCAA	82
Rho	GCAGAAGTGAAGGCTCTCAATCCCA	AACGCACTCTCCAGATACAGACACG	106
MGST	ATGCTCCAGACCAATCCTGATGTAG	CACCACAAAGGGAATGATGTTCTCC	107
Theta	AACGACATCCAGTTCGACTACAAGA	TGGTCTGGAGTGTGGAACTTCTCAG	179

qPCR conditions were as follows: 94°C/4 min; 40 cycles of 94°C/20 s, 60–62°C/20 s, 72°C/25 s; 72°C/5 min. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that a single PCR product was detected. Each sample was run in three tubes, and PCR reactions without the addition of the template used as blanks. The absolute standard curve of each isoform was constructed in the range of 10^5–10^{11} plasmid molecules. After completion of the PCR amplification, data were analyzed with the OpticonMonitor software 2.03 version (MJ research), and the molecular copy of each GST isoform was derived from corresponding threshold value compared with the standard curve.

Statistics

A total of 10 different bighead carp ($n = 10$) was used for each experiment. The error bars in all figures represent the mean ($n = 10$) + SE of mean.

RESULTS

Molecular Clone and Phylogenetic Relationship

The corenucleotide of GST isoforms of alpha, kappa, mu, mGST, pi, rho, and theta have been cloned (the Genbank accession numbers of these sequences were GQ120527–GQ120533). These GST isoforms have not been reported in bighead carp before except GST alpha and rho. To investigate the phylogenetic relationship of bighead carp GST isoenzymes with different classes of GST enzymes from other vertebrates, a phylogenetic tree was constructed with deduced amino acid sequences (Figure 1). Different GSTs included in phylogenetic analysis formed their own clades. The bighead carp GST sequences show the highest similarity with those from common carp (*Cyprinus carpio*) and zebrafish (*Danio rerio*). However, at present an exact phylogenetic relationship is difficult to predict, due to deficient of GSTs sequences information. Since only one isoform from each class GST was found in bighead carp as yet, further research is needed to find more members of GST family.

Tissue Distribution of GST Transcripts

We quantified the constitutive mRNA transcription of GST isoforms (alpha, kappa, mu, mGST, pi, rho, and theta) in eight different tissues (liver, kidney, spleen, intestine, muscle, heart, brain, and gill). All the GST isoforms can be detected in different organs, whereas the mRNA expression of certain isoforms showed tissue predominant expression.

The copy number of GST isoforms, alpha, pi, and rho, (molecules/pg total RNA) in bighead carp was calculated from the corresponding standard curve (Figure 2). The expression of GST alpha was highest in intestine, followed by spleen and heart.

The tissue distribution pattern of GST pi showed some similarities with GST alpha, but expression of GST pi was with appreciable levels in gill whereas GST alpha expression was minimal. The copy numbers of GST rho isoform in liver, intestine, spleen, heart, and brain were all above 400 molecules/pg RNA, and it was scarcest in gill with only 23 molecules/pg RNA.

In bighead carp, the expression of GST kappa was highest in muscle about 400 molecules/pg RNA, whereas its expression in liver, kidney, spleen, heart, and brain were at the same level about 200 molecules/pg RNA (Figure 3). GST mu expression was highest in spleen followed by brain and heart. The expression of mGST was higher in intestine and liver about 500 molecules/pg RNA and showed a relatively similar level in kidney, gill, muscle, heart, and brain less than 200 molecules/pg RNA (Figure 3). The GST theta was expressed in all the tissues studied, and the expression of GST theta was relatively high in spleen, intestine, liver, kidney, heart, and brain, but its copy numbers were less than 100 molecules/pg RNA in all the tissues (Figure 3).

Figure 4 shows a composite of the tissue expression of all GST isoforms in the tissues studied. From this overall view, it is apparent that the majority of the GST isofoms are expressed in liver, kidney, intestine, and spleen whereas a few are highly expressed in heart and brain. GST pi was far highly expressed in almost all the studied tissues except muscle. GST alpha and rho were also expressed at a relatively high level in several organs. This means that GST pi, alpha, and rho may play an important role in detoxification of xenobiotics.

DISCUSSION

Many drugs and xenobiotics enhance the expression of GSTs in an isoenzyme-specific way. Differences in expression of specific GST isoenzymes within tissues might reflect various mechanisms of biological regulation and have been proposed as one underlying cause of organ-selective toxicity. Profiling GSTs has been an established method that used to predict potential sites of toxicity and metabolism in response to exposure to particular environmental pollutants [27,28]. In the present study, Q-PCR was used to measure the molecule numbers of GST isoenzymes instead of enzyme kinetic analysis because it is difficult to define a
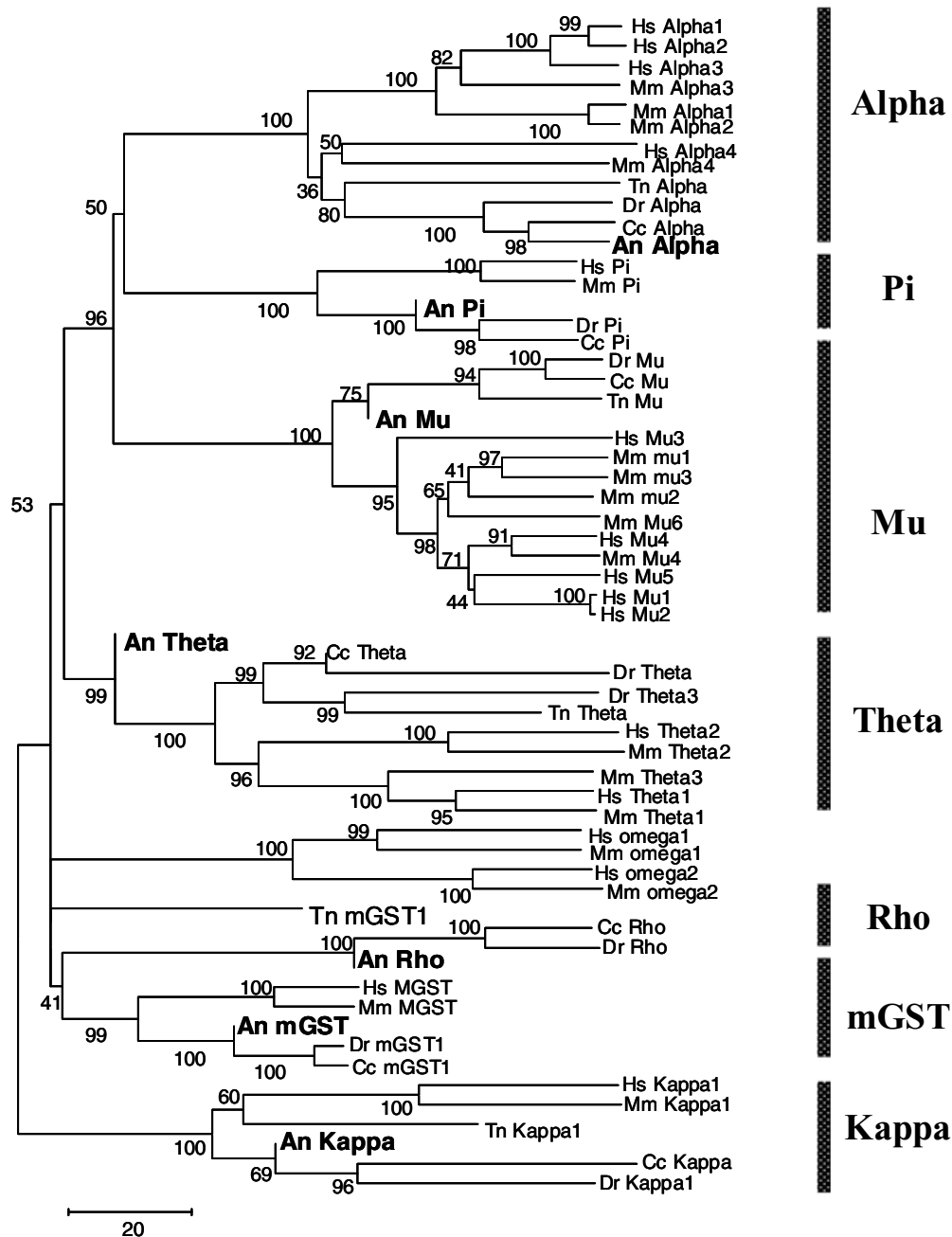
FIGURE 1. Phylogenetic tree of piscine GSTs and their homologue molecules from mammals. Multiple sequence alignments were performed using the CLUSTALX program. The phylogenetic tree based on the results of alignments was obtained by using the Mega 3.0 program. A bootstrap analysis was performed using 1000 replicates to test the relative support for particular clades. The GenBank accession numbers of sequences used are listed in Table 1.

distinction between GST classes by specific substrates because of broad and overlapping substrates [13,29]. The Q-PCR method is of greatly sensitive to detection and quantification of gene expression levels, in particular for low abundance mRNA [30,31,32].

The present study cloned seven bighead carp GST isoforms and examined their expressions in a variety of tissues, to obtain a global constitutive expression profile of these enzymes in various tissues. The information obtained from the study could be distilled into two generalized principles: (1) multiple GST isoenzymes were ubiquitously expressed in all tissues, and some express at a similar pattern in different tissues; (2) some GST isoforms were expressed in a more predominant
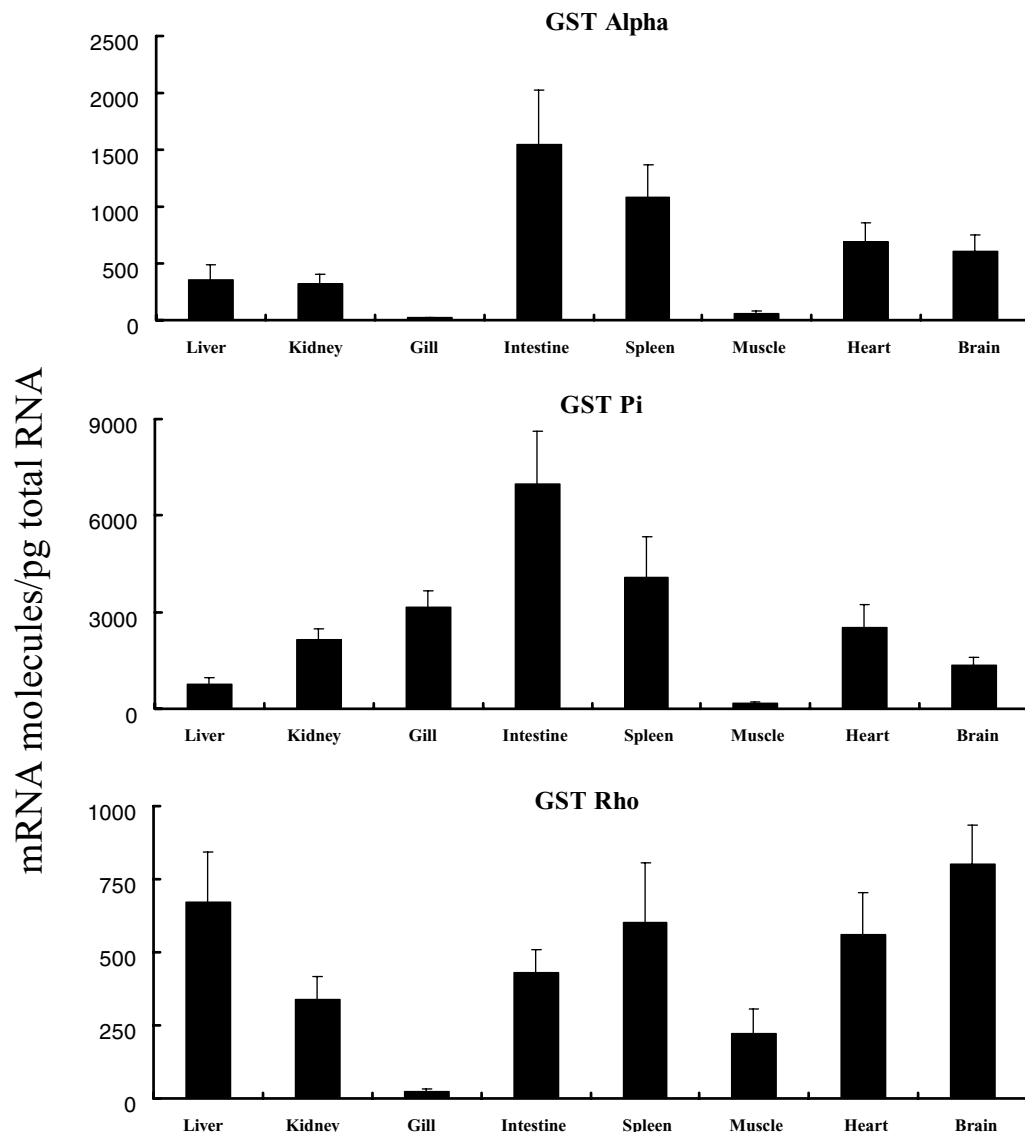
FIGURE 2. The copy numbers of bighead carp GST alpha, pi, and rho isoform (molecules/pg total RNA) were calculated by each standard curve in liver, kidney, spleen, muscle, intestine, brain, heart, and gill of bighead carp.

pattern than others, such like that GST pi was far highly expressed in almost all the studied tissues.

In the present study, many GST isoforms had high constitutively expression in liver, kidney, intestine, and spleen. The fish liver, kidney, and intestine have long been thought to be important organs involved in detoxification of xenobiotics [33,34]. Knight et al. [12] examined the constitutive mRNA expression of 19 different GST enzymes in 14 different tissues in mice and found that most GSTs expression was high in the liver, kidney, and large intestine that was consistent with our results [35]. Higher expression of certain GST isoforms in spleen was an interesting finding in the present study, because spleen is generally considered to be an immunity related tissue and not involved in xenobiotic

metabolism. Sheweita et al. [36] reported that the expression of GST isoenzymes was decreased in the spleen at 2, 4, 6, 8, and 10 weeks in *Schistosoma haematobium* infected hamsters. Thus, we may speculate that although spleen is not an organ involved in significant absorption or metabolism, the expression of specific GST isoforms in the spleen may offer protection against environmental xenobiotics.

GST pi was found to have the highest expression in almost all the studied tissues except muscle in this experiment; the other two isoforms, GST alpha and rho were also highly expressed in liver, kidney, intestine, spleen, heart, and brain of bighead carp. High expression of GST pi, alpha, and/or rho was similar with pervious studies on mammals and other fish species.
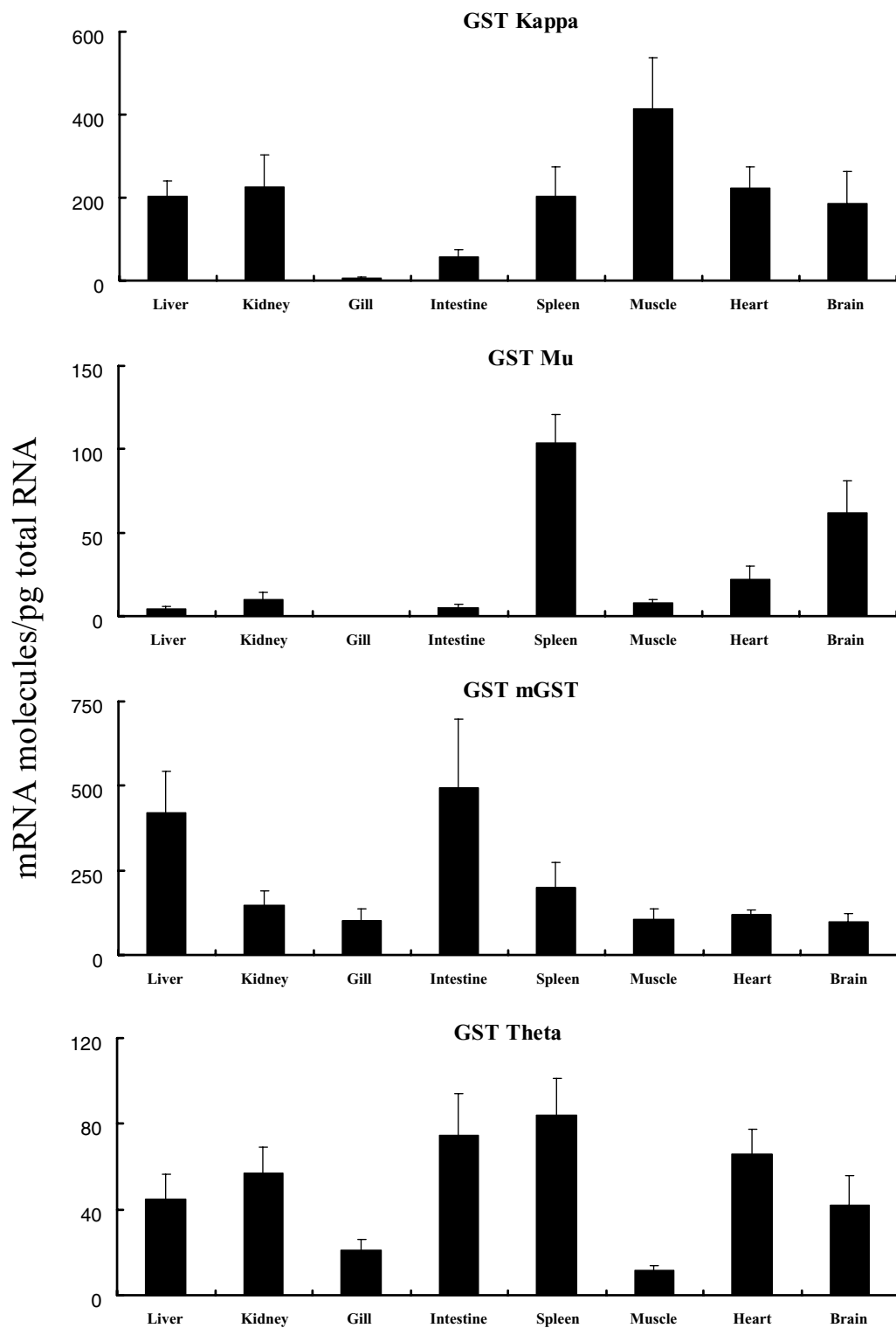
FIGURE 3. The copy numbers of bighead carp GST kappa, mu, theta, and mGST (molecules/pg total RNA) were calculated by each standard curve in liver, kidney, spleen, muscle, intestine, brain, heart, and gill of bighead carp.
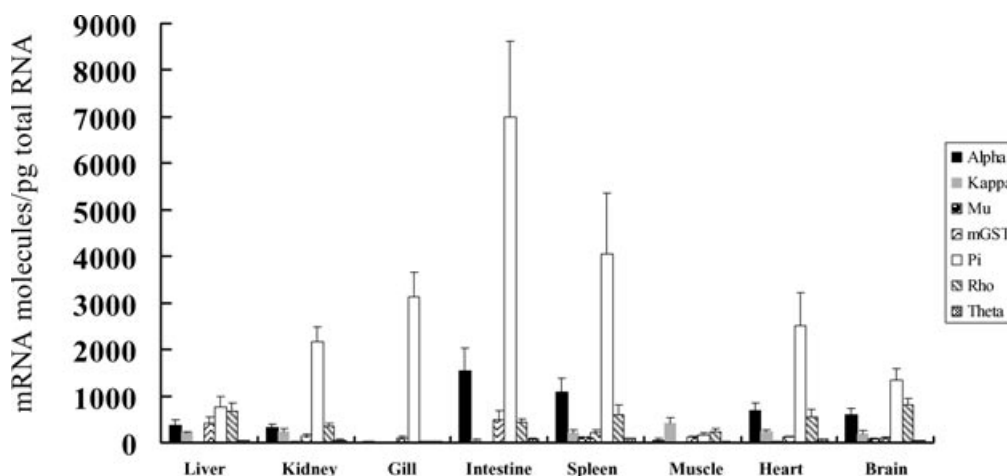
FIGURE 4. A composite of the tissue expression of all GST isoforms in the tissues studied in liver, kidney, spleen, muscle, intestine, brain, heart, and gill of bighead carp.

Coles et al. [37] quantified GST protein expression in GI tracts of human subjects using HPLC and examined interindividual variability/consistency of organ-specific patterns of expression. The authors found Gstp1, Gsta1, and Gsta2 as major, and Gstm1 and Gstm3 as minor constituents. Fu and Xie [13] examined the copy number of nine GST isoforms in the liver of common carp. The authors found that the copy numbers of GST alpha, rho, and pi isoforms were higher than others and mGST2 is the scarcest type GST. Knight et al. [12] also reported the higher expression of GST alpha3, pi, and mu1 isoform in liver of mice, and the expression lever of pi was much higher in males than in females, whereas in other organs the expression pattern of GST isoforms were different. The expression profiles described by Kim et al. [22] in pufferfish have some similarities as well differences from that in bighead carp in the present study. The authors studied the tissue distribution pattern of GST alpha, mu, theta, and mGST3 in nine different organs. All the GSTs showed the highest expression in liver, but contrary to bighead carp, the highest expression of GST theta was found in most organs. This discrepancy may be due to the differences between species, the reagents used, and also GST isoforms examined.

The expression of GST pi has always been thought to play an important role in mammals, while its function in fish has not been paid much attention to. In human, GST pi is the most widely distributed enzyme of all GSTs and the most abundant form in many tissues except in the liver [38]. It is dominating, for example, in the lungs [39] and in the rectum [40], also in lymphocytes its activity is higher as that of other GSTs [41]. Approximately, 50% of the Caucasians carry a mutation in the GSTP gene [42]. While, in fish, pharmacologists and toxicologists pay more attention to the

expression of GST alpha. Because the GST alpha class is the only GST class with Se-dependent glutathione peroxidase activity, one might expect that the highest level of these isoforms would be in liver where most of metabolism occurs. The constitutive levels of expression of xenobiotic-metabolizing enzymes in a tissue determine its ability to handle xenobiotic load [12]. Thus, GST pi and rho may play an important role in protecting cellular macromolecules against electrophiles and products of oxidative stress in fish as well.

In summary, the present experiment cloned seven bighead carp GST isoforms and examined their expressions in a number of tissues. The majority of GSTs had the highest constitutive expression in the intestine, liver, kidney, and spleen. These findings are in agreement with the roles of these tissues in xenobiotic metabolism. We also found that GST pi, alpha, and rho were highly expressed in most organs, indicating that these GST isoforms may play an important role in detoxification of xenobiotic. However, the specific roles of individual GST enzymes in each tissue still remain a matter of speculation and need more research to clarify.

ACKNOWLEDGMENTS

REFERENCES

1. Muir DC, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ. Arctic marine ecosystem contamination. Sci Total Environ 1992;122:75–134.

2. Garrigues P, Narbonne JF, Lafaurie M, Ribera D, Lemaire P, Raoux C, Michel X, Salaun JP, Monod JL, Romeo M. Banking of environmental samples for short-term biochemical and chemical monitoring of organic contamination in coastal marine environments: the GICBEM experience (1986–1990). Groupe Interface Chimie Biologie des Ecosystemes. Mar Sci Total Environ 1993; 139:225–236.

3. Nacci DE, Kohan M, Pelletier M, George E. Effects of benzo[a]pyrene exposure on a fish population resistant to the toxic effects of dioxin-like compounds. Aquat Toxicol 2002;57:203–215.

4. Chen J, Xie P, Zhang D-W, Ke Z-X, Yang H. In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic *Microcystis* blooms. Aquaculture 2006;26:1026–1038.

5. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005;45: 51–88.

6. Leaver MJ, Wright J, George SG. Structure and expression of a cluster of glutathione S-transferase genes from a marine fish, the plaice (*Pleuronectes platessa*). Biochem J 1997;321:405–412.

7. Keen JH, Jakoby WB. J Biol Chem 1978;253:5654–5657.

8. Litowsky I, Abramovitz M, Homma H, Niitsu Y. Drug Metab Rev 1988;19:305–318.

9. Morel F, Rauch C, Petit E, Piton A, Theret N, Coles B, Guillouzo A. Gene and protein characterization of the human gulutathiones S-transferase kappa and evidence for a peroxiaomal localization. J Biol Chem 2004;279:16246–16253.

10. Ladner JE, Parsons JF, Rife CL, Gilliland GL, Armstrong RN. Parallel evolutionary pathways for glutathione transferases: structure and mechanism of the mitochondrial class kappa enzyme rGSTK1-1. Biochemistry 2004;43: 352–361.

11. Robinson A, Huttley GA, Booth HS, Board PG. Modelling and bioinformatics studies of the human kappa class glutathione transferase predict a novel third transferase family with homology to prokaryotic 2-hydroxychromene-2-carboxylate isomerases. Biochem J 2004;379:541–552.

12. Knight TR, Choudhuri S, Klaassen CD. Constitutive mRNA expression of various glutathione S-transferase isoforms in different tissues of mice. Toxicol Sci 2007;100:513–524.

13. Fu J, Xie P. The acute effects of microcystin LR on the transcription of nine glutathione S-transferase genes in common carp *Cyprinus carpio* L. Aquat Toxicol 2006;80:261–266.

14. Hayes JD, Pulford DJ. The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoforms to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30:45–600.

15. Konishi T, Kato K, Araki T, Shiraki K, Takagi M, Tamaru Y. A new class of glutathione S-transferase from the hepatopancreas of the red sea bream *Pagrus major*. Biochem J 2005;388:299–307.

16. Martinez-Lara E, Leaver M, George S. Evidence from heterologous expression of glutathione *S*-transferases A and A1 of the plaice (*Pleuronectes platessa*) that their endogenous role is in detoxification of lipid peroxidation products. Mar Environ Res 2002;54:263–266.

17. Doi AM, Pham RT, Hughes EM, Barber DS, Gallagher EP. Molecular cloning and characterization of a glutathione *S*-transferase from largemouth bass (*Micropterus salmoides*) liver that is involved in the detoxification

18. Gupta S, Medh RD, Leal T, Awasthi YC. Selective expression of the three classes of glutathione S-transferase isoforms in mouse tissues. Toxicol Appl Pharmacol 1990;104:533–542.

19. Pandey S, Parvez S, Sayeed I, Haque R, Bin-Hafeez B, Raisuddin S. Biomarkers of oxidative stress: a comparative study of river Yamuna fish, *Wallago attu* (Bl. & Schn.). Sci Total Environ 2003;309:105–115.

20. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus Bloch*. Ecotoxicol Environ Saf 2003;56:295–301.

21. Sarkar A, Ray D, Shrivastava AN, Sarker S. Molecular biomarkers: their significance and application in marine pollution monitoring. Ecotoxicology 2006;15:333–340.

22. Kim JH, Raisuddin S, Rhee JS, Lee YM, Han KN, Lee JS. Molecular cloning, phylogenetic analysis and expression of a MAPEG superfamily gene from the pufferfish *Takifugu obscurus*. Comp Biochem Physiol C Toxicol Pharmacol 2008;149:358–362.

23. Opuszynski K, Shireman JV. Food habits, feeding behavior and impact of triploid bighead carp, *Aristichthys nobilis*, in experimental ponds. J Fish Bio 1995;42:517–530.

24. Xie P, Liu JK. Practical success of biomanipulation using filter-feeding fish to control cyanobacteria blooms: a synthesis of decades of research and application in a subtropical hypereutrophic lake. Sci World J 2001;1:337–356.

25. Liang YL. The apparatus of filtering and feeding of silver carp and bighead carp. J Dalian Fish College 1981;1:13–33 (in Chinese).

26. Tang Y. Evaluation of balance between fishes and available fish food s in multispecies fish culture ponds in Taiwan. Trans Am Fish Soc 198; 99:708–717.

27. Mitchell AE, Morin D, Lakritz J, Jones AD. Quantitative profiling of tissue and gender-related expression of glutathione *S*-transferase isoforms in the mouse. Biochem J 1997;325:207–216.

28. Sherratt P J, Williams S, Foster J, Kernohan N, Green T, Hayes JD. Direct comparison of the nature of mouse and human GST t1–1 and the implications on dichloromethane carcinogenicity. Toxicol Appl Pharmacol 2002;179:89–97.

29. Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 2001;360:1–16.

30. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;30:e36.

31. Wang X-W, Seed B. A PCR primer bank for quantitative gene expression analysis. Nucleic Acids Res 2003;31: e154.

32. Miao W, Yu T, Orias E, Wan M-L, Fu C-J. Identification of differentially expressed genes in *Tetrahymena thermophila* in response to Dichlorodiphenyltrichloroethane (DDT) by suppression subtractive hybridization. Environ Microbiol 2006;8:1122–1129.

33. Cazenave J, Bistoni MA, Pesce SF, Wunderlin DA. Differential detoxification and antioxidant response in diverse organs of *Corydoras paleatus* experimentally exposed to microcystin-RR. Aquat Toxicol 2006;76:1–12.

of 4-hydroxynonenal. Biochem Pharmacol 2004;67:2129–2139.

34. Woo S, Yum S, Kim DW, Park HS. Transcripts level responses in a marine medaka (*Oryzias javanicus*) exposed to organophosphorus pesticide. Comp Biochem Phy C 2009;149:427–432.

35. Thomson RE, Bigley AL, Foster JR, Jowsey IR, Elcombe CR, Orton TC, Hayes JD. Tissue-specific expression and subcellular distribution of murine glutathione *S*-transferase class kappa. J Histochem Cytochem 2004;52:653–662.

36. Sheweita SA, Mostafa MH, Ebid F, El-Sayed W. Changes in expression and activity of glutathione S-transferase in different organs of *Schistosoma haematobium* infected hamster. J Biochem Mol Toxicol 2002;17:138–145.

37. Coles BF, Chen G, Kadlubar FF, Radominska-Pandya A. Interindividual variation and organ-specific patterns of glutathione S-transferase alpha, mu, and pi expression in gastrointestinal tract mucosa of normal individuals. Arch Biochem Biophys 2002;403:270–276.

38. Suzuki T, Coggan M, Shaw DC, Board PG. Electrophoretic and immunological analysis of human glutathione-*S*-transferase isozymes. Ann Hum Genet 1987;51: 95–106.

39. Saarikoski ST, Voho A, Reinikainen M, Anttila S, Karjalainen A, Malaveille C, Vainio H, Husgafvel-Pursiainen K, Hirvonen A. Combined effect of polymorphic GST genes on individual susceptibility to lung cancer. Int J Cancer 1998;77:516–521.

40. Nijhoff WA, Grubben MJ, Nagengast FM, Jansen JB, Verhagen H, van Poppel G, Peters WH. Effects of consumption of Brussels sprouts on intestinal and lymphocytic glutathione-*S*−transferases in humans. Carcinogenesis 1995;16:2125–2128.

41. Seidegard J, Ekstrom G. The role of human glutathione transferases and epoxide hydrolases in the metabolism of xenobiotics. Environ Health Perspect 1997;105:791–799.

42. Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR. Identification of genetic polymorphisms at the glutathione-*S*-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 1997;18:641–644.