◀Research Note▶

Cloning and Expression of Cellular Glutathione Peroxidase (GPX1) in Japanese Quail (*Coturnix japonica*)

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Glutathione peroxidase 1 (cellular glutathione peroxidase, EC 1.11.1.9 (GPX1)) is an antioxidant enzyme that plays an important role in scavenging reactive oxygen species to protect cells from oxidative damage. To clone the quail GPX1, total RNA was extracted from mature female quail liver. Degenerated primers were designed based on conserved sequences among five species (human, monkey, cattle, rat, and mouse) to amplify the quail fragment GPX1 with reverse transcription polymerase chain reaction. The cDNA sequence of partial Japanese quail GPX1 is 212 nucleotides in length. The rapid amplification of cDNA ends system (5' and 3' RACE) was used to obtain the full-length Japanese quail GPX1 cDNA. The 843-nucleotide quail GPX1 cDNA contains a 153 amino acid open reading frame, a 7 bp 5'-untranslated region, and a 377 bp 3'-untranslated region containing the poly A tail. It contains putative selenocysteine residue which is encoded by an unusual stop codon TGA. Comparison of amino acid sequences showed that Japanese quail GPX1 shares 65–74% identity with GPX1 of other animals, suggesting that our clone is an authentic member of GPX1. GPX1 mRNA was expressed in all tissues examined including kidney, adrenal gland, liver, small follicle, small intestine, heart, and breast muscle; the highest level was detected in liver. This quail GPX1 cDNA will provide a tool for further studies of GPX1 expression in the quail.

Key words: antioxidant enzyme, cellular glutathione peroxidase, Japanese quail, mRNA expression, selenocysteine

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Introduction

Glutathione peroxidase (GPX) is a generic name for a family of multiple isozymes. There are four known GPXs that contain selenocysteine at the active site classical or cellular GPX (GPX-1; EC 1.11.1.9), gastrointestinal GPX (GPX-2), plasma GPX (GPX-3), and phospholipid GPX (GPX-4). Additionally, at least two other proteins (GPX5, GPX6) have over 40% sequence identity to cellular GPX but do not contain selenocysteine (Arthur, 2000; Herbert *et al.*, 2007). In mammals, GPX together with superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) constitutes an enzymatic antioxidant system which recycles reactive oxygen species (ROS) and limits their toxicity. ROS are generated as byproducts of aerobic metabolism and include molecules such as superoxide anions (O_2^-), hydroxy radicals ($OH^\cdot$), and hydroperoxides (H_2O_2). ROS can lead to oxidative damage to proteins, nucleic acids, and lipids, resulting in cellular dysfunction (Fang *et al.*, 2002). The damage induced by ROS is a main source of oxidative stress in living organisms (Han *et al.*, 2005). GPX

plays an important role in cellular antioxidant defense by reducing H_2O_2 or various hydroperoxides using glutathione as a reducing agent to form water or corresponding alcohols respectively (Herbette *et al.*, 2007).

Poultry are an important food resource for humans. They have high production efficiency and are improved continuously. That means they have high metabolic rates with ROS that are generated as byproducts of aerobic metabolism (Boveris and Chance, 1973). Thus, antioxidant defense systems are important to the health status of poultry and consequently to their productivity. Cellular glutathione peroxidase or GPX1 was the first identified selenoprotein (Rotruck *et al.*, 1976) and is the most abundant biochemical form of body selenium (Se) (Behne and Wolters, 1983). It has been widely considered to be a major antioxidant enzyme that plays an important role in protecting cells from attack by ROS (Lei and Cheng, 2005). There are no reports on the nucleotide sequence of GPX1 in any avian species. Japanese quail is an important agricultural species for meat and egg production in many countries, and is also a useful model for the extrapolation of data to chickens and other commercially important poultry species (Jones, 1996). Therefore, it is appropriate to determine the nucleotide sequence of the GPX1 gene in Japanese quail.

In this study, we first cloned and characterized GPX1

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cDNA from the liver of Japanese quail. Then the GPX1 mRNA expression was examined in various tissues of the quail.

Materials and Methods

RNA Isolation and First-Strand cDNA Synthesis

Total RNA was extracted from mature female quail liver with RNAiso (Takara Bio, Japan) according to the manufacturer's instructions. The concentration of total RNA was estimated by measuring UV light absorbance at 260 nm using a spectrophotometer (Gene Quant; Pharmacia Biotech, Uppsala, Sweden). The first-strand cDNA was synthesized from 2 µg of total RNA using SuperScript™ First-Strand Synthesis System for RT-PCR kit (Invitrogen, CA, USA) according to the manufacturer's instructions.

Cloning of Quail GPX1

Full-length GPX1 of Japanese quail was obtained by reverse transcription polymerase chain reaction (RT-PCR), and by 3' and 5' rapid amplification of cDNA (RACE). Degenerated primers were designed based on highly conserved GPX1 nucleotide sequences of human (GenBank accession number: NM000581), mouse (NM008160), rat (NM030826), monkey (AB120997) and cattle (BC149308), which were compared using the CLUSTAL-W multiple alignment algorithm (<http://align.genome.jp/>). The primers of QGPX1-F1 or QGPX1-F2, and those of QGPX1-R1 (Table 1), were used to amplify the first Japanese quail GPX1 cDNA fragment. All PCR amplifications were performed in a reaction mixture containing ExTaq polymerase (Takara Bio, Japan) on a TaKaRa PCR thermal cycler. PCR reactions were performed after 1 min of preparatory denaturation at 94°C as follows: 35 cycles of denaturation at 94°C for 30 sec, annealing at 51°C for 30 sec, and elongation at 72°C for 30 sec, followed by a 1 min extension at 72°C and cooling to 4°C. The PCR products were subjected to agarose gel electrophoresis, and the band of the expected size was purified using the Rapid PCR Purification System (Marligen Biosciences, MD, USA) according to the manufacturer's instructions. Purified DNA was subcloned into pT7 Blue T-Vector (Novagen, WI, USA) using the TA Cloning Kit (Invitrogen, CA, USA) and se-

quenced by dye-terminator chemistry on an Applied Biosystems Model 310 sequencer using the dideoxy-mediated chain-termination method (Sanger *et al.*, 1977).

Based on the partial sequence information of Japanese quail GPX1 cDNA, new primers were designed to amplify the 3' end and 5' end of Japanese quail GPX1 (Table 1). 3'-RACE (rapid amplification of 3' cDNA end) was performed using the 3' RACE System for Rapid Amplification of cDNA Ends (Invitrogen, CA, USA), and 5'-RACE (rapid amplification of 5' cDNA end) was performed using the 5' RACE System for Rapid Amplification of cDNA Ends, Version 2.0 (Invitrogen, CA, USA). To amplify the 3' end of quail GPX1, cDNA was made from messenger RNA by reverse transcription with SuperScrip II reverse transcriptase primed with Oligo-dT adapter primer (AP solution). Gene-specific primer QGPX1-3 RACE-F1 and Universal Amplification Primer (UAP) were used to amplify the 3' end. The PCR reaction conditions were the same as those described above, except the annealing temperature was changed to 62°C. To amplify the 5' end, first-strand cDNA was synthesized using gene-specific primer 1 (GSP1). PCR amplified dC-tailed cDNA using Abridged Anchor Primer (AAP) and gene-specific primer 2 (GSP2). The primary PCR product was reamplified using UAP and gene-specific primer 3 (GSP3). The PCR reaction conditions were the same as those described above, except the annealing temperature was changed to 58–62°C. Amplified 3'- and 5'-RACE PCR products were separated by 1% agarose gel electrophoresis. The bands of expected size were eluted from the gel using the Rapid Gel Extraction System (Marligen Biosciences, MD, USA) according to the manufacturer's instructions and sequenced as described above.

Sequence Analysis

The GPX1 gene sequence was analyzed and compared using the BLAST search program of GenBank (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). A multiple sequence alignment of the GPX1 gene was created using CLUSTAL W (Thomson *et al.*, 1994). Phylogenetic analysis of the aligned sequences was performed using the neighbor-joining method of the MEGA 4.0 program (Tamura *et al.*, 2007). Bootstrap analysis (500 bootstrap

Table 1. Oligonucleotides used in the study

Name	Oligonucleotide sequence	Length
QGPX1-F1	5'-CAGTTYGGRCA YCAGGAGAA-3'	20 nt
QGPX1-F2	5'-SAAYSARGAGATYCTGAA-3'	18 nt
QGPX1-R1	5'-CAGGAACCTTCTCRAAGYTCCAGG-3'	23 nt
QGPX1-3RACE-F1	5'-CTGTTCGCCCTTCCTGAGAGA-3'	21 nt
GSP1	5'-TCTCTCAGGAAGGCGAACAG-3'	20 nt
GSP2	5'-GTTACCTCGCACTTTTCG-3'	19 nt
GSP3	5'-TGAAGTTGGGTTTGAAGCCG-3'	20 nt
GPX1-F	5'-CAGTTCGGGCATCAGGAGAA-3'	20 nt
GPX1-R	5'-CGAGGAACTTGCTCGAAAGTTACCAGG-3'	27 nt
β-actin-F	5'-ACAATGTACCCTGGCATTGCT-3'	21 nt
β-actin-R	5'-TCGTCTTGT TTTATGCGCATT-3'	21 nt

replications) was employed to test the reliability of the topologies of the phylogenetic trees in the present study. The homology between the Japanese quail GPX1 amino acid sequence and other GPX1 amino acid sequences was calculated by using the FASTA program (<http://www.ebi.ac.uk/fasta33/>). The isoelectric point (pI) and molecular weight (M_w) of Japanese quail GPX1 protein were calculated using the ExPASy program (www.expasy.org/tools/pi_tool.html).

Expression Analysis of GPX1 mRNA in Tissues

PCR assays were designed to normalize GPX1 gene expression levels to β -actin mRNA. One microliter of first-strand cDNA was used for PCR amplification. The total reaction volume was 50 μ l containing 1 μ l of cDNA, 10 pmol of each forward and reverse primer, 4 μ l of dNTPs (2.5 mM each), 5 μ l of 10x PCR buffer and 1.25 u of Taq DNA-polymerase (Takara Bio, Japan). GPX1 mRNA was amplified using the GPX1-F and GPX1-R primers; β -actin mRNA was amplified using the β -actin-F and β -actin-R primers. PCR reactions for GPX1 were performed after 1 min of preparatory denaturation at 94°C as follows: 40 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec, and elongation at 72°C for 30 sec, followed by a 1 min extension at 72°C and cooling to 4°C. PCR reactions for β -actin were performed after 1 min of preparatory denaturation at 94°C as follows: 30 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and elongation at 72°C for 30 sec, followed by a 1 min extension at 72°C and cooling to 4°C. These numbers of PCR cycles ensured quantification within the exponential phase of amplification. Equal amounts of RT-PCR reactions (9 μ l) were loaded on standardized 1% agarose gels containing 0.1 μ l/ml ethidium bromide. The gel images were digitalized by a gel documentation system (Kodak Laboratories, NY, USA) and GPX1 mRNA bands were quantified by NIH Image J software. GPX1 mRNA levels were normalized to the β -actin mRNA.

Results

Cloning and Analysis of Japanese Quail GPX1 cDNA

The nucleotide and predicted amino acid sequences of the cDNA shown in Fig. 1 are presumed to represent Japanese quail GPX1. The putative transcription initiation site found by 5'-RACE is located at nucleotide (nt) position 1. The initiation site of translation was placed at nt 8, inferred by conceptual translation of the cDNA sequence in all three reading frames and by alignment with the known sequences of GPX1 proteins available in GenBank. The putative Japanese quail GPX1 cDNA (Fig. 1) is found to contain a 459 bp open reading frame (ORF) and an in-frame TAA stop codon at the 3'-end of the coding region. The ORF is flanked by a 359 bp 3'-untranslated region followed by the putative poly (A) tail. Two polyadenylation consensus sequences (5'-AATAAA-3') are located between the stop codon and the poly (A) tail. As shown in Fig. 1, the deduced GPX1 is composed of 153 amino acid residues. The theoretical molecular

weight (M_w) of the protein sequence is calculated to be 16.72 kDa and the isoelectric point (pI) to be 8.69. The predicted amino acid sequence exhibited characteristic glutathione peroxidase signature residues LGFPCNQF (residues 64–71). Japanese quail GPX1 contained two selenocysteine residues (residues 39 and 148) identified as Selenocysteine (U). The coding region of the Japanese quail GPX1 cDNA shares 65–74% identity with the GPX1 sequences from rat, mouse, dog, human, monkey, rabbit, pig, and cattle (Table 2).

Figure 2 shows the alignment of the Japanese quail GPX1 deduced amino acid sequence with this of the GPX1 of other animals. All GPX1 cDNAs had a TGA codon that encodes U which is conserved in all animals. Characteristic glutathione peroxidase signature residues (LGFPCNQF) are conserved in Japanese quail. Residues constituting the catalytic triad (U-39, Q-74 and W-142) are also conserved in Japanese quail GPX. The conserved sequences correspond largely to those parts of the protein that form the active site in bovine GPX (Epp *et al.*, 1983). These indicated that essential catalytic properties are similar between mammalian and Japanese quail GPX1.

For phylogenetic characterization, the Japanese quail GPX1 protein sequence was compared to several GPX1 sequences from various organisms. A phylogenetic tree was constructed by the neighbor-joining method using the MEGA 4 program. Phylogenetic analysis showed that Japanese quail GPX1 is clustered with the GPX1 of other organisms (Fig. 3).

GPX1 mRNA Expression Analysis

To examine the GPX1 transcription levels in Japanese quail, the GPX1 mRNA expression pattern was investigated in different tissues by RT-PCR using gene-specific primers (Fig. 4). GPX1 mRNA was widely expressed in all tissues examined including kidney, adrenal gland, liver, small intestine, heart, and breast muscle. The highest expression was observed in liver, and the lowest in the small follicle (Fig. 4).

Discussion

In this study, the full-length Japanese quail GPX1 gene sequence was cloned and characterized from the liver of mature female quail. The Japanese quail GPX1 protein contained an amino acid selenocysteine encoded by the opal termination codon (TGA). This is consistent with the fact that GPX belongs to a group of proteins known in mammals to contain selenocysteine (Sukena *et al.*, 1987). This unusual amino acid occurs at the active site of GPX and other proteins known as selenoproteins such as thioredoxin reductase and selenoprotein P (Hoffmann *et al.*, 2007). Phylogenetic analysis showed that the Japanese quail GPX1 is grouped with other GPX1. Hence, the identification of glutathione peroxidase residue LGFPCNQF, the presence of the conserved selenocysteine residue, and phylogenetic grouping with several GPX1 in the currently described protein suggested that this protein is GPX1.

To observe the distribution of this gene in Japanese

1 GGCAGCAATGGCAGCAAAGGGGCTGGCGGGGATCGTGGCGCGGCCGCTGGGCGCAGCGGAG 61
M A A K G L A G I V A R P L G A A E

62 CCGCTGGCGCTGGATTTCGCTGCGGGGCAAAGTGTCTGGTTCGTCAACGTGGCGTCGCTT 121
P L A L D S L R G K V L L V V N V A S L

122 TGAGGCACCACCGCGGATTTCCTGCAGCTCAACGAGCTGCAGCAGCGCTACGGGCCC 181
U G T T T R D F L Q L N E L Q Q R Y G P

182 CGCGGGCTGCGTGTGCTCGGCTTCCCCTGCAACCAGTTCGGGCATCAGGAGAACGCCACC 241
R G L R V L G F P C N Q F G H Q E N A T

242 AACGAGGAGATCCTGCGCTCGCTGGAGTTCGTGCGGCCCGGCAACGGCTTCAAACCCAAC 301
N E E I L R S L E F V R P G N G F K P N

302 TTCACCATGTTTCGAAAAGTGCAGAGTGAACGGGAAGGGAGCCCACCCGCTGTTTCGCCTTC 361
F T **M** F E K C E V N G K G A H P L F A F

362 CTGAGAGAGGCGCTGCCGTTCCCCACGACGACCCGTCGGCGCTGATGACCAACCCGCAG 421
L R E A L P F P H D D P S A L M T N P Q

422 TACATCATCTGGTACCCCCGTAGTGCCTTGAACGACGTCTACCTGGTTAACTTTTCGAGCAAG 481
Y I I **W** Y P R S A U T T S T **W** *

482 TTCTTCGTAGACCCCGAGGGCGTCCCCTTCCGCCGCTACAGCCGCCACTTCGAGACCATC 541
542 AAAC TG CAGGACGACATCGAGCTGCTGCTCCGGAAGGTGCCCAAGGAGGCTCTGCAATAA 601
602 **AGG**AAAGCCTGCAGCTCTGTTATATGAAGGGCTGCTGCTCCGGCTGATCTCCACCATCC 661
662 GCACATGCTTTGCTTGTGACGGCGCATCTTCCAAATCCTCGAAGTGTGCCCGATGTTTTC 721
722 AAAGCTGCGTGTGAAATGTTTGGGGGATCTCAGCTTGGGGCAGCAGCCTATGGGGCTGGT 781
782 GATGGCATGGGAGTCTATGAAATAAAGACCAATCTATCTGCAAAAAAAAAAAAAAAAAAAAA 841
842 AA 843

Fig. 1. The nucleotide sequence of cDNA for Japanese quail GPX1 and its amino acid sequence. The translation initiation (ATG) and stop (TAA) codons are double-underlined. The TGA codon that is assumed to encode selenocysteine (U) is bold and underlined. The motifs of polyadenylation signal are also bold. The data have been submitted to GenBank under the accession number AB371709.

Table 2. Homology between Japanese quail and other GPX1 amino acid sequences

Sequence from EMBL GenBank (accession number)	Number of amino acids	Percentage identity*
Rat (P04041)	201	73.9
Dog (Q6PXE0)	145	73.9
Mouse (Q5RJH8)	201	73.1
Human (P07203)	203	71.3
Pongo (Q4AEI3)	201	71.3
Pig (Q8MJ14)	206	68.1
Rabbit (P11909)	200	65.8
Cattle (P00435)	205	65.1

* Percentage amino acid identity is calculated by the FASTA program.

quail, GPX1 mRNA expression was studied by RT-PCR using β -actin as a control. Expression was found in all tissues examined and was highly expressed in liver. This

observation agrees with previous studies of mammalian species, such as rat (Ho and Howard, 1992) and Japanese monkey (Fukuhara and Kageyama, 2003), that showed that GPX1 is widely expressed in many tissues and highly expressed in liver. The GPX1 mRNA was especially abundant in tissues known to have high metabolic activity. This localization is consistent with a role for this protein in removing ROS generated through internal body metabolism. Its expression in many tissues suggests that this antioxidant enzyme may be important in providing local cellular protection. Ojano-Dirain *et al.* (2004) have reported that H_2O_2 levels are higher in the duodenal mitochondria of low-feed-efficiency broiler breeders than in high-feed-efficiency broiler breeders. This may indicate that the relationship between H_2O_2 and antioxidant enzyme is involved in the phenotypic expression of feed efficiency in broiler breeders. GPX1 is a major and ubiquitously expressed antioxidant enzyme present in cytosol and mitochondria. It is involved in the detoxification of

Rat	MSAARLSAVAQS-----TVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTTRDYT	55
Mouse	MCAARLSAAAQS-----TVYAFSARPLTGGEFVSLGSLRGKVLLIENVASLUGTTTRDYT	55
Human	MCAARLAAAAA---AQSVMYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTTVRDYT	57
Monkey	MCAARLVAAA-----AQSVMYFSAARPLAGGEPVSLGSLRGKVLLIENVASLUGTTTVRDYT	55
Rabbit	MCAARMAAAA-----QSVYSFSAHPLAGGEPVNLGSLRGKVLLIENVASLUGTTTVRDYT	54
Cattle	MCAAQRSAAALAAAAPRTVYAFSARPLAGGEPFNLSSLRGKVLLIENVASLUGTTTVRDYT	60
Pig	MCAAQRSAAALAAVAPRSVYAFSARPLAGGEPISLGSRLRGKVLLIENVASLUGTTTVRDYT	60
Quail	MAAKGLAGIV-----ARPLGAAEPLALDSLGRKVLLVNVASLUGTTTRDFL	47
	* * * * *	
Rat	EMNDLQKRLGPRGLVVLGFPCNQFGHQENGNKEEILNSLKVVRPGGGFEPNFTLFEKCEV	115
Mouse	EMNDLQKRLGPRGLVVLGFPCNQFGHQENGNKEEILNSLKVVRPGGGFEPNFTLFEKCEV	115
Human	QMNELQRRLLGPRGLVVLGFPCNQFGHQENAKNEEILNSLKVVRPGGGFEPNFTLFEKCEV	117
Monkey	QMNELQRRLLGPRGLVVLGFPCNQFGHQENAKNEEILNSLKVVRPGGGFEPNFTLFEKCEV	115
Rabbit	QMNELQERLLGPRGLVVLGFPCNQFGHQENAKNEEILNSLKVVRPGGGFEPNFTLFEKCEV	114
Cattle	QMNDLQRRLLGPRGLVVLGFPCNQFGHQENAKNEEIPNCLKVVRPGGGFEPNFTLFEKCEV	120
Pig	QMNELQRRLLGPRGLVVLGFPCNQFGHQENAKNGEILNCLKVVRPGGGFEPNFTLFEKCEV	120
Quail	QLNELQQRYGPRGLRVLGFPCNQFGHQENATNEEILRSLEFVRPGNGFKPNFTMFEKCEV	107
	* * * * *	
Rat	NGEKAHPLETFRLNALPAPSDDPALMTDPKYIIWSPVCR-NDISWNFEKFLVGPDPGVV	174
Mouse	NGEKAHPLETFRLNALPAPSDDPALMTDPKYIIWSPVCR-NDIAWNFEKFLVGPDPGVV	174
Human	NGAGAHPLFAFLREALPAPSDDATAALMTDPKLITWSPVCR-NDVAWNFEKFLVGPDPGVPL	176
Monkey	NGAGAHPLFAFLREALPAPSDDATAALMTDPKLITWSPVCR-NDVAWNFEKFLVGPDPGVPL	174
Rabbit	NGAKASPLFAFLREALPPSDDPALMTDPKFITWCPVCR-NDVSWSEKFLVGPDPGVV	173
Cattle	NGEKAHPLEFAFLREVLPPTSDDATAALMTDPKFITWSPVCR-NDVSWNFEKFLVGPDPGVV	179
Pig	NGANAHPLFAFLREALPTPSDDATAALMTDPKFITWSPVCR-NDIAWNFEKFLVGPDPGVPL	179
Quail	NGKGAHPLEFAFLREALPFPHDDPSALMTNPQYIIWYPRSAUTTSTW-----	153
	* * * * *	
Rat	RRYSRRFRTIDIEPDIEALLSKQPSNP	201
Mouse	RRYSRRFRTIDIEPDIEALLSQSGNS	201
Human	RRYSRRFQTIDIEPDIEALLSQGPSA	203
Monkey	RRYSRRFQTIDIEPDIEALLSQGPSA	201
Rabbit	RRYSRRFPTIDIEPDIEALLSKGSGGA	200
Cattle	RRYSRRFLTIDIEPDIEALLSQGASA-	205
Pig	RRYSRRFLTIDIEPDIEALLSQEPSSA	206
Quail	-----	

Fig. 2. Comparison of GPX1 amino acid sequences of Japanese quail and GPX1 of the human (accession no. NM000581), monkey (AB120997), rat (NM030826), mouse (NM008160), cattle (BC149308), pig (AF532927) and rabbit (NM001085444). Dashes indicate the amino acid gaps that are necessary to align these sequences. The conserved residues in all sequences are indicated by an asterisk (*). Glutathione peroxidase signature residues are bold and underlined. The catalytically important residues are bold.

hydrogen and lipid peroxide (Brigelius-Flohe and Flohe, 2003). It has been widely considered a major antioxidant enzyme that plays an important role in protecting cells from attack by ROS (Lei and Cheng, 2005). De Hann *et al.* (1998) demonstrated a role for GPX1 in protecting against oxidative stress by showing that Gpx1 *-/-* mice are highly sensitive to the oxidant paraquat. Liddell *et al.* (2006) demonstrated that GPX1 contributes to the rapid clearance of H₂O₂ by mouse astrocytes and that both GPX1 and a high concentration of glutathione are required to protect these cells from iron-dependent peroxide damage. These findings reinforce the notion that this antioxidant enzyme may be essential for all tissues.

In addition, glutathione peroxidases have been proposed

as possible tools for bio-monitoring environmental toxicants and stressors. Their differential expression as compared to normal expression pattern indicates exposure to cellular stress and adverse cellular effects, thus serving as biomarkers of these effects (Li *et al.*, 2003; Zhang *et al.*, 2004).

In industrial poultry production, while marked improvements have been made in both high production rates (meat and eggs) and feed efficiency (poultry), high incidences of mortality and metabolic disorders threaten the viability of the industry. Metabolic disorder is associated with increased metabolism, rapid growth rate or high egg production that results in the failure of a body system because of an increased workload on that organ or system

Fig. 3. The phylogenetic tree of GPXs constructed from the protein-encoding cDNA sequences by the neighbor-joining method using the MEGA 4 program. Bootstrap value (%) obtained with 500 replicates is shown at each node.

Fig. 4. RT-PCR detection of GPX1 and β -actin mRNA expression in various tissues of Japanese quail, including kidney, adrenal gland, liver, small follicle, small intestine, heart and breast muscle. Data are expressed as means $\pm$ SD of 3 replications. Error bars indicate SD.

(Julian, 2005). Therefore, the study of the GPX1 gene at the molecular level as an antioxidant defense system may lead to a better understanding and solution of the problems confronting to poultry production in the future.

In this study, we determined the nucleotide sequence of GPX1 cDNA and presented the tissue distributions of GPX1 mRNA expression in Japanese quail. These data will be useful in further studies relating to GPX1 gene expression in Japanese quail.

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