Biochemical and genetic characterization of a murine class Kappa glutathione S-transferase

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The class Kappa family of glutathione S-transferases (GSTs) currently comprises a single rat subunit (rGSTK1), originally isolated from the matrix of liver mitochondria [Harris, Meyer, Coles and Ketterer (1991) Biochem. J. **278**, 137–141; Pemble, Wardle and Taylor (1996) Biochem. J. **319**, 749–754]. In the present study, an expressed sequence tag (EST) clone has been identified which encodes a mouse class Kappa GST (designated mGSTK1). The EST clone contains an open reading frame of 678 bp, encoding a protein composed of 226 amino acid residues with 86 % sequence identity with the rGSTK1 polypeptide. The mGSTK1 and rGSTK1 proteins have been heterologously expressed in *Escherichia coli* and purified by affinity chromatography. Both mouse and rat transferases were found to exhibit GSH-conjugating and GSH-peroxidase activities towards model substrates. Analysis of expression levels in a range of mouse and rat tissues revealed that the mRNA encoding these enzymes is expressed predominantly in heart, kidney, liver and skeletal muscle. Although other soluble GST isoenzymes are believed to reside primarily within the cytosol, subcellular fractionation of mouse liver demonstrates that this novel murine class Kappa GST is associated with mitochondrial fractions. Through the use of bioinformatics, the genes encoding the mouse and rat class Kappa GSTs have been identified. Both genes comprise eight exons, the protein coding region of which spans approx. 4.3 kb and 4.1 kb of DNA for *mGSTK1* and *rGSTK1* respectively. This conservation in primary structure, catalytic properties, tissue-specific expression, subcellular localization and gene structure between mouse and rat class Kappa GSTs indicates that they perform similar physiological functions. Furthermore, the association of these enzymes with mitochondrial fractions is consistent with them performing a specific conserved biological role within this organelle.

Key words: catalytic activity, class Kappa glutathione S-transferase (GST), expression, gene structure, mitochondria.

INTRODUCTION

The glutathione S-transferases (GSTs) are encoded by two evolutionarily distinct multigene families, termed membrane-associated proteins involved in eicosanoid and glutathione metabolism (‘MAPEG’) or microsomal GSTs [1,2] and the soluble GSTs [3]. In mammals, the latter family comprises at least eight classes of transferase, designated Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta and Zeta [3–5]. These soluble enzymes function as dimers and catalyse the conjugation of GSH with compounds containing an electrophilic centre [6]. Besides GSH-conjugating activity, GSTs can also serve as peroxidases, isomerases and thiol transferases. Furthermore, a number of non-catalytic functions have been documented for GSTs, including binding of non-substrate ligands [7] and modulation of signal transduction pathways [8,9].

Although the soluble GSTs were traditionally thought to function primarily as detoxication enzymes, it is now apparent that certain transferases contribute to other aspects of metabolism. For example, a clearly defined role for class Sigma GSTs has been established in the context of prostaglandin D_2 synthesis [10–13], whereas the identification of the human class Zeta GST as maleylacetoacetate isomerase revealed a novel role for this enzyme in the degradation of tyrosine [14,15].

The class Kappa family of GSTs was proposed by Pemble et al. [16] to accommodate a unique rat enzyme (designated rGSTK1-1), which was originally purified as a 13–13 homodimer from the matrix of liver mitochondria [17]. Although the rGSTK1 subunit was initially classified as a class Theta GST on the basis of limited N-terminal sequence information [17], elucidation of its complete primary structure through cDNA cloning showed the absence of sequence similarity to any of the other recognized classes of transferase [16]. Despite the atypical primary structure of rGSTK1, the purified enzyme exhibited substantial GSH-conjugating activity towards the model substrates, 1-chloro-2,4-dinitrobenzene (CDNB) and ethacrynic acid (EA). Although this enzyme, therefore, exhibits catalytic features in common with soluble GSTs, these have evolved in the absence of any significant similarity in primary structure.

The original purification of rGSTK1-1 from the hepatic mitochondrial matrix suggested that this enzyme could possess a previously unrecognized role in the maintenance of mitochondrial integrity. However, the lack of antisera specific for the class Kappa transferase prevented a more informative analysis of the relative

Abbreviations used: BDNB, 1-bromo-2,4-dinitrobenzene; BLAT, basic local alignment tool; CDNB, 1-chloro-2,4-dinitrobenzene; CHP, cumene hydroperoxide; EA, ethacrynic acid; EPNP, 1,2-epoxy-3-(4′-nitrophenoxy)propane; EST, expressed sequence tag; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione S-transferase; 4-HNE, 4-hydroxynon-2-enal; IDNB, 1-iodo-2,4-dinitrobenzene; IPTG, isopropyl β-D-thiogalactoside; LDH, lactate dehydrogenase; MnSOD, manganese superoxide dismutase; ORF, open reading frame; ROS, reactive oxygen species; RT, reverse transcription; tBHP, to-butyl hydroperoxide; tPBO, *trans*-4-phenylbut-3-en-2-one.

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The nucleotide sequence data reported in this paper have been submitted to the GenBank®, EMBL, DDBJ and GSDB Nucleotide Sequence Databases under the accession number AY279096.

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distribution of the protein between cytosol and mitochondria. Early studies by Ryle and Mantle [18] suggested that the majority of GST activity in mitochondria can be accounted for by loose association of cytoplasmic forms. By contrast, emerging evidence suggests that transferases belonging to the Alpha and Mu classes of GSTs are present in both cellular compartments [19–21]. A rat liver microsomal GST has also been shown to associate with the outer mitochondrial membrane [22]. Determination of the subcellular localization of class Kappa GSTs is likely to prove central to elucidating the physiological role of these enzymes.

Despite the realization that rGSTK1 is distinct in terms of its primary structure and apparent localization, the rat enzyme represents the only member of this class of transferase characterized to date. Although a human cDNA has been identified which exhibits a high level of sequence identity with rGSTK1 [16], this putative human protein has not been studied at the biochemical level. Furthermore, given that our knowledge concerning rGSTK1 is limited to sequence information and enzymology, there is essentially no information about the organization and regulation of the *rGSTK1* gene. Collectively, the lack of such information precludes our ability to predict the likely endogenous function of the enzyme, and hinders efforts to examine the evolutionary relationship between class Kappa GSTs and the other soluble transferase families.

The present study describes the identification and characterization of a novel murine class Kappa transferase subunit, designated mGSTK1. The expression of mGSTK1 and rGSTK1 mRNAs has been examined in a range of tissues. In addition, antisera have been raised against the mouse transferase. Immunoblotting of hepatic subcellular fractions reveals that mGSTK1 is associated with the mitochondrial fraction, rather than the cytosolic fraction. Furthermore, the genes encoding mGSTK1 and rGSTK1 have been identified using bioinformatics. Collectively, these data demonstrate that the primary structure, catalytic properties, tissue-specific expression, subcellular localization and genomic organization of class Kappa GSTs are conserved between mouse and rat. These observations are consistent with a common mitochondrially associated endogenous function for this class of transferase in these species.

MATERIALS AND METHODS

Reagents

Unless stated otherwise, all chemicals were obtained from Sigma–Aldrich (Poole, Dorset, U.K.). The GST substrate 1-iodo-2,4-dinitrobenzene (IDNB) was purchased from Fluka (Poole, Dorset, U.K.). Restriction endonucleases were obtained from Roche Diagnostics Ltd (Lewes, East Sussex, U.K.). Oligonucleotides were from MWG Biotech (Ebersberg, Germany) and are listed in Table 1.

Identification of class Kappa GSTs using bioinformatics

The mRNA encoding rGSTK1 has been described previously [16]. A mouse (*Mus musculus*) cDNA encoding the class Kappa GST was identified by a BLAST search of the expressed sequence tag (EST) database through the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov), using the rGSTK1 mRNA sequence as a search template. The EST clone (IMAGE 5043079) was derived from mouse kidney RNA and supplied by the U.K. Human Genome Mapping Project Resource Centre (Hinxton, Cambs., U.K.) in the pCMV•SPORT6 vector [23].

Table 1 Sequences of oligonucleotide primers used in the present study

Where appropriate, restriction sites are underlined.

Primer	Sequence
MGSTK1-S	5′-CTTGCTCTTCAGCTACCTAAAGCTC-3′
MGSTK1-AS	5′-CACTTGTGAGGCACAGAAGTAAAGG-3′
RGSTK1-S	5′-GCTTCACGTTCGCTTCTCTCTC-3′
RGSTK1-AS	5′-GGCCACAGAAGACAGCTTCATC-3′
MKP15-S	5′-CTCTCACCACTGTCATATGGGGCCGGC-3′
MKP15-AS	5′-GGGCCACATCAGATGGATCCATCAAAG-3′
RKP15-S	5′-CTCTCTCCACTACCATATGGGGCCGGC-3′
RKP15-AS	5′-CAGCTTCATCAGCTCGAGATGTCAAAG-3′
MKSEQ1	5′-CCAGAGATGCTGGAGAAGGTGTCC-3′
RKSEQ1	5′-GAAGATATCACGGAGTCCCAGAAC-3′
MKMTC-S	5′-CTTGCTCTTCAGCTACCTAAAGCTCTTCGGC-3′
MKMTC-AS	5′-GCACTTGTGAGGCACAGAAGTAAAGGCAGG-3′
RKMTC-S	5′-GCTTCACGTTCGCTTCTCTCTCCACTACAG-3′
RKMTC-AS	5′-GGCCACAGAAGACAGCTTCATCAGATGGAG-3′

Genes encoding class Kappa GSTs were identified using the basic local alignment tool (BLAT) search facility [24] available through the University of California Santa Cruz (Genome) website (http://www.genome.ucsc.edu), using the mGSTK1 and rGSTK1 mRNA sequences to search against the February 2002 and November 2002 data assemblies respectively.

Cloning of class Kappa GSTs

Sequence information derived from cDNA clones and genomic data was used to design oligonucleotide primers to allow amplification of the mGSTK1 and rGSTK1 open reading frames (ORFs) by reverse transcription (RT)-PCR. The mGSTK1 ORF was amplified from skin RNA (OriGene Technologies, Rockville, MD, U.S.A.) using primers MGSTK1-S and MGSTK1-AS (Table 1). The rGSTK1 ORF was amplified from RNA prepared from RL-34 rat liver epithelial cells using primers RGSTK1-S and RGSTK1-AS.

Both ORFs were amplified using the OneStep RT-PCR system (Qiagen Ltd, Crawley, West Sussex, U.K.). Reactions were performed in a 50 μl volume containing OneStep RT-PCR buffer (10 μl), 200 ng of each primer, 400 μM each of dATP, dCTP, dGTP and dTTP, 2 μl of enzyme mix (a blend of Omniscript™ and Sensiscript™ reverse transcriptases and the heat-activated DNA polymerase HotStarTaq™) and 500 ng of RNA. Amplification was carried out using a preheated ThermoHybaid PCR Sprint Temperature Cycling System (ThermoHybaid, Ashford, Middlesex, U.K.). RT was performed at 50 °C for 30 min. Samples were then heated to 95 °C for 15 min and amplification was performed over 40 cycles through denaturation at 94 °C for 30 s, primer annealing at 63 °C for 30 s and extension at 72 °C for 1 min. The reaction was completed with a single incubation at 72 °C for 10 min. The resulting PCR products were ligated into the pCR2.1 cloning vector (Invitrogen, Paisley, Scotland, U.K.).

Sequence analysis

Sequence analysis was carried out by the DNA Sequencing Laboratory, Department of Molecular and Cellular Pathology, Ninewells Hospital, University of Dundee, Scotland, U.K. The sequence of IMAGE clone 5043079 was determined using the T7 and SP6 promoter sites present within pCMV•SPORT6. The sequence of the mGSTK1 and rGSTK1 ORFs were determined

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using M13 forward and reverse priming sites present within the vector.

The fidelity of the RT-PCR reaction used to amplify the ORFs was confirmed by comparing the sequence of the amplified product with that of IMAGE clone 5043079 (in the case of mGSTK1), the previously documented cDNA sequence (in the case of rGSTK1) and genomic sequence (for both mGSTK1 and rGSTK1).

The predicted amino acid sequences of the proteins encoded by the ORFs were analysed using the GeneJockey II sequence analysis software (BIOSOFT, Cambridge, U.K.). Multiple sequence alignments were performed using the PILEUP program within the GCG Wisconsin package version 8.1 software. The BOXSHADE program (http://www.ch.embnet.org/software/BOX-form.html) was used to display sequence alignments.

Heterologous expression and protein purification

Both mGSTK1 and rGSTK1 were expressed using the pET15b vector (Novagen, Madison, WI, U.S.A.). The mGSTK1 and rGSTK1 ORFs were first amplified from the pCR2.1 holding vectors using MKP15-S/MKP15-AS and RKP15-S/RKP15-AS primer pairs respectively. These primers were designed to incorporate *Nde*I/*Bam*HI (mGSTK1) and *Nde*I/*Xho*I (rGSTK1) restriction sites into the 5′- and 3′-ends of the ORFs. Reactions were carried out in a 50 μl volume containing reaction buffer (5 μl), 1 mM $MgSO_4$, 200 ng of each primer, 400 μM each of dATP, dCTP, dGTP and dTTP, 100 ng of recombinant plasmid and 2.5 units of Platinum® *Pfx* DNA polymerase (Invitrogen). The amplification reaction was carried out in a ThermoHybaid PCR Sprint Temperature Cycling System. The template DNA was denatured initially through incubation at 94 °C for 2 min. Each ORF was then amplified over 30 cycles through denaturation at 94 °C for 30 s, primer annealing at 63 °C for 30 s, followed by extension at 68 °C for 1 min. The reaction was completed with a single incubation at 68 °C for 5 min. The resulting PCR products were digested with the appropriate restriction enzymes and subcloned into the pET15b expression vector. The fidelity of the PCR was confirmed by sequencing each insert in pET15b. The mouse insert was sequenced using the T7 priming site present in pET15b and the internal sequencing primer MKSEQ1, whereas the rat insert was sequenced using the T7 priming site and the internal sequencing primer RKSEQ1.

Recombinant pET15b containing the mGSTK1 ORF was used to transform *Escherichia coli* strain BL21 (DE3) pLysS (Novagen, Nottingham, U.K.), whereas the recombinant plasmid containing the rGSTK1 ORF was used to transform *E. coli* strain Rosetta™ (DE3) pLysS (Novagen). Transformed colonies were grown to exponential phase at 37 °C in Luria–Bertani broth containing ampicillin (50 μg/ml) and chloramphenicol (34 μg/ml). Expression from the pET15b vector was induced at 37 °C through the addition of 1 mM isopropyl β-D-thiogalactoside (IPTG). Bacteria were then harvested by centrifugation at 6000 ***g*** for 20 min at 4 °C and the cell pellets stored at − 70 °C until required.

The mGSTK1 and rGSTK1 proteins were routinely prepared from frozen bacterial pellets obtained from 50 ml of culture. The bacterial pellet was thawed on ice before being resuspended in 10 ml of buffer A [5 mM imidazole, 0.5 M NaCl, 20 mM Tris/HCl (pH 7.9) containing 0.01 % (v/v) Igepal] at 25 °C. Lysozyme was added to a final concentration of 50 μg/ml and the bacterial suspension incubated at 37 °C for 10 min before being snap-frozen in liquid N_2. The samples were then defrosted rapidly at 37 °C and sonicated on ice (three separate 30 s bursts, each of 16 μm amplitude). The insoluble cell debris was removed by centrifugation at 12 000 ***g*** for 20 min at 4 °C. The resulting supernatant was retained and filtered under vacuum (0.45 μm pore size) before being applied on to a 1 ml HiTrap™ nickel-agarose chelating column (Amersham Biosciences, Little Chalfont, Bucks., U.K.) at a flow rate of 3.8 ml/min. All purification steps were carried out at 4 °C. In order to remove non-specifically bound material, the column was washed with buffer A containing increasing concentrations of imidazole, as follows: 5 ml of 5 mM imidazole, 30 ml of 30 mM imidazole, 5 ml of 50 mM imidazole and 1 ml of 80 mM imidazole. The recombinant protein was eluted using 5 ml of 250 mM imidazole. The protein was dialysed for 24 h at 4 °C against two changes, each of 2 litres, of 0.5 M sodium phosphate buffer (pH 7.0) containing 1 mM dithiothreitol. Glycerol was added to a final concentration of 25 % (v/v) and the recombinant proteins stored at − 70 °C.

Biochemical assays

Protein concentrations were measured by the method of Bradford [25] with reagents purchased from Bio-Rad Laboratories (Hemel Hempstead, Herts., U.K.).

All GST enzyme activity assays were conducted at 37 °C. In all instances, the non-enzymic reaction was measured and subtracted from the overall reaction rate. Activity towards CDNB, 1-bromo-2,4-dinitrobenzene (BDNB) and IDNB, as well as GSH-peroxidase activity with cumene hydroperoxide (CHP) and t-butyl hydroperoxide (tBHP), were measured at 340 nm with a Cobas Fara II centrifugal analyser (Hoffmann-La Roche Ltd, Basel, Switzerland). Enzyme activities were also determined manually with a Varian Cary 50 Bio UV-visible spectrophotometer (Walton-on-Thames, Surrey, U.K.) at the following wavelengths and pH conditions: EA, 270 nm and pH 6.5; 4-hydroxynon-2-enal (4-HNE), 230 nm and pH 7.5; *trans*-4-phenylbut-3-en-2-one (tPBO), 290 nm and pH 6.5; and 1,2-epoxy-3-(4′-nitrophenoxy)propane (EPNP), 360 nm and pH 6.5.

Tissue-specific expression of class Kappa GSTs

The tissue-specific expression of mGSTK1 and rGSTK1 was examined using Multiple Tissue cDNA (MTC™) panels (Clontech, Palo Alto, CA, U.S.A.). These panels comprised pooled first-strand cDNA preparations isolated from eight identical mouse or rat tissues. Each panel is supplied following normalization to four different housekeeping genes. The relative abundance of mGSTK1 and rGSTK1 cDNA in each tissue sample was determined by PCR using MKMTC-S/MKMTC-AS and RKMTC-S/RKMTC-AS primer pairs respectively (Table 1). The relative expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the mouse and rat samples was also examined, using primers supplied with the panels. The PCR reaction was carried out in a 50 μl volume containing reaction buffer (5 μl), 200 μM each of dATP, dCTP, dGTP and dTTP, 0.4 μM of each primer, 1 ng of each tissue cDNA and TITANIUM™ *Taq* DNA polymerase (1 μl). The template was denatured initially through incubation at 94 °C for 30 s. Amplification was then achieved by denaturation at 94 °C for 30 s, followed by primer annealing/extension at 68 °C for 30 s. Agarose-gel electrophoresis was used to examine PCR products after successive rounds of amplification in order to ensure the linearity of the reaction. Products of the mGSTK1, rGSTK1, mGAPDH and rGAPDH reactions were analysed after 31, 31, 27 and 29 cycles respectively. The products of the mGSTK1 and

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rGSTK1 reactions were sequenced to verify amplification of the desired targets.

Electrophoresis and immunochemical analyses

Antisera against the purified histidine-tagged mGSTK1 recombinant protein were raised in female New Zealand White rabbits using an immunization protocol similar to that described previously [26]. Antisera specific for mouse class Pi GST and rat lactate dehydrogenase (LDH) have been described previously [27,28], whereas manganese superoxide dismutase (MnSOD) antibodies were kindly provided by Dr Lesley I. McLellan, Biomedical Research Centre, University of Dundee, Scotland, U.K.

Discontinuous SDS/PAGE was performed according to the method of Laemmli [29] in 12 % (w/v) polyacrylamide resolving gels, using the Mini-Protean III Cell apparatus (Bio-Rad). Resolved proteins were either visualized by staining with Coomassie Brilliant Blue R250 or transferred to a Hybond™-P PVDF membrane (Amersham Biosciences) prior to analysis by Western blot. Membranes were incubated with the appropriate primary antibody and binding of the immobilized proteins was detected with either goat anti-(rabbit IgG) or rabbit anti-(sheep IgG) antibody conjugated with horseradish peroxidase (Bio-Rad) and enhanced chemiluminescence (Amersham Biosciences).

Isolation of mitochondrial and cytosolic fractions from mouse liver

Mitochondria were isolated as described by Chappell and Hansford [30]. Animals were killed by exposure to a rising concentration of CO_2. Livers were removed from the abdominal cavity of the animal, ensuring removal of the gall bladder, and immediately transferred to a beaker of ice-cold 1.15 % KCl. The following procedures were carried out at 4 °C. The livers were weighed and thoroughly rinsed in STE buffer [250 mM sucrose, 5 mM Tris-base and 2 mM EGTA (pH 7.4)]. Thereafter, livers were finely chopped in 40 ml of STE buffer and the tissue was homogenized with six passes using a Safe-Grind™ plastic-coated Potter-Elvehjem tissue grinder (Jencons-PLS, Leighton Buzzard, U.K.). The resulting homogenates were centrifuged at 800 ***g***, and the supernatants obtained stored on ice. The pellet was then resuspended in 20 ml of STE buffer using the Safe-Grind™ Plastic-coated Potter-Elvehjem tissue grinder. This homogenate was again centrifuged at 800 ***g*** and the supernatant removed. The two supernatants were pooled and

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 M   G   P   A   P   R   I   L   E   L   F   Y   D   V   L   S   P   Y   S   W    20
ATG GGG CCG GCG CCG CGC ATC CTG GAA CTC TTC TAC GAC GTG CTG TCC CCG TAC TCC TGG   60

 L   G   F   E   V   L   C   R   Y   Q   H   L   W   N   I   K   L   Q   L   R    40
CTG GGC TTT GAG GTC CTA TGC AGA TAC CAA CAC CTC TGG AAT ATA AAG CTG CAG TTG CGG  120

 P   T   L   I   A   G   I   M   K   D   S   G   N   Q   P   P   A   M   V   P    60
CCC ACT TTA ATC GCT GGG ATC ATG AAA GAC AGC GGA AAC CAA CCA CCT GCT ATG GTT CCC  180

 R   K   G   Q   Y   I   F   K   E   I   P   L   L   K   Q   F   F   Q   V   P    80
CGA AAA GGC CAG TAC ATC TTC AAA GAG ATT CCT CTC CTG AAG CAG TTC TTC CAG GTT CCC  240

 L   N   I   P   K   D   F   F   G   E   T   V   K   K   G   S   I   N   A   M   100
CTC AAC ATA CCC AAG GAT TTC TTT GGT GAG ACT GTG AAG AAA GGA AGT ATA AAT GCC ATG  300

 R   F   L   T   T   V   S   M   E   Q   P   E   M   L   E   K   V   S   R   E   120
CGC TTC CTC ACC ACT GTA AGC ATG GAG CAA CCA GAG ATG CTG GAG AAG GTG TCC AGA GAG  360

 I   W   M   R   V   W   S   R   D   E   D   I   T   E   Y   Q   S   I   L   A   140
ATA TGG ATG CGT GTA TGG TCT CGA GAT GAA GAT ATC ACG GAG TAT CAG AGC ATT CTG GCT  420

 A   A   V   K   A   G   M   S   T   A   Q   A   Q   H   F   L   E   K   I   S   160
GCA GCA GTG AAG GCT GGA ATG TCC ACA GCG CAA GCC CAA CAC TTT CTG GAG AAG ATC TCC  480

 T   Q   Q   V   K   N   K   L   I   E   N   T   D   A   A   C   K   Y   G   A   180
ACA CAA CAG GTG AAG AAC AAG CTC ATT GAG AAC ACG GAT GCA GCC TGC AAA TAT GGG GCC  540

 F   G   L   P   T   T   V   A   H   V   D   G   K   T   Y   M   L   F   G   S   200
TTT GGG CTA CCC ACC ACT GTT GCC CAT GTG GAT GGT AAA ACC TAC ATG TTA TTT GGG TCT  600

 D   R   L   E   L   L   A   Y   L   L   G   E   K   W   M   G   P   V   P   P   220
GAC CGC TTG GAG TTG CTA GCT TAC CTG CTA GGA GAG AAG TGG ATG GGC CCT GTA CCC CCA  660

 T   A   N   A   R   L   *                                                       226
ACT GCG AAT GCC AGA CTT TAA GACTGCCTTACAGAGTAATCTTTGATTTCTCCATCTGATGTGGCCCTGGGA  732

GCTGGAGCAAGAGTCCACCTTTAGCTGTGCCTGCCTTTACTTCTGTGCCTCAGAAGTGCCTTTTGAAAAGCCTTAAATT  811

CTGCATTCCCATAAAATAAAGTTGATGCCACCAGACAAAAAAAAAAAAAAAAA                            864
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Figure 1 cDNA sequence and primary structure of mGSTK1

The deduced amino acid sequence is shown in single-letter amino-acid notation above each codon. Amino acid numbering includes the initiator methionine. The translation termination codon is marked with an asterisk.

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mGSTK1    1  MGPAPRILELFYDVLSPYSWLGFEVLCRYQHLWNIKLQLRPTLIAGIMKDSGNQPPAMVP
rGSTK1    1  MGPAPRVLELFYDVLSPYSWLGFEVLCRYQHLWNIKLKLRPALLAGIMKDSGNQPPAMVP

mGSTK1   61  RKGQYIFKEIPLLKQFFQVPLNIPKDFFGETVKKGSINAMRFLTTVSMEQPEMLEKVSRE
rGSTK1   61  HKGQYILKEIPLLKQLFQVPMSVPKDFFGEHVKKGTVNAMRFLTAVSMEQPEMLEKVSRE

mGSTK1  121  IWMRVWSRDEDITEYQSILAAAVKAGMSTAQAQHFLEKISTQQVKNKLIENTDAACKYGA
rGSTK1  121  LWMRIWSRDEDITESQNILSAAEKAGMATAQAQHLLNKISTELVKSKLRETTGAACKYGA

mGSTK1  181  FGLPTTVAHVDGKTYMLFGSDRLELLAYLLGEKWMGPVPPTANARL
rGSTK1  181  FGLPTTVAHVDGKTYMLFGSDRMELLAYLLGEKWMGPVPPTLNARL
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Figure 2 Alignment of mGSTK1 and rGSTK1 amino acid sequences

The deduced primary structure of mGSTK1 was aligned with that of rGSTK1. Numbering of amino acids includes the initiator methionine residue. Identical amino acid residues are shown against a black background, whereas similar residues are shown against a grey background.

isolation of mitochondrial and cytosolic fractions was achieved through successive centrifugation of the resulting supernatants. Centrifugation at 4 °C was performed as follows: 800 ***g*** for 4 min, 12 000 ***g*** for 10 min and 100 000 ***g*** for 20 min. The final supernatant was retained as the cytosolic fraction. The corresponding mitochondrial pellet was rinsed in 30 ml of STE buffer and centrifuged at 12 000 ***g*** for 10 min at 4 °C before being resuspended in STE buffer (1 ml/g of wet weight of original liver).

RESULTS

Identification of a novel murine class Kappa GSTs

To date, the class Kappa family of GSTs has not been studied in species other than rat. The EST database was therefore searched for cDNA clones encoding a putative mouse class Kappa GST. This search identified numerous clones that exhibited significant nucleotide sequence similarity with the rGSTK1 cDNA. One such clone (IMAGE 5043079) was sequenced and found to be approx. 870 bp in length (Figure 1). A 678 bp ORF was identified within this sequence, encoding a protein composed of 226 amino acids, with a calculated molecular mass of 25 707 Da. The cDNA was also found to contain limited sequence upstream from the predicted translational initiation codon and approx. 180 bp of sequence downstream of the TAA termination codon. The latter included a consensus polyadenylation signal (AATAAA) located approx. 16 bp upstream from the polyadenylated tail. The sequence of this ORF was independently verified by RT-PCR using mouse skin RNA as a template.

In order to compare the predicted primary structure of this mouse polypeptide (designated mGSTK1) with that of rGSTK1, the two amino acid sequences were aligned (Figure 2). This comparison revealed that rGSTK1 and mGSTK1 are identical in size and exhibit striking similarities in primary structure. At the protein level, the overall sequence identity between mGSTK1 and rGSTK1 was found to be 86 %. Of the 31 amino acid differences which exist between these two proteins, ten of these are represented by conservative substitutions and 21 are represented by non-conservative changes. It is interesting to note that the primary structure of these proteins is particularly well conserved at both the N- and C-termini. Only a single conservative substitution occurs between amino acid residues 1–35 (Val^{7} in rGSTK1 to Ile^{7} in mGSTK1), whereas two substitutions occur between amino acid residues 174–226 (the non-conservative replacement of Leu^{222} in rGSTK1 with Ala^{222} in mGSTK1, and the conservative replacement of Met^{203} in rGSTK1 with Leu^{203} in mGSTK1).

Heterologous expression and catalytic properties of class Kappa GSTs

The conservation in primary structure between mGSTK1 and rGSTK1 subunits suggested that the enzymes may share similar catalytic properties. In order to test this hypothesis, both proteins were expressed in *E. coli*. The ORF encoding the mouse protein was amplified by RT-PCR from skin RNA and subcloned into the pET15b expression vector. This vector incorporates a polyhistidine tag at the N-terminus of the protein to facilitate subsequent purification through nickel-agarose affinity chromatography. Primers specific for rGSTK1 were used to amplify the ORF encoding the rat enzyme by RT-PCR, using RNA isolated from rat liver RL-34 epithelial cells. The rGSTK1 ORF was also subcloned into the pET15b expression vector. Heterologous expression of both proteins was attempted initially in *E. coli* strain BL21 (DE3) pLysS. In the case of the mouse protein, transcriptional induction with IPTG resulted in robust expression of a protein with an apparent molecular mass of around 29 kDa (Figure 3a). Surprisingly, although parallel induction of *E. coli* strain BL21 (DE3) pLysS transformed with the rGSTK1 expression vector resulted in the selective expression of a protein of comparable molecular mass, the level of expression obtained was significantly lower relative to that observed with the mouse protein (results not shown). Expression of rGSTK1 was therefore attempted in *E. coli* Rosetta™ (DE3) pLysS cells, as this host strain is designed to enhance the expression of eukaryotic proteins by providing tRNAs for codons rarely used in the bacterium. Induction of this strain with IPTG resulted in significantly higher levels of expression (Figure 3b). Although expression of both mGSTK1 and rGSTK1 was detectable as early as 1 h after induction, higher levels of expression were observed at later time points.

Both mGSTK1 (Figure 4a) and rGSTK1 (Figure 4b) were routinely purified from populations of *E. coli* following 2.5 h induction with IPTG. Robust expression of each recombinant protein was observed in cell lysates prepared from induced cultures (Figures 4a and 4b, lane 1). Further analysis of these lysates revealed that both mGSTK1 and rGSTK1 were present almost exclusively in the soluble fraction (Figures 4a and 4b, lane 2 compared with lane 3). Each recombinant protein was purified to apparent homogeneity by nickel-agarose affinity chromatography (Figures 4a and 4b, lane 5). The approximate yield of mGSTK1

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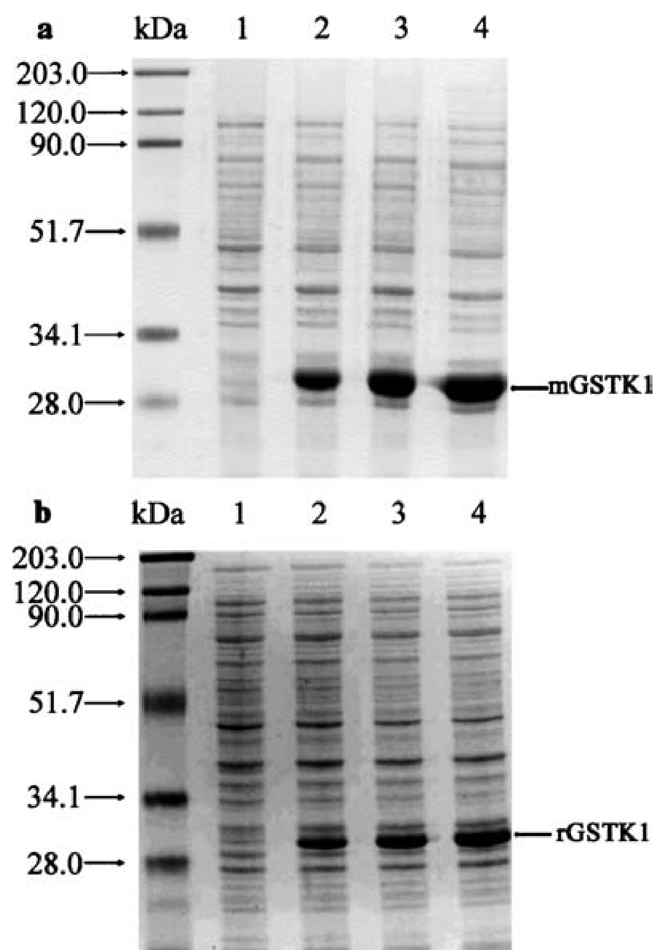

Figure 3 Heterologous expression of recombinant mGSTK1 and rGSTK1

Both mGSTK1 (**a**) and rGSTK1 (**b**) were expressed with a polyhistidine N-terminal tag to facilitate their purification. mGSTK1 was expressed in *E. coli* strain BL21 (DE3) pLysS, whereas rGSTK1 was expressed in *E. coli* strain Rosetta^{TM} (DE3) pLysS. Bacterial lysates were prepared at various times following induction with IPTG and analysed by SDS/PAGE. The samples were loaded as follows: lane 1, bacterial lysates prepared immediately prior to IPTG induction; lanes 2, 3 and 4, bacterial lysates prepared 1 h, 2 h and 3 h after IPTG induction respectively. Molecular-mass markers (in kDa) were included on each gel and are indicated on the left.

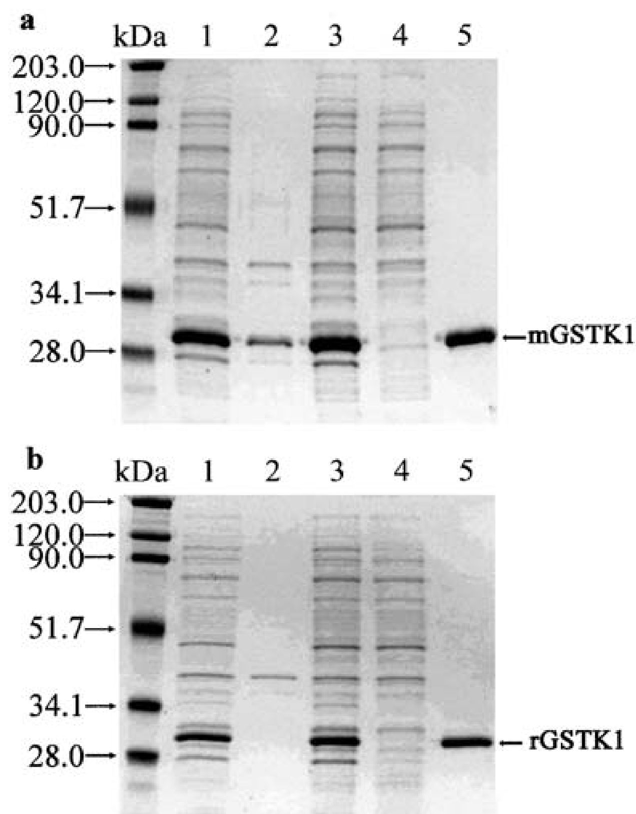

Figure 4 Purification of recombinant mGSTK1 and rGSTK1

Both mGSTK1 and rGSTK1 were expressed with a polyhistidine N-terminal tag using the pET15b expression vector. mGSTK1 was expressed in *E. coli* strain BL21 (DE3) pLysS, whereas rGSTK1 was expressed in *E. coli* strain Rosetta^{TM} (DE3) pLysS. Expression of both proteins was induced with IPTG for 2.5 h at 37 °C. Purification of mGSTK1 (**a**) and rGSTK1 (**b**) was achieved through nickel-agarose affinity chromatography. Samples isolated at various stages during the purification protocol were analysed by SDS/PAGE. Samples were loaded as follows: lane 1, whole-cell lysates prepared from *E. coli* induced to express the recombinant protein; lane 2, insoluble pellet isolated from bacterial lysate; lane 3, soluble fraction of bacterial lysate; lane 4, material which failed to bind the nickel-agarose purification column; lane 5, purified recombinant protein. Molecular-mass markers (in kDa) were included on each gel and are indicated on the left.

from *E. coli* induced for 2.5 h with IPTG was typically 7 mg protein/100 ml culture, whereas that of rGSTK1 was typically 3.5 mg protein/100 ml.

The ability to isolate high quantities of pure protein facilitated investigations into the catalytic properties of class Kappa GSTs. In the first instance, the GSH-conjugating activity of each recombinant protein was examined using a range of aryl halide substrates (Table 2). These analyses revealed that both mGSTK1-1 and rGSTK1-1 exhibited high transferase activity towards BDNB, CDNB and IDNB. In each case, the specific activity of mGSTK1-1 was higher than that of rGSTK1-1. Relatively low GSH-conjugating activity was also exhibited by each protein towards EA. By contrast, neither enzyme displayed detectable transferase activity towards 4-HNE, EPNP or tPBO. The ability of the recombinant proteins to reduce hydroperoxides was examined next. These analyses revealed that both mGSTK1-1 and rGSTK1-1 reduced CHP. Again, the specific activity exhibited by rGSTK1-1 towards CHP was lower than that of mGSTK1-1. The mouse enzyme was also found to possess low peroxidase activity towards tBHP. Collectively, these data confirm that, despite differences in the specific activities of the two proteins, mGSTK1-1 and rGSTK1-1 possess similar substrate specificities. The relatively high activity exhibited by mGSTK1-1 and rGSTK1-1 towards a number of the substrates examined suggests that these enzymes may contribute to detoxication processes *in vivo*.

Tissue-specific expression of class Kappa GSTs

The tissue-specific expression of class Kappa GSTs has not been investigated to date. In order to gain insights into the potential endogenous functions of this class of transferase, the relative expression of mGSTK1 and rGSTK1 has been examined in a range of tissues. For these investigations, panels of normalized cDNA samples derived from mouse (Figure 5a) and rat (Figure 5b) organs were analysed by semi-quantitative PCR. As shown in Figure 5 (panel i), PCR analyses yielded a single product of the expected size (862 bp and 770 bp for mGSTK1 and rGSTK1 respectively. Analysis of the expression profiles obtained revealed that, in both mouse and rat, the class Kappa GST is expressed at highest levels in heart, kidney, liver and skeletal muscle. Lower levels of expression were observed in brain and lung.

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Table 2 Substrate specificity of GST isoenzymes

The specific activity of GST isoenzymes towards a number of model substrates is shown. Results for mGSTK1-1 and rGSTK1-1 were determined using at least two preparations of recombinant protein and represent means ± S.D. for at least three determinations. Data for mGSTA1-2, mGSTA3-3, mGSTA4-4, mGSTM1-1, mGSTM1-2, mGSTM1-4, mGSTM3-3, mGSTM4-4, mGSTP1-1 and mGSTP2-2 are taken from Hayes and Pulford [6]. The activity of mGSTS1-1 was determined by Kanaoka et al. [35]. The catalytic properties of the class Theta enzymes were documented by Whittington et al. [36] and Coggan et al. [37]. ND, Not detected. – , data not available.

	Specific activity (μmol/min per mg of protein)								
Isoenzyme	CDNB	BDNB	IDNB	CHP	tBHP	4-HNE	EA	EPNP	tPBO
mGSTK1-1	154.8 ± 25.0	134.4 ± 12.4	93.5 ± 17.7	1.05 ± 0.04	0.03 ± 0.01	ND	0.29 ± 0.08	ND	ND
rGSTK1-1	54.4 ± 17.0	67.0 ± 2.5	33.3 ± 2.6	0.09 ± 0.02	< 0.01	ND	0.44 ± 0.12	ND	ND
mGSTA1-2	3.1	–	–	1.06	–	–	0.86	ND	ND
mGSTA3-3	15.1	–	–	11.6	–	1.1	0.10	0.23	0.01
mGSTA4-4	12.0	–	–	0.70	–	55.4	1.90	2.76	0.01
mGSTM1-1	148.0	–	–	0.10	–	6.0	0.12	0.48	0.04
mGSTM1-2	81.0	–	–	–	–	–	–	–	0.39
mGSTM1-4	74.0	–	–	–	–	–	–	–	0.08
mGSTM3-3	22.2	–	–	–	–	–	0.01	–	0.08
mGSTM4-4	60.0	–	–	–	–	–	–	0.12	–
mGSTP1-1	119.0	–	–	0.14	–	2.6	4.30	0.77	0.01
mGSTP2-2	0.12	–	–	0.01	–	–	0.04	ND	ND
mGSTS1-1	3.0	–	–	–	–	–	–	–	–
mGSTT1-1	0.03	–	–	2.9	1.7	–	ND	90.6	–
mGSTT3-3	0.52	–	–	1.13	ND	–	0.017	5.48	–

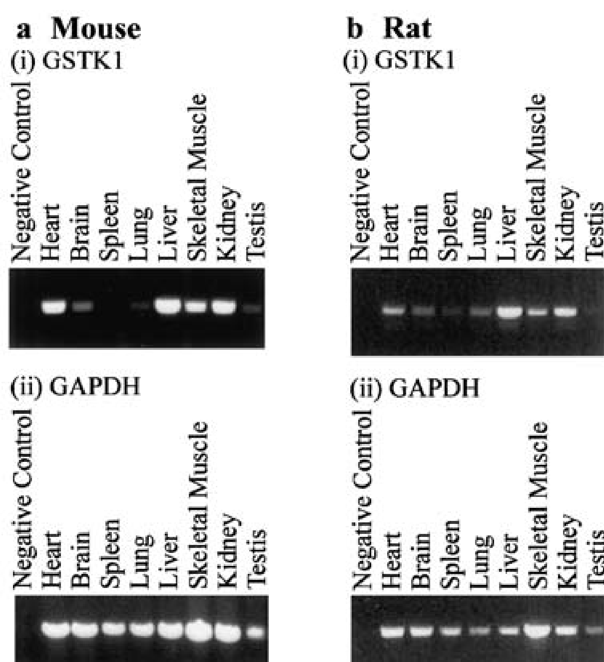

Figure 5 Tissue-specific expression of mGSTK1 and rGSTK1

Normalized panels of cDNAs isolated from various mouse (**a**) and rat (**b**) tissues were analysed for GSTK1 (i) and GAPDH (ii) expression by PCR. Amplification of mGSTK1 and rGSTK1 was performed over 31 cycles. The expression of GAPDH mRNA was examined as a control. Amplification of GAPDH was performed over 27 and 29 cycles for mouse and rat respectively. Reaction products were analysed by agarose-gel electrophoresis.

Among the tissues examined, lowest levels of class Kappa GST mRNA were observed in spleen and testes. The GAPDH control shown in Figure 5 (panel ii) confirms that the class Kappa GST expression profile obtained reflects genuine differences in the relative abundance of the mRNA encoding the transferase. Collectively, these data suggest that the biological function of class Kappa GST is conserved between these species.

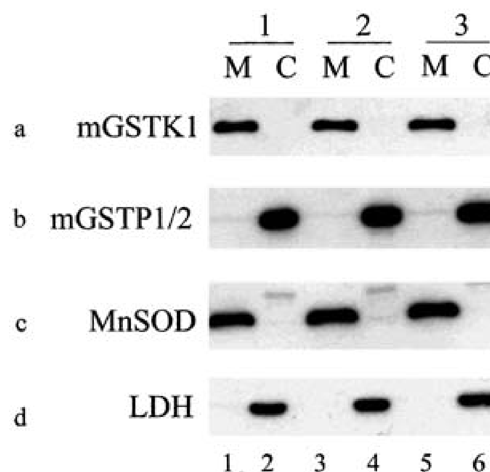

Figure 6 Subcellular localization of mGSTK1

Mitochondrial (M) and cytosolic (C) fractions were prepared by centrifugation from livers isolated from three mice (individual preparations numbered 1–3). Protein samples were resolved by SDS/PAGE and analysed by Western blotting using antisera specific for mouse class Kappa GST (**a**), mouse class Pi GST (**b**), MnSOD (**c**) or rat LDH (**d**). Lanes 1, 3, and 5 contain mitochondrial fractions isolated from animals 1–3, whereas lanes 2, 4 and 6 contain the corresponding cytosolic fractions.

Subcellular localization of mGSTK1

Given that rGSTK1-1 was originally isolated from the matrix of liver mitochondria [17], it seemed likely that the mouse class Kappa GST would also be present in this organelle. Mitochondrial and cytosolic fractions were, therefore, isolated by differential centrifugation from liver homogenates prepared from three individual animals. Western-blot analysis of fractions using antibodies specific for predominantly mitochondrial (MnSOD) and cytosolic (LDH) proteins confirmed the purity of these fractions (Figures 6c and 6d). Parallel experiments were performed using antisera raised against the mouse class Kappa GST (Figure 6a). Significantly, mGSTK1 protein was only detected in mitochondrial fractions. In marked contrast, class Pi GST was

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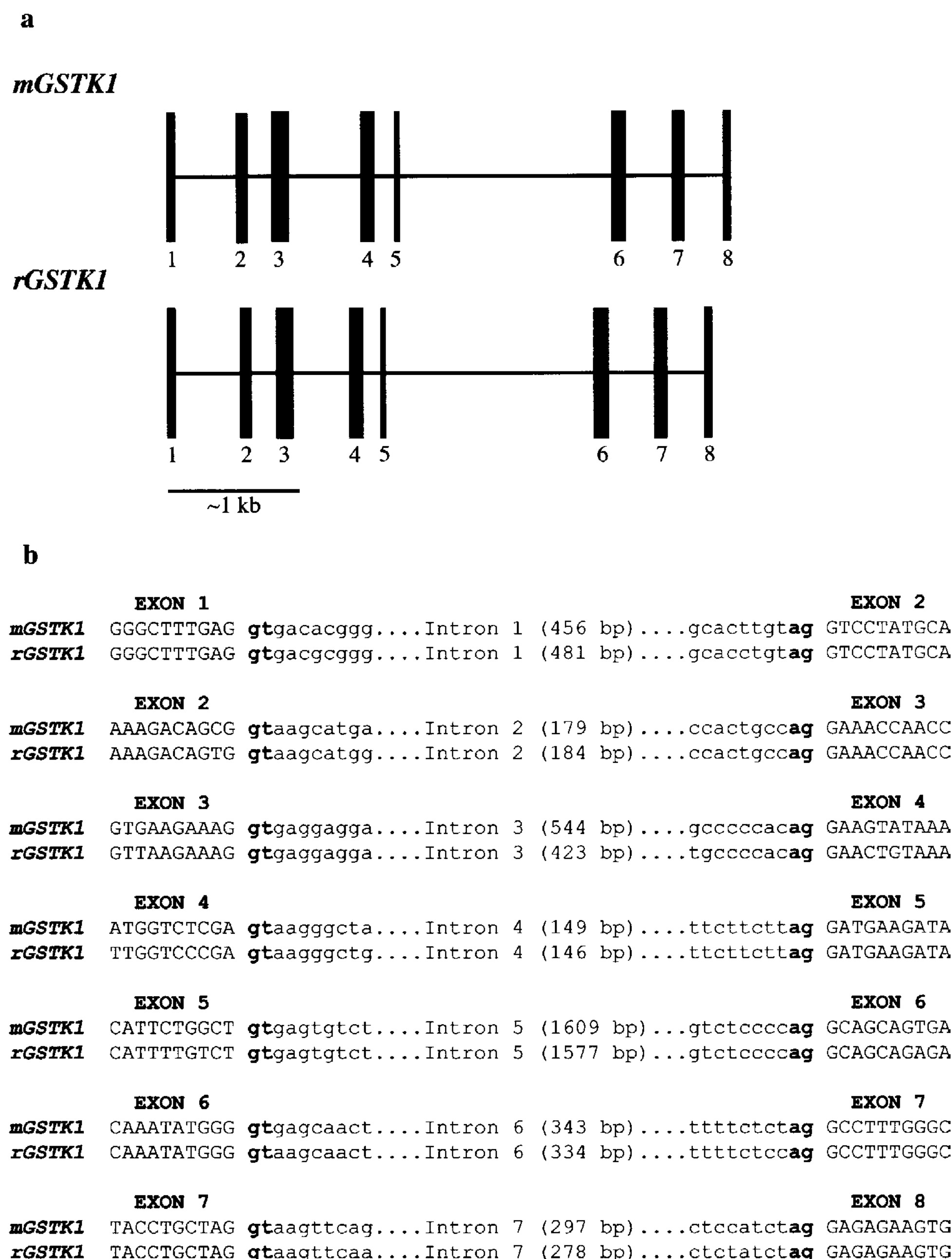

Figure 7 Class Kappa GST gene structure

Genes encoding mGSTK1 and rGSTK1 were identified using the BLAT search facility available through the University of California Santa Cruz (Genome) website (www.genome.ucsc.edu). The corresponding mRNA sequence was used as the search template. Gene structures are drawn to scale (**a**). Exons are represented by boxes, whereas introns are represented by lines. Only protein-coding regions of exons are shown. The sequence flanking each of the exon–intron boundaries in *mGSTK1* and *rGSTK1* have been aligned (**b**). Sequences located within exons and introns are shown in upper and lower case respectively. The gt-ag splice donor–acceptor sites are shown in bold.

only detected in cytosolic extracts (Figure 6b). These data clearly demonstrate that the class Kappa GST is selectively enriched in mouse hepatic mitochondrial isolates. This conservation in subcellular localization between mouse and rat suggests that class Kappa GSTs possess a conserved function within mitochondria.

Identification of genes encoding class Kappa GSTs

Genes encoding class Kappa GSTs have not been identified previously. The mGSTK1 and rGSTK1 mRNA sequences were, therefore, used as templates to search against genome sequence data. Regions of the mouse and rat genome exhibiting high levels of sequence identity with the mRNA templates were identified using the BLAST search facility. This search identified loci exhibiting 99.9 % and 100 % identity with the mGSTK1 and rGSTK1 mRNA sequences respectively (Figure 7a). The protein coding region of the *mGSTK1* gene spans approx. 4.3 kb of DNA and is located on chromosome 6. The gene is composed of eight exons, with the translation initiation codon present in exon one and the translation termination codon present in exon eight. The protein coding sequence of the mGSTK1 mRNA is identical with that encoded by the *mGSTK1* gene identified in

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the present study. One nucleotide present in the 3′-untranslated region of the mRNA template differs from that predicted from the genomic sequence. This may represent inter-strain variation. The *rGSTK1* gene is located on chromosome 4 and the protein coding region of the gene spans approx. 4.1 kb of DNA. The rat gene was also found to comprise eight exons, with translation initiation and termination codons again located within exons one and eight respectively. In addition to this conservation in gross aspects of genomic organization between *mGSTK1* and *rGSTK1*, detailed comparisons between the two genes revealed further similarities (Figure 7b). Of particular relevance is the fact that the positions of the predicted splice junctions are conserved between *mGSTK1* and *rGSTK1*. Analysis of the sequence flanking these splice junctions reveals that each conforms to the gt-ag rule [31]. Intron size is also well conserved between the two genes. Although the introns are generally relatively small (< 550 bp), in both *mGSTK1* and *rGSTK1*, intron 5 is the largest (1609 bp and 1577 bp for *mGSTK1* and *rGSTK1* respectively). These analyses demonstrate that, in addition to the conservation in the primary structure, catalytic properties, tissue-specific expression and subcellular localization of these two transferases, the enzymes are also the products of two highly similar genes.

DISCUSSION

When the existence of Kappa class GSTs was first proposed in 1996 [16], only a single enzyme, originally purified from rat liver mitochondria, was assigned to this gene family. Although a cDNA encoding a putative human class Kappa GST was also identified in the paper that first named this transferase family [16], the human protein has not been studied. Subsequently, class Kappa GSTs have not been characterized and little is known about their functional and structural relationship with other GST families. To date, rGSTK1 has not been heterologously expressed and, as it has only been purified from rat liver mitochondria in relatively small amounts, its substrate specificity has not been described in great detail. The tissue-specific expression and subcellular distribution of class Kappa GSTs have not been documented. Finally, the absence of any information about the organization and chromosomal localization of class Kappa GST genes has hindered assessment of their relationship with other transferase genes.

Catalytic properties of class Kappa GSTs

Due to the fact that the rat class Kappa GST does not bind GSH–agarose affinity matrices [17], in the present study, both mGSTK1 and rGSTK1 were expressed with a polyhistidine N-terminal tag to facilitate subsequent purification. Investigations into the substrate specificity of mGSTK1-1 and rGSTK1-1 revealed that both transferases exhibit high activity towards a range of aryl halides. Interestingly, the specific activity of rGSTK1-1 was consistently lower than that of mGSTK1-1, despite the high level of amino acid sequence identity between these enzymes. Similar differences have, nevertheless, been observed between highly similar mammalian class Sigma GST isoenzymes [13]. The specific activity of rGSTK1-1 in a standard assay using CDNB as substrate was found to be 54.4 μmol/min per mg of protein. This value compares favourably with that of 82 units/mg documented previously for the native enzyme [17].

Both of the class Kappa transferases were found to possess GSH-peroxidase activity towards CHP. Again, it was found that the rat isoenzyme was less active than mGSTK1-1. The low activity of rGSTK1-1 towards CHP and tBHP may account for the earlier conclusion that class Kappa transferases lack GSH-peroxidase activity.

The original preparation of GSTK1-1 from rat liver exhibited high activity towards EA (26 units/mg). By contrast, in the present study, both mouse and rat class Kappa GSTs displayed substantially lower activity towards this substrate (0.29 μmol/min per mg of protein and 0.44 μmol/min per mg of protein for mGSTK1-1 and rGSTK1-1 respectively). The data presented in Table 2 clearly demonstrate that the apparent specific activity of the native enzyme preparation is unusually high, whereas the data obtained with recombinant mGSTK1-1 and rGSTK1-1 appear broadly consistent with the activities exhibited by other soluble rodent transferases. The original purification of rGSTK1-1 entailed reversed-phase HPLC, and it remains possible that the use of acetonitrile caused the transferase to adopt an altered conformation that conferred the atypical activity towards EA.

Biological functions of class Kappa GSTs

Notwithstanding these considerations, studies on both native and recombinant class Kappa transferases demonstrate that the enzymes exhibit activity towards a number of model GST substrates and they therefore probably contribute to detoxication processes *in vivo*. In order to elucidate further the putative endogenous role of class Kappa GSTs, the distribution of the transferase has been examined on both a tissue-specific and intracellular level. In mouse and rat, GSTK1 mRNA is expressed at high levels in liver and kidney, consistent with a role for the transferase in detoxication. It is important to note that class Kappa GSTs are also highly expressed in heart, skeletal muscle, lung and brain; organs that are exposed to substantial levels of reactive oxygen species (ROS). Interestingly, other classes of GSTs are also abundant in certain of these tissues. For example, the class Omega GST is expressed at highest levels in mouse liver, heart and lung [32]. The significance of expression in these tissues may also involve other functions of GSTs besides that of detoxication.

Whereas class Kappa GSTs appear similar to other soluble transferases in terms of their catalytic properties and tissue-specific expression, the association of mGSTK1-1 with mitochondrial fractions suggests that this enzyme may possess an independent endogenous function. Although the presence of class Alpha and Mu GSTs within mitochondria has been documented, these transferases are localized predominantly within the cytosol [19–21]. The identification of mGSTK1-1 as a mitochondria-associated enzyme may reflect a previously underestimated role for GSTs within this organelle. Certainly, the mitochondrial respiratory chain represents a major source of ROS. As such, mitochondrial GSH and GSH-dependent enzymes represent an important defence mechanism against ROS-induced toxicity. The data presented herein suggest that class Kappa GSTs may represent a novel component of this defence system. Although rGSTK1-1 was originally isolated from the matrix of rat liver mitochondria, further experiments will be required to elucidate the precise location of class Kappa GSTs within this organelle. Such intra-mitochondrial localization data will undoubtedly provide further insights into the likely biological role of these enzymes.

The mechanism through which class Kappa GSTs are directed to the mitochondria is yet to be studied. Although many polypeptides are targeted to the mitochondria by an N-terminal presequence which is subsequently cleaved when the protein enters the mitochondria, there is no evidence for such a sequence

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within class Kappa GSTs. The transferases are by no means unique in this regard, given that other mitochondria-targeted proteins contain internal non-cleavable targeting sequences [16,33]. Detailed mutational analysis of class Kappa GST polypeptides will be required to elucidate any such mitochondrial targeting sequence.

Organization and chromosomal localization of GST genes

Genes encoding class Kappa GSTs had not been identified at the outset of this study. Through analysis of mouse and rat genome data, genes encoding mGSTK1 and rGSTK1 have been identified. In terms of genomic organization, the present study demonstrates that, in both mouse and rat, *GSTK1* genes are composed of eight exons, with the translational start codon present in the first exon.

For both *mGSTK1* and *rGSTK1*, determining the size of exon 1 upstream of the translation initiation codon will require subsequent elucidation of the transcriptional start site. It is noteworthy that in certain class Alpha and Sigma GST genes, exon 1 encodes only 5′-untranslated sequence, with the translation initiation codon present in exon 2. The possibility that there may exist an additional exon upstream of that designated exon 1 in the present study was thus considered. Analysis of the sequence upstream of the translational start site in mGSTK1 and rGSTK1 EST clones does not provide evidence for the existence of an additional upstream exon. Furthermore, the 1.5 kb region immediately upstream of exon 1 in the *mGSTK1* gene drives transcription of a reporter gene which lacks any native promoter sequence (results not shown). Thus the distribution of mRNA sequence over eight exons, coupled with the fact that the region upstream of the first exon possesses promoter activity, provides compelling evidence for the *GSTK1* gene structure presented in Figure 7.

Interestingly, mouse genome analysis identified a second sequence with a high level of sequence identity with mGSTK1 mRNA. This sequence localizes to mouse chromosome 19 and contains a complete ORF encoding a predicted protein composed of 227 amino acids. Alignment of this predicted protein sequence with that of mGSTK1 reveals that they are 77 % identical. The ORF of this predicted protein appears to be encoded by a single exon. Furthermore, the consensus polyadenylation signal present within exon 8 of *mGSTK1* is not conserved in the chromosome 19 sequence. This absence of a polyadenylation signal may inhibit mRNA processing. Finally, EST clones derived from the mRNA relating to this predicted protein could not be identified. Collectively, these observations support the notion that this sequence represents a pseudogene. It is noteworthy that a similar rGSTK1-related sequence was identified on rat chromosome 2, although sequence information over this region of the rat genome is incomplete. Interestingly, a putative reverse-transcribed pseudogene related to class Omega GSTs was recently identified in human [34].

Conclusions

The present study describes the identification and characterization of a novel murine class Kappa GST subunit, designated mGSTK1. The catalytic properties and tissue-specific expression of this mouse enzyme have been examined and compared with parallel data obtained for the rat class Kappa GST. Immunoblotting of hepatic subcellular fractions demonstrates that, unlike other soluble transferases, the mouse class Kappa GST is present at substantially higher levels in mitochondrial fractions than in cytosol. Finally, bioinformatics have been used to identify the genes encoding mGSTK1 and rGSTK1. The data presented herein provide important information as to the putative endogenous functions of this class of transferase and provide novel insights into the relationship between class Kappa GSTs and the other members of this superfamily.

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