Purification and characterization of a recombinant human Theta-class glutathione transferase (GSTT2-2)

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A cDNA encoding the human Theta-class glutathione transferase GSTT2-2 was expressed in *Escherichia coli* as a ubiquitin fusion protein. The co-translational removal of the ubiquitin by a cloned ubiquitin-specific protease, Ubp1, generates enzymically active GSTT2-2 without any additional N-terminal residues. The recombinant isoenzyme was purified to apparent homogeneity by DEAE anion-exchange, gel filtration, dye ligand chromatography and high resolution anion-exchange chromatography on Mono Q FPLC. The recombinant enzyme had significant activity with a range of substrates, including cumene hydroperoxide and 1-menapthyl sulphate. The activity of GSTT2-2 with a range of secondary lipid peroxidation products such as the *trans*,*trans*-alka-2,4-dienals and *trans*-alk-2-enals, as well as its glutathione peroxidase activity with organic hydroperoxides, suggest that it may play a significant role in protection against the products of lipid peroxidation.

INTRODUCTION

The glutathione transferases (GSTs) represent a group of Phase II detoxication enzymes that catalyse the nucleophilic attack of reduced glutathione on electrophilic centres of endogenous and xenobiotic compounds. Cytosolic GSTs from mammals can be divided into four distinct classes: Alpha, Mu, Pi and Theta, which can be distinguished from one another by their N-terminal amino acid sequence and immunochemical properties [1–4]. Distinct from the Alpha-, Mu- and Pi-class GSTs, the mammalian Theta-class GSTs are not retained by glutathione or *S*-hexylglutathione affinity matrices and they show very low or lack activity with the model substrate 1-chloro-2,4-dinitrobenzene (CDNB). Phylogenetic studies of the GST family have identified Theta-like GSTs in mammals, fish, plants, insects, yeasts, fungi and bacteria [5]. In addition to these, a GST 13-13 isolated from the matrix of rat liver mitochondria [6] also appeared to have N-terminal amino acid sequence similarity with the Theta class. The existence of Theta-like GSTs across a broad range of species suggests a longer evolutionary history for the Theta class as compared with the Alpha, Mu and Pi classes [5].

A common characteristic of the Theta-class GSTs is their affinity towards organic hydroperoxide species. Two Theta-class GSTs, namely GST 5-5 from rat liver and GST 5*-5* from rat liver nucleus, were reported to detoxify DNA hydroperoxides [7]. GST Yrs-Yrs, the rat orthologue of GSTT2-2, exhibits glutathione peroxidase activity towards a variety of polyunsaturated fatty acid hydroperoxides [8] and, in a previous study, cumene hydroperoxide was shown to be a good substrate for GSTT2-2 [4]. Thus the glutathione peroxidase activity of the Theta-class GSTs further strengthens the suggestion that their major role may be in the protection of cells against oxygen toxicity and that they might have evolved as a protective mechanism when organisms adapted to an aerobic environment.

Little is known about the substrate specificity of GSTT2-2. By the use of a recently cloned cDNA [9], we have now expressed recombinant GSTT2-2 in *Escherichia coli* in sufficient quantity to allow a more extensive characterization of its properties.

MATERIALS AND METHODS

Materials

Compounds used in the preparation of culture broth and buffers were purchased from Difco Laboratories (Detroit, MI, U.S.A.), Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Ajax Chemical Co. (Sydney, Australia). Restriction endonucleases and their buffers were purchased from Pharmacia Australia. *Taq* polymerase is a product of Promega. A Sequenase™ Version 2.0 DNA sequencing kit was obtained from USB. Oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer (Applied Biosystems Inc., Foster City, CA, U.S.A.) by the Australian National University Biomolecular Resource Facility. [α-^{32}P]dATP and [α-^{33}P]dATP were purchased from Bresatec (Adelaide, Australia). A random priming DNA labelling kit was obtained from Amersham. Nitrocellulose membrane and a silver staining kit were purchased from Bio-Rad Laboratories. Chemicals used in enzyme assays were purchased from Aldrich Chemical Co., Sigma Chemical Co. and Boehringer Mannheim. Glutathione reductase is a product of Sigma Chemical Co. 1-Menapthyl sulphate was synthesized by the method of Clapp and Young [10].

Enzyme assays

GST assays using *trans*,*trans*-alka-2,4-dienals and *trans*-alk-2-enals were described by Brophy et al. [11]. The determination of GST activity with 1-menapthyl sulphate was essentially as described by Gillham [12], and glutathione peroxidase activity was determined by the procedure described by Beutler [13]. GST activity with dichloromethane as a substrate was determined at 37 °C in a 1.5 ml Eppendorf tube containing 40 mM dichloro-

Abbreviations used: GST, glutathione transferase; CDNB, 1-chloro-2,4-dinitrobenzene.

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methane, 100 mM GSH, 0.5 M sodium phosphate buffer, pH 6.5, and 2 μg of purified enzyme. Formaldehyde released after 60 min was determined spectrophotometrically at 415 nm, by the method of Nash [14], using known concentrations of formaldehyde as standards. Glutathione transferase assays using CDNB, 1,2-dichloronitrobenzene, ethacrynic acid, 1,2-epoxy-3-(4-nitrophenoxy)propane, 4-phenylbut-3-en-2-one and 2-cyano-1,3-dimethyl-1-nitrosoguanidine as substrates were previously described in detail by Mannervik and Widersten [15]. Activity with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole was determined spectrophotometrically at 419 nm in sodium acetate buffer, pH 5.0, as described by Ricci et al. [16].

Heterologous expression of GSTT2-2

GSTT2-2 was expressed in *E. coli* as a ubiquitin fusion protein and co-translationally cleaved from ubiquitin by a co-expressed yeast ubiquitin-specific protease (Ubp1) by methods previously described in detail by Baker et al. [17]. A cDNA clone termed pHT4, encoding the entire coding sequence of GSTT2, was previously isolated by Tan et al [9]. Two oligonucleotide primers, HT2ExA (5′ GTGCCGCGGTGGTATGGGCCTAG-AGCTGT 3′) and HTExB (5′ GGAAGCTTTTGTTGCTAAT-CTCCTGG 3′) were designed to amplify the coding region of GSTT2 from pHT4 and create *Sac*II and *Hin*dIII sites at the 5′ and 3′ ends respectively. PCR was performed on a capillary thermal cycler (Corbett Research) for 30 cycles (95 °C, 10 s; 50 °C, 10 s; 72 °C, 60 s) in a 20 μl reaction containing 200 μM dNTPs, 10 pmol of each primer, 1.5 units of *Taq* polymerase (Promega) and 1 × *Taq* polymerase buffer (Promega). The 790 bp PCR product was digested with *Sac*II and *Hin*dIII and cloned into the expression vector pRB269 [17]. The resulting plasmid pRBKL3 produced a ubiquitin–GSTT2 fusion protein. The cDNA cloned in pRBKL3 was sequenced entirely to confirm that there were no errors introduced by the amplification step. When pRBKL3 was transfected into a strain of *E. coli* expressing the yeast ubiquitin-specific protease Ubp1 from the plasmid pCG001 [18], the N-terminal ubiquitin moiety was co-translationally removed from the fusion protein, generating recombinant GSTT2-2 without any additional residues.

Enzyme purification

A 40 litre culture of *E. coli* containing pRBKL3 and pCG001 was grown in a large-scale fermenter. Growth media contained standard Luria Broth supplemented with glucose to a final concentration of 6.3 g/l. Ampicillin and chloramphenicol were added for selection to a final concentration of 100 μg/ml and 34 μg/ml respectively. The culture was allowed to grow until exponential phase (A_{600} between 0.7 and 0.9), then isopropyl β-D-thiogalactoside was added to a final concentration of 0.1 mM and the culture was allowed to grow for another 3 h. Bacterial cells were harvested by centrifugation and resuspended in buffer A (10 mM Tris, 1 mM EDTA, 0.5 mM β-mercaptoethanol, pH 8.25). Cells were then passed through a Ribi cell disruptor and stored frozen in 50 ml aliquots at −20 °C if not used immediately.

A 50 ml sample of cell lysate was diluted with buffer A to 150 ml and centrifuged at 26000 ***g*** for 20 min. The supernatant was applied to a 29 cm × 2.5 cm DEAE-cellulose column equilibrated in buffer A. The column was developed by addition of a NaCl gradient from 0 to 150 mM. Fractions containing activity towards cumene hydroperoxide were pooled and concentrated on an Amicon concentrator with a Diaflo PM10 ultrafilter (Amicon Corp., Lexington, MA, U.S.A.). The concentrated material was loaded on to a 60 cm × 3 cm Sephacryl S-200 gel filtration column pre-equilibrated in buffer B (10 mM sodium phosphate, 1 mM EDTA, 0.5 mM β-mercaptoethanol, pH 6.8). Fractions containing activity towards cumene hydroperoxide were again pooled and loaded on to a 8 cm × 1.5 cm Orange A Matrex gel column (Amicon Corp.) pre-equilibrated in the same buffer. The column was developed using a KCl gradient from 0 to 1 M in buffer B. Fractions possessing activity towards cumene hydroperoxide were pooled and concentrated using a Diaflo PM10 ultrafilter. While concentrating, the sample was desalted and the buffer was changed to buffer A. The concentrated material was loaded on to a high resolution Mono Q HR 5/5 FPLC column and developed using two linear salt gradients of 0–20 mM and 20–200 mM NaCl in buffer A. Fractions containing activity towards cumene hydroperoxide were pooled. Purified GSTT2-2 was examined on SDS/PAGE (12% gels) by the method of Laemmli [19] The gels were stained using a Silver Staining Plus kit (Bio-Rad), following the manufacturer's protocol. Protein concentration was determined using method described by Bradford [20]. Table 1 (see below) presents a summary of the purification steps.

Enzyme characterization

The isoelectric point of GSTT2-2 was estimated by isoelectric focusing on Phast gels (Amrad, Pharmacia) and by chromatofocusing on a Mono P HR 5/20 FPLC column (Amrad). The pH optimum was determined with cumene hydroperoxide as a substrate using a previously described range of buffers [21]. N-terminal amino acid sequencing was performed on an Applied Biosystems 477A automated protein sequencer.

RESULTS

Construction of the GSTT2 expression vector

The expression of GSTT2 cDNA was initially attempted using the expression vector pKK233-2. However, the level of protein expression was extremely low. Subsequently an alternative expression system, utilizing the ubiquitin protein fusion system, was adopted. The expression of eukaryotic protein as a fusion protein with ubiquitin has been shown to enhance the levels of protein expression in a bacterial system [17]. Figure 1 outlines the construction of the ubiquitin–GSTT2 fusion expression vector. The expression vector pRB269 contains a ubiquitin cDNA downstream of a *tac* promoter. A multi-cloning site is available immediately after the ubiquitin cDNA for the insertion of cDNAs to be expressed as ubiquitin fusions. The coding region of GSTT2 was amplified from the pHT4 clone using a forward primer which contained a *Sac*II site and a reverse primer containing a *Hin*dIII site. These restriction sites allow the insertion of the GSTT2 cDNA downstream of the ubiquitin gene without any additional residues between the C-terminal glycine of ubiquitin and the N-terminal methionine of GSTT2-2. We initially constructed a ubiquitin–GSTT2 fusion expression vector excluding the initial methionine of GSTT2. However, the level of protein expression was extremely low and the initial methionine of GSTT2 was subsequently encoded in the pRBKL3 expression vector. The ubiquitin-fused GSTT2 was cleaved by the co-expressed Ubp1 at the ubiquitin–GSTT2 junction, yielding a mature GSTT2-2 recombinant protein. N-terminal amino acid sequencing of the purified enzyme did not identify any ubiquitin sequence, suggesting that the ubiquitin moiety of the fusion protein had been completely removed by the action of Ubp1. However, the amino acid sequence data yielded two residues of about equal concentration at each position, suggesting that two proteins were being sequenced. Further analysis of the data

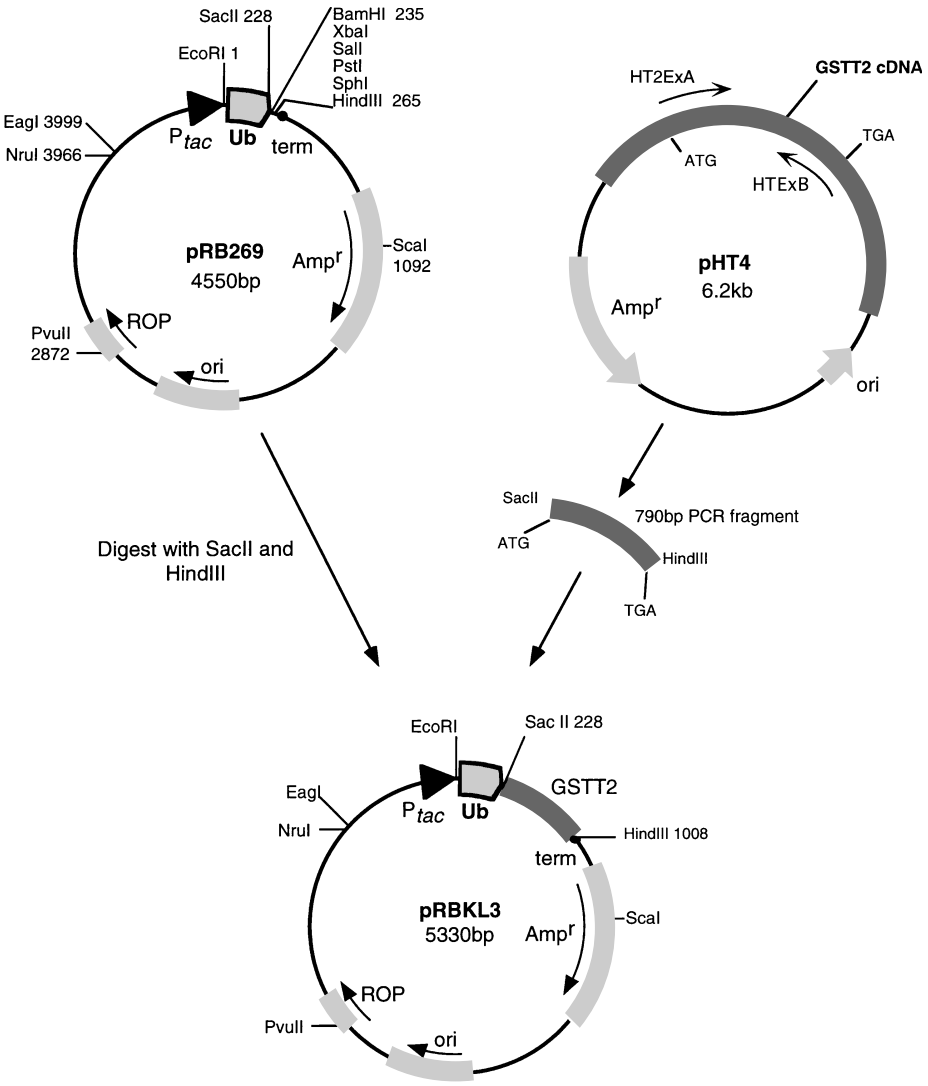

Figure 1 Construction of the GSTT2 expression vector

The coding region of GSTT2 cDNA was amplified from the plasmid pHT4, containing the GSTT2 cDNA [9]. The forward primer contained a *Sac*I site and the reverse primer contained a *Hin*dIII site for directional cloning of the amplified product into the pRB269 expression vector. The recombinant protein was produced as a ubiquitin–GSTT2 fusion protein and was subsequently cleaved by the co-expressed ubiquitin protease Ubp1.

indicated that approx. 50% of the preparation had an N-terminal methionine residue followed by the expected GSTT2 sequence [9], while in the remaining 50% the N-terminal methionine had been removed and the GSTT2 sequence started one residue out of frame. In our experience, it is not uncommon for N-terminal methionine residues to be removed during expression in *E. coli*.

The recombinant GSTT2-2 was expressed at a level of 1.6 mg/l of culture. Although this rate of expression was able to produce sufficient protein for the present studies, the level of protein expression appears to be low when compared with that of 60 mg/l for GSTP1-1 in the same expression vector [17].

Purification of recombinant GSTT2-2

Recombinant GSTT2-2 was purified to apparent homogeneity by a series of chromatographic steps involving anion exchange on DEAE-cellulose, gel filtration on Sephacryl S-200, dye-ligand chromatography on Orange A Matrex gel, and high resolution anion-exchange chromatography on Mono Q FPLC. In all purification steps the protein concentration was monitored by absorbance at 280 nm, and GSTT2-2 activity was monitored by the measurement of glutathione peroxidase activity with cumene hydroperoxide. Crude bacterial lysate was diluted to 3 vol. and after centrifugation was loaded on to an anion-exchange DEAE-cellulose column. The column was washed with 4 bed volumes of buffer A before elution with a NaCl gradient from 0 to 150 mM. Fractions containing activity towards cumene hydroperoxide were eluted between 50 and 66 mM NaCl.

Sephacryl S-200 was used to separate proteins by size, and in this case it also served as a convenient step to desalt and exchange buffers (from buffer A to buffer B). The separation profile from Sephacryl S-200 yielded four major protein peaks. Fractions containing activity towards cumene hydroperoxide fell only within peak 2 (Figure 2). Samples were pooled and loaded on to Orange A Matrex gel. The Orange A retained approx. 15%

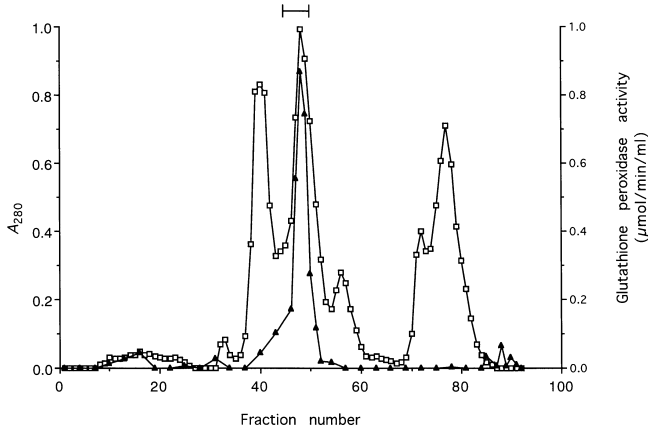

Figure 2 Gel-filtration chromatography of recombinant GSTT2-2 on Sephacryl S-200

Pooled fractions from the DEAE-cellulose column were concentrated and applied immediately to a Sephacryl S-200 gel-filtration column (60 cm × 3 cm). The column was eluted with buffer B at a flow rate of 1.0 ml/min. Samples were collected as 2 ml fractions and GSTT2-2 activity was determined using cumene hydroperoxide (▲). Protein concentration was monitored at A_{280} (□). Active fractions in protein peak 2, indicated by the horizontal bar, were pooled for further purification.

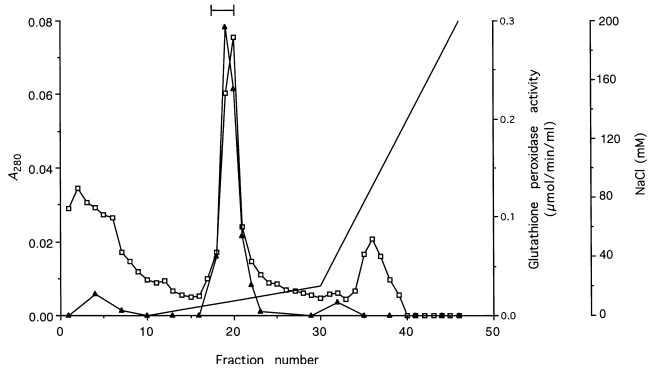

Figure 3 Purification of recombinant GSTT2-2 by Mono Q FPLC anion-exchange chromatography

Pooled fractions from Orange A Matrex gel chromatography were desalted, buffer-exchanged from buffer B to buffer A during concentration and loaded on to a Mono Q HR 5/5 FPLC column. The column was pre-equilibrated with buffer A, and two NaCl gradients of 0–20 mM and 20–200 mM were used to develop the column at a flow rate of 0.5 ml/min. Fractions of 1.0 ml were collected, and GSTT2-2 activity towards cumene hydroperoxide (▲) and absorbance at A_{280} (□) were monitored. Fractions that contained activity towards cumene hydroperoxide were pooled as indicated by the horizontal bar.

of the protein which exhibited activity towards cumene hydroperoxide. However, about 30% of the protein which was active towards cumene hydroperoxide flowed through the column, presumably as a result of overloading. Therefore fractions 13–32, containing the 30% flow-through protein were re-chromatographed after the column had been re-equilibrated. A gradient of KCl from 0 to 1 M was used to develop the column. Fractions found to be active with cumene hydroperoxide were eluted between 0.26 and 0.52 M KCl. The final purification step utilized high resolution anion-exchange chromatography on a Mono Q FPLC column. The fractions which contained activity towards cumene hydroperoxide were eluted from the Mono Q column as a single peak between 4 and 12 mM NaCl (Figure 3). The purity

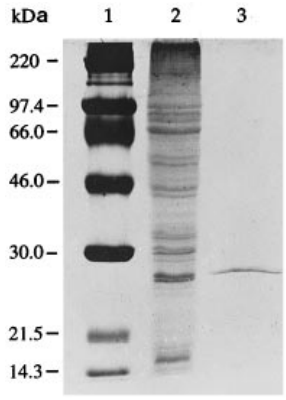

Figure 4 SDS/PAGE analysis of recombinant GSTT2-2

Recombinant GSTT2-2 protein was analysed by SDS/PAGE in a 12% gel. GSTT2-2 was purified to apparent homogeneity. The molecular mass of GSTT2-2 on SDS/PAGE by comparison with the standard markers is about 27 kDa. Lane 1, molecular mass markers; lane 2, crude bacteria lysate expressing GSTT2-2; lane 3, purified GSTT2-2.

of the protein recovered from Mono Q was analysed on SDS/PAGE (12% gel). Silver staining of the gel showed that there were no contaminating proteins (Figure 4). The purified protein had a molecular mass of about 27 kDa when compared with the standard markers, which was in accordance with the calculated molecular mass of 27489 Da. A summary of the purification steps and the yield of purified protein is given in Table 1.

The pH optimum of GSTT2-2 as determined with cumene hydroperoxide was found to be in the pH range 7.6–8.3 (Figure 5). Isoelectric focusing in polyacrylamide gels and chromatofocusing experiments on a Mono P FPLC column indicated that GSTT2-2 had a isoelectric point between 5.2 and 5.3 (results not shown).

Substrate specificity of GSTT2-2

Table 2 outlines the specific activity of GSTT2-2 with a variety of substrates. GSTT2-2 showed peroxidase activity towards cumene hydroperoxide and t-butyl hydroperoxide. However, there was no detectable activity towards hydrogen peroxide. It is notable that GSTT2-2 was active towards unsaturated aldehyde compounds such as the alka-2,4-dienals and alk-2-enals. Furthermore, the activity with these substrates increased with the length of the carbonyl carbon chain. In line with the earlier report of Hussey and Hayes [4], the enzyme was also found to be active towards 1-menapthyl sulphate. The specific activity of the recombinant GSTT2-2 with both 1-menapthyl sulphate and cumene hydroperoxide was comparable with that of the enzyme purified from human liver. GSTT2-2 has no detectable activity towards dichloromethane, unlike the other human Theta-class isoenzyme GSTT1-1 [22]. Finally, low but detectable levels of activity were also demonstrated with ethacrynic acid and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole as substrates.

DISCUSSION

GSTT2-2 was previously purified from human liver [4]. A cDNA clone thought to encode GSTT2 was subsequently cloned from a λgt11 human liver cDNA library [9] and the amino acid sequence derived from the cDNA clone was found to be identical with the N-terminal amino acid sequence provided by Hussey

Table 1 Summary of the purification of recombinant human GSTT2-2

The specific activity of GSTT2-2 (μmol/min per mg of protein) was determined using cumene hydroperoxide as substrate.

Fraction	Volume (ml)	Total protein (mg)	Specific activity (μmol/min per mg)	Total activity (μmol/min)	Yield (%)
Crude extract	125	1812.50	0.035	63.37	100.0
DEAE-cellulose	77	97.79	0.272	26.57	41.9
Sephacryl S-200	26	7.49	1.866	13.97	22.0
Orange A affinity matrix	20	0.76	2.175	1.65	2.6
Mono Q FPLC	2	0.068	6.885	0.47	0.7

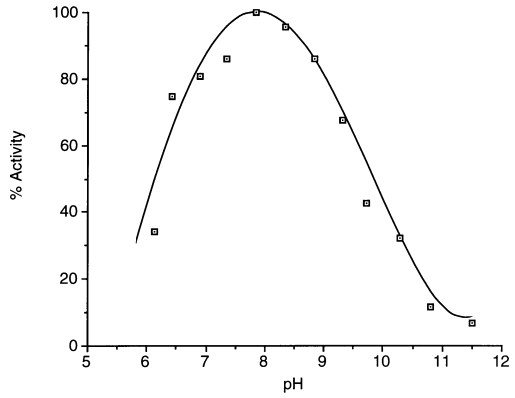

Figure 5 pH profile of GSTT2-2 with cumene hydroperoxide as substrate

Activity is expressed as a percentage of the maximum attained.

Table 2 Activity of recombinant human GSTT2-2 with various substrates

Results are means $\pm$ S.D., calculated from four samples. ND, not detectable.

Substrate	Specific activity (μmol/min per mg of protein)
Cumene hydroperoxide	6.885 ± 0.482
t-Butyl hydroperoxide	0.406 ± 0.042
Hydrogen peroxide	ND
1-Menaphtyl sulphate	0.317 ± 0.028
Ethacrynic acid	0.290 ± 0.020
7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	0.057 ± 0.004
Dichloromethane	ND
1,2-Dichloronitrobenzene	ND
CDNB	ND
1,2-Epoxy-3-(4-nitrophenoxy)propane	ND
4-Phenylbut-3-en-2-one	ND
2-Cyano-1,3-dimethyl-1-nitrosoguanidine	ND
Hexa-2,4-dienal	ND
trans,trans-Hepta-2,4-dienal	ND
trans,trans-Nona-2,4-dienal	$(50 \pm 3) \times 10^{-3}$
trans,trans-Deca-2,4-dienal	$(113 \pm 15) \times 10^{-3}$
trans-Hex-2-enal	ND
trans-Oct-2-enal	$(68.2 \pm 4.8) \times 10^{-3}$
trans-Non-2-enal	$(119.6 \pm 9.6) \times 10^{-3}$

and Hayes [4]. The GSTT2-2 purified from human liver by Hussey and Hayes [4] was active towards both cumene hydroperoxide and 1-menapthyl sulphate, and the recombinant enzyme produced in the present study has similar activities with these substrates, confirming that the cDNA reported by Tan et al. [9] encodes the isoenzyme described by Hussey and Hayes [4].

We tested different expression plasmids that have previously achieved relatively high levels of expression of recombinant GSTs in *E. coli*. However, the highest expression level that we obtained in pRBKL3 was low in comparison with the levels we reported previously with other GSTs [17,23,24]. In these cases, the expressed GSTs were rapidly purified in high yield by glutathione affinity chromatography. The apparently low level of GSTT2-2 expression appears to reflect the low yield of the enzyme during the extensive purification steps required for Theta-class GSTs (see Table 1, and [4]). In the present case, the final yield of purified protein does not appear to be a good measure of protein expression in bacterial cells.

In the present study we tested the catalytic activity of GSTT2-2 with a number of compounds that have been shown to be substrates for other GSTs. Of particular interest is the activity of GSTT2-2 with secondary lipid peroxidation products such as the alka-2,4-dienals and alk-2-enals. Aldehydic products have been identified in rat liver microsomes as a result of ADP-Fe^{2+}-stimulated lipid peroxidation [25]. These products are known to inhibit several microsomal and plasma membrane enzymes, as well as inhibiting DNA and protein synthesis [25]. The activity of GSTT2-2 with these lipid peroxidation products, together with its glutathione peroxidase activity, points to an important role for this enzyme in the protection of cells against the toxic products of oxygen and lipid peroxidation. The specific activity of GSTT2-2 doubled when the carbonyl carbon chain length of alka-2,4-dienals and alk-2-enals increased from eight to nine or from nine to ten carbon atoms. Appreciable increases in specific activity with chain length of alka-2,4-dienals and alk-2-enals were also documented for a GST isolated from the tapeworm *Moniezia expansa* [11] and for the human Mu-class GSTs [24]. This increased activity may reflect the increased hydrophobicity of the longer-carbon-chain compounds, allowing easier access to the hydrophobic active site. The structure of a Theta-class GST from *Lucilia cuprina* revealed that the insect GST has a deeper active site than mammalian GSTs. Furthermore, amino acid sequence alignment has suggested that the extended α-helix 5 in the mammalian Theta-class isoenzymes would result in an even deeper active site in these enzymes [26]. Because of the apparent increased affinity of GSTT2-2 for substrates with increased carbon chain length, and the inability of GSTT2-2 to bind to conventional glutathione/agarose affinity matrices, we attempted to purify GSTT2-2 using a GSH–agarose matrix with a very long spacer arm, equivalent to 20 carbon atoms. Unfortunately, this attempt was no more successful than conventional glutathione affinity matrices (K.-L. Tan and P. G. Board, unpublished work).

The conjugating activity of GSTT2-2 with 1-menapthyl sulphate suggests the possibility of its action against a wide range of reactive sulphate esters, including those of arylmethanols such as

7,12-dihydroxymethylbenz[*a*]anthracene and 5-hydroxymethylchrysene, as in the case of its rat orthologue GST Yrs-Yrs [2]. Conjugation of these compounds may be an important mode of protection against hepatocarcinogenesis. Activity was also detected with ethacrynic acid as a substrate. The level of activity obtained appears to be intermediate between those of the human Alpha- and Pi-class GST isoenzymes [15].

We could not detect any significant activity with dichloromethane as a substrate. In contrast, the alkyl halides appear to be good substrates for GSTT1-1 and the rat 5-5 Theta-class isoenzymes [22,27]. A genetically determined deficiency of GSTT1-1 occurs in approx. 16% of European Caucasians [22,28,29]. The absence of any activity of GSTT2-2 with alkyl halides may make the deficiency of GSTT1-1 of greater physiological significance following exposure to this class of compounds. Very few of the substrates tested with GSTT2-2 gave significant levels of activity, and the natural substrate for this enzyme has yet to be identified. However, the activities observed with 1-menapthyl sulphate, cumene hydroperoxide, alka-2,4-dienals and the alk-2-enals suggest classes of compounds that may include important natural substrates.

The role of GSTT2-2 in cellular metabolism is still largely unknown. Its distinct characteristics when compared with GSTT1-1 or members of the other GST classes may indicate that it has a novel functional role. The broad tissue distribution of GSTT2-2 (K.-L. Tan and P. G. Board, unpublished work) suggests that its role is probably not highly specialized as would be indicated by restricted expression in a specific tissue. Therefore a role in protection against organic peroxides or lipid peroxidation products seems highly likely.

We are grateful to Dr. Gordon Barlin and Mr. Steve Ireland for the preparation of 1-menapthyl sulphate, and to Dr. Denis Shaw and Dr. Peter Milburn for their assistance in protein sequencing. We thank Dr. Rohan Baker for providing the ubiquitin expression plasmids. K.-L.T. received a scholarship from the Australian Government under the AusAid scholarship scheme, and an ANU Ph.D. scholarship from the Australian National University, Canberra, Australia.

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Received 13 November 1995/8 January 1996; accepted 15 January 1996