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Characterization of a human class-Theta glutathione *S*-transferase with activity towards 1-menaphthyl sulphate

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A purification scheme is described for a glutathione *S*-transferase (GST) from human liver that catalyses the conjugation of 1-menaphthyl sulphate (MS) with GSH; the method devised results in an approx. 500-fold increase in specific activity towards MS. The human enzyme which metabolizes MS is a homodimer comprising subunits of M_r 25100, and immunochemical experiments have shown it to be a member of the class-Theta GSTs. Automated Edman degradation of this enzyme has confirmed that it is a Theta-class GST but the amino acid sequence obtained differs from that of GST θ described previously [Meyer, Coles, Pemble, Gilmore, Fraser & Ketterer (1991) Biochem. J. **274**, 409–414]. We have therefore designated the enzyme that catalyses the conjugation of MS with GSH GST T2–2* (in the absence of complete amino acid sequence data, the T1 and T2 subunits are provisionally designated T1* and T2*); the evidence which indicates that GST θ (which should possibly now be called GST T1–1*) and GST T2–2* represent distinct isoenzymes is discussed.

INTRODUCTION

The glutathione *S*-transferases (GSTs) are a complex group of detoxification enzymes that catalyse the conjugation of GSH with a broad spectrum of electrophiles (Ketterer, 1988; Mannervik & Danielson, 1988). The conjugation of toxic electrophiles with GSH not only decreases their ability to modify macromolecules (Chasseaud, 1979) but allows their elimination from the cell via the glutathione *S*-conjugate efflux pump (Sies & Ketterer, 1988; Ishikawa, 1989; Ishikawa *et al.*, 1989).

In all mammals a large number of GST isoenzymes exist, each with distinct catalytic and structural properties. Knowledge of the complexity of this supergene family is incomplete. The earliest reports of chromatographic purification of GST employed either 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP) or 1-menaphthyl sulphate (MS) as substrates (Fjellstedt *et al.*, 1973; Gillham, 1973). The subsequent development of a general purification scheme by Habig *et al.* (1974), which allowed the isolation and characterization of several of the major rat liver GSTs, revealed that many isoenzymes are inactive with either EPNP or MS. By contrast, it was found that most GSTs catalyse the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with GSH and therefore CDNB was adopted as the substrate of choice to monitor GST purification (Clark *et al.*, 1973; Habig *et al.*, 1976). Interestingly, the enzymes that detoxify EPNP and MS are essentially inactive with CDNB and the widespread exclusive use of this latter substrate has resulted in the GSTs that metabolize EPNP and MS being largely ignored until relatively recently [see Hiratsuka *et al.* (1990) and Meyer *et al.* (1991)].

Molecular studies of GSTs have shown that they can be divided, on the basis of sequence identity, into at least five separate classes (Pickett & Lu, 1989; Hiratsuka *et al.*, 1990). One of these is membrane-bound and designated microsomal GST (Morgenstern *et al.*, 1982, 1985; McLellan *et al.*, 1989) and the remaining four are cytosolic proteins, called class Alpha, Mu, Pi and Theta GSTs (Mannervik *et al.*, 1985; Meyer *et al.*, 1991). In addition to structural similarities, GSTs that are members of the same class, but isolated from different species, also often exhibit common functional properties (Mannervik *et al.*, 1985).

By contrast with the other multigene families of GST, relatively little is known about the class-Theta enzymes, particularly in the human. This family, which is responsible for the detoxification of MS and EPNP, merits detailed investigation because it appears to play a critical role in the prevention of hepatocarcinogenesis by sulphate esters formed *in vivo* from hydroxymethylarenes such as 5-hydroxymethylchrysene (HCR) or from 7,12-dihydroxymethylbenz[*a*]anthracene (DHBA) (Okuda *et al.*, 1986; Watabe *et al.*, 1986). A GST from rat liver that detoxifies reactive sulphate esters, including those formed from HCR and DHBA, has been purified and as it comprised two identical subunits it was designated Yrs–Yrs (Hiratsuka *et al.*, 1990). This enzyme was shown to be active with MS but not EPNP. A cDNA encoding GST Yrs–Yrs has been described by Ogura *et al.* (1991). Although at least three class-Theta GSTs exist in the rat (referred to as 5–5, 12–12 and 13–13 by Ketterer and his colleagues) it appears probable that only one of these transferases, possibly 12–12, is active with MS (Harris *et al.*, 1991; Meyer *et al.*, 1991).

It is not known how many class-Theta GSTs exist in the human. To date, a single Theta-class transferase, called GST θ, has been isolated from human liver (Meyer *et al.*, 1991). Although Meyer *et al.* (1991) have reported that this transferase is active with EPNP, it is not known whether it is active with MS. In the rat, separate isoenzymes are responsible for metabolizing EPNP and MS. It therefore appears that if human tissue extracts are able to catalyse the conjugation of MS with GSH, this activity is probably attributable to a Theta-class GST distinct from GST θ.

We have found that human liver cytosol exhibits MS–GSH-conjugating activity. In the present paper we describe the purification of the enzyme responsible for catalysing this reaction. Characterization of this human transferase indicated that it is a member of the Theta class, but it appears to be distinct from the GST θ preparation described by Meyer *et al.* (1991).

EXPERIMENTAL

Materials

All chemicals used were of analytical grade and are readily

Abbreviations used: GST, glutathione *S*-transferase; MS, 1-menaphthyl sulphate; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; CDNB, 1-chloro-2,4-dinitrobenzene; HCR, 5-hydroxymethylchrysene; DHBA, 7,12-dihydroxymethylbenz[*a*]anthracene; HMBA, 7-hydroxymethyl-12-methylbenz[*a*]anthracene.

available commercially. The h.p.l.c. solvents were from Rathburn Chemicals Ltd., Peeblesshire, Scotland. MS was supplied by Professor F. Oesch (Section on Biochemical Pharmacology, University of Mainz, Mainz, Germany).

Tissue

Several human liver specimens were studied, each of which was obtained less than 16 h *post mortem* and stored at −80 °C before use. Macroscopic examination of these specimens at autopsy indicated that there was no evidence of any abnormality in the samples used for study.

Protein determination

Protein concentrations were measured using the method of Bradford (1976) adapted for use on the Cobas Fara (Roche Diagnostics, Welwyn Garden City, Herts., U.K.) centrifugal analyser.

Sodium determination

Sodium concentrations were estimated using flame photometry with an IL 343 photometer [Instrumentation Laboratory (UK) Ltd., Warrington, Cheshire, U.K.].

Enzyme assays

Enzymic activity assays for all substrates were carried out at 37 °C. The methods of Habig & Jakoby (1981) were used for the measurement of GST activity with CDNB, EPNP and *p*-nitrobenzyl chloride. Peroxidase activity, towards cumene hydroperoxide, was measured using an adaptation of the method of Wendel (1981) as described elsewhere (Hussey *et al.*, 1991). The method of Gillham (1971) was used for the measurement of GST activity with the substrate MS.

Enzyme purification

A portion of human liver (approx. 600 g) was allowed to thaw at room temperature and a 25% (w/v) homogenate was prepared in ice-cold 10 mM-Tris/HCl buffer, pH 8.25, containing 1 mM-EDTA and 0.5 mM-dithiothreitol. The 100000 *g* supernatant, referred to as cytosol, was obtained after centrifugation of the homogenate for 60 min, at 4 °C. The cytosol was applied immediately to two columns (4.4 cm × 90 cm) of DEAE-cellulose (Chromatography Services Ltd., Merseyside, Cheshire, U.K.) equilibrated in the homogenization buffer. The two columns, which were run in parallel, were developed with a 0–300 mM-NaCl gradient, formed in the same buffer. Those fractions that contained GST activity towards MS were combined and applied immediately to a column (3.3 cm × 45 cm) of Bio-Rad HT-grade hydroxyapatite (Bio-Rad Laboratories, Hemel Hempstead, Herts., U.K.) equilibrated in 10 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.5 mM-dithiothreitol. A 10–250 mM-sodium phosphate gradient was used to develop the column and the eluate was monitored for enzyme activity. The fractions with activity for MS were combined and dialysed against three changes, each of 2 litres, of 10 mM-sodium phosphate buffer, pH 6.8, containing 1 mM-EDTA and 0.5 mM-dithiothreitol (at 4 °C). The dialysed material was subjected to dye-ligand chromatography on Matrex Gel Orange A (Amicon Ltd., Stonehouse, Glos., U.K.). This column (1.6 cm × 26 cm) was pre-equilibrated with 10 mM-sodium phosphate/1 mM-EDTA/0.5 mM-dithiothreitol, pH 6.8, and protein was eluted with a 0–1.0 M gradient of KCl formed in the running buffer. Those fractions that had been retained by the Matrex Gel Orange A column and possessed activity for MS, were pooled and dialysed extensively against 20 mM-Tris/HCl buffer, pH 8.0, containing 0.5 mM-dithiothreitol. The sample was loaded (in 5 ml portions) on to a Mono Q f.p.l.c. column equilibrated with the 20 mM-Tris/HCl, pH 8.0, buffer. The column was developed with a salt gradient, formed in two linear stages consisting of 0–0.15 M-NaCl formed over 60 min followed immediately by 0.15–1.0 M-NaCl formed over 10 min.

Protein concentration, GST activity with MS as substrate and GSH peroxidase activity with cumene hydroperoxide as substrate were measured in all the chromatographic fractions collected.

Electrophoresis

SDS/PAGE was performed using the discontinuous buffer system of Laemmli (1970). The resolving gel contained 12% (w/v) polyacrylamide and 0.32% (w/v) *NN'*-methylene-bisacrylamide (Hayes & Mantle, 1986*b*).

Immunoblotting

Western blotting was performed by the method of Towbin *et al.* (1979) as described in detail by Hayes & Mantle (1986*a*). Antisera raised against purified human GST were obtained as described previously (Hayes & Mantle, 1986*a*). Antiserum raised in sheep against rat GST E was provided by Dr. W. B. Jakoby (The National Institute of Health, Bethesda, MD, U.S.A.).

Reversed-phase h.p.l.c.

Reversed-phase h.p.l.c. using a μ-Bondapak C_{18} column (Millipore, Harrow, Middx, U.K.), was used to isolate the human Theta-class GST subunits. This method has been described in detail elsewhere (Hayes *et al.*, 1989). The column was developed, over 60 min, with a linear 40–55% (v/v) acetonitrile gradient in aq. 0.1% (v/v) trifluoroacetic acid.

CNBr cleavage

A portion of GST (60 μg), which had been purified under reducing conditions, was subjected to reversed-phase h.p.l.c. The protein in the peak recovered from the μ-Bondapak C_{18} column was freeze-dried before being dissolved in 0.15 ml of aq. 70% (v/v) formic acid containing approx. 2 mg of CNBr. Digestion was allowed to proceed in the dark for 20 h before the reaction mixture was diluted with 10 vol. of water and freeze-dried. The cleaved protein was redissolved in 0.2 ml of aq. 0.1% (v/v) trifluoroacetic acid and the peptides were resolved using an Applied Biosystems 130A Microbore Separation System with an Aquapore RP300 reversed-phase column (7 μm particle size; 2.1 mm × 30 mm) (Applied Biosystems, Warrington, Cheshire, U.K.). The column was developed with a linear 0–70% acetonitrile gradient in aq. 0.1% (v/v) trifluoroacetic acid formed over 45 min.

Amino acid sequencing

Automated amino acid sequencing of GST subunits and CNBr-cleaved peptides was performed on an Applied Biosystems 477A instrument with a 120A on-line phenylthiohydantoin analyser as described elsewhere (Hayes *et al.*, 1989).

RESULTS

Purification of GST from human liver

A GST which has a high activity towards the aralkyl sulphate ester MS was purified from human liver cytosol using four chromatographic steps; namely, anion-exchange chromatography on DEAE-cellulose, hydroxyapatite chromatography,

Table 1. Purification of a human hepatic Theta-class GST

For experimental details see the text. GST activity was measured with MS at 37 °C.

Fraction	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg)	Recovery (%)
Cytosol	38160	53136±2988	1.39±0.08	100
Cytosol after 24 h at 4 °C	38160	11178±1548	0.29±0.04	21
DEAE-cellulose	4030	5304±377	1.32±0.09	9.9
Hydroxyapatite	950	1724±24	1.82±0.02	3.2
Matrex Gel Orange A	3.7	258±7.5	70.1±2.04	0.5
Mono Q f.p.l.c.	0.2	97±3.8	497.2±19.4	0.17

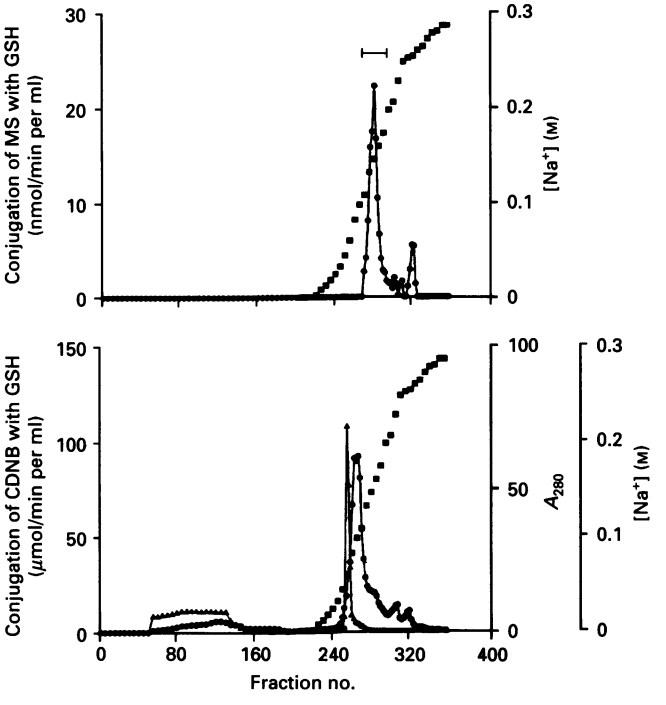

Fig. 1. Elution of human liver cytosolic GST from DEAE-cellulose

Anion-exchange chromatography on DEAE-cellulose was performed at pH 8.25 in 10 mM-Tris/HCl buffer, containing 1 mM-EDTA and 0.5 mM-dithiothreitol. Human liver cytosol was prepared and applied immediately to columns (4.4 cm × 90 cm) containing DE-52. After washing with approx. 1 litre of the equilibration buffer, a 0–300 mM-NaCl gradient (■) was used to develop the anion exchanger. The GST activity towards MS (●) and CDNB (▲) and the absorbance at 280 nm (○) was measured in each fraction (each of 11.5 ml). Those fractions that were combined for further purification are indicated by the horizontal bar.

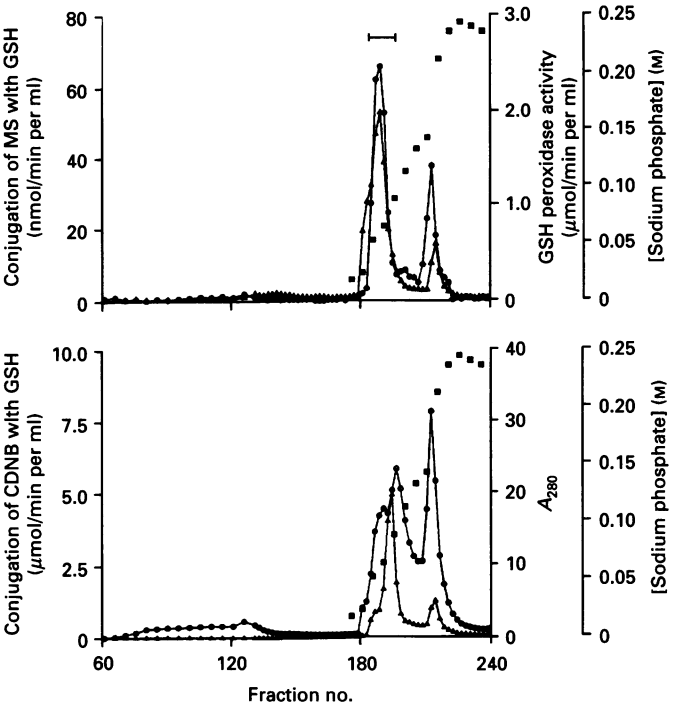

Fig. 2. Hydroxyapatite chromatography of human hepatic GST

Material from the DEAE-cellulose column was subjected to chromatography on a column (3.3 cm × 45 cm) of hydroxyapatite equilibrated with 10 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.5 mM-dithiothreitol. A 10–250 mM-sodium phosphate gradient (■) was used to develop the column and the eluate was monitored for GST activity towards MS (●) and CDNB (▲). Glutathione peroxidase activity towards cumene hydroperoxide (△) and absorbance at 280 nm (○) were also measured in each fraction. The horizontal bar indicates the active fractions that were combined for further purification.

dye-ligand chromatography on Matrex Gel Orange A, and finally anion-exchange f.p.l.c. on Mono Q.

The specific activity of human liver cytosol with MS as substrate was found to decrease significantly with storage at 4 °C. After storage at 4 °C for 24 h the activity of hepatic 100000 *g* supernatants towards MS was typically decreased to about 20% of that recovered in freshly prepared liver cytosol (see e.g. Table 1). Because of this dramatic loss of activity the sample was applied immediately, without prior dialysis, to two columns containing DEAE-cellulose. These two columns were developed in parallel and the elution profiles obtained, one of which is shown in Fig. 1, were closely similar. A peak of GST activity towards MS, which was not co-eluted with a peak of activity towards CDNB, was resolved between 110 and 160 mM-NaCl on the salt gradient.

The fractions collected after hydroxyapatite chromatography of the DEAE-cellulose-purified material were assayed for glutathione peroxidase activity, with cumene hydroperoxide as substrate, as well as for GST activity, towards MS and CDNB. The GST activity towards MS was eluted as two peaks on the sodium phosphate gradient; one peak was eluted between 40 and 70 mM-sodium phosphate and a second peak was eluted between 130 and 170 mM-sodium phosphate. Both of these peaks contained glutathione peroxidase activity. Those fractions in the first peak of GST activity towards MS (i.e. eluted at 40–70 mM-sodium phosphate) that were not coincident with activity towards CDNB were combined for further purification, as shown in Fig. 2.

The third purification step involved chromatography on Matrex Gel Orange A (Fig. 3). Approximately one-third of the GST activity towards MS failed to be retained by this column. However, the remaining GST activity towards MS was eluted as a single peak, between 0.2 and 0.3 M-KCl on the gradient, that was well resolved from the GST activity towards CDNB. Those fractions active towards MS were also found to contain glutathione peroxidase activity for cumene hydroperoxide. The material with MS–GSH-conjugating activity recovered from the Matrex Gel Orange A column was subjected to a final purification

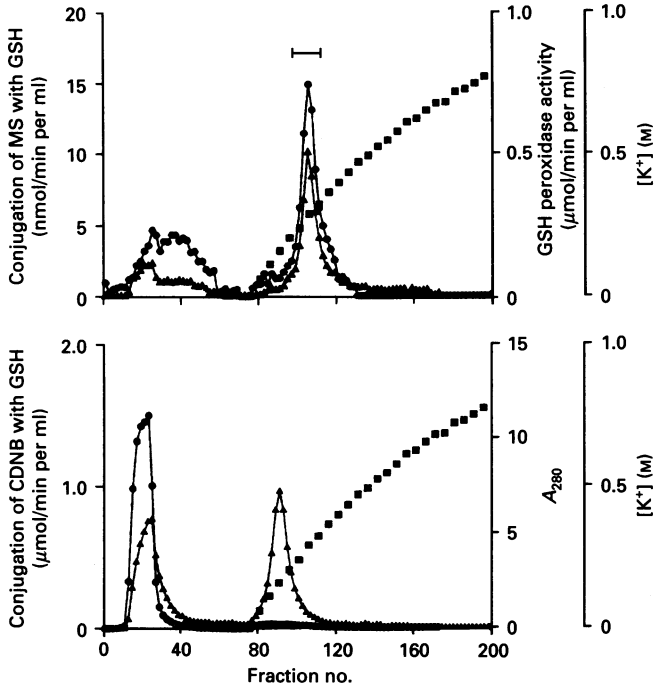

Fig. 3. Dye-ligand chromatography of human GST with activity for MS

The preparation of human Theta-class GST recovered from hydroxyapatite was subjected to chromatography on a column (1.6 cm × 26 cm) of Matrex Gel Orange A equilibrated with 10 mM-sodium phosphate buffer, pH 6.8, containing 1 mM-EDTA and 0.5 mM-dithiothreitol. The sample was dialysed extensively against the equilibration buffer before application to the column. After the formation of a 0–1.0 M-KCl gradient (■), the fractions (3.4 ml) collected were analysed for GST activity using MS (●) and CDNB (▲) as substrates, glutathione peroxidase activity using cumene hydroperoxide (△) as substrate and absorbance at 280 nm (○). The peak of GST activity towards MS resolved on the salt gradient was pooled as indicated by the horizontal bar.

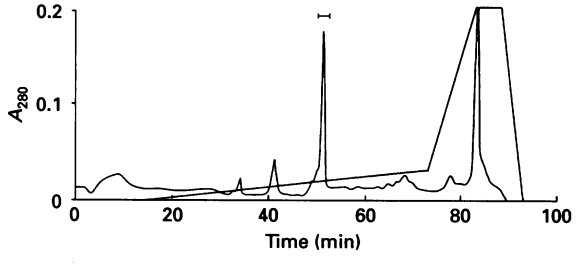

Fig. 4. Purification of a human hepatic Theta-class GST by anion-exchange f.p.l.c.

The material purified by DEAE-cellulose, hydroxapatite and dye-ligand chromatography was dialysed extensively against 20 mM-Tris/HCl buffer, pH 8.4 at 4 °C, containing 0.5 mM-dithiothreitol, before application to Mono Q f.p.l.c. The column was eluted at 0.75 ml/min and developed with a 0–1.0 M-NaCl gradient in the dialysis buffer; this gradient was established in two steps as shown by the straight line. Fractions (0.75 ml) were collected and the absorbance at 280 nm was monitored continuously. Those fractions that contained GST activity towards MS were pooled as indicated by the horizontal bar.

step on Mono Q f.p.l.c. (Fig. 4). The enzyme activities towards MS and cumene hydroperoxide were found to be co-eluted from the Mono Q column as a single peak between 80 and 90 mM-NaCl on the salt gradient.

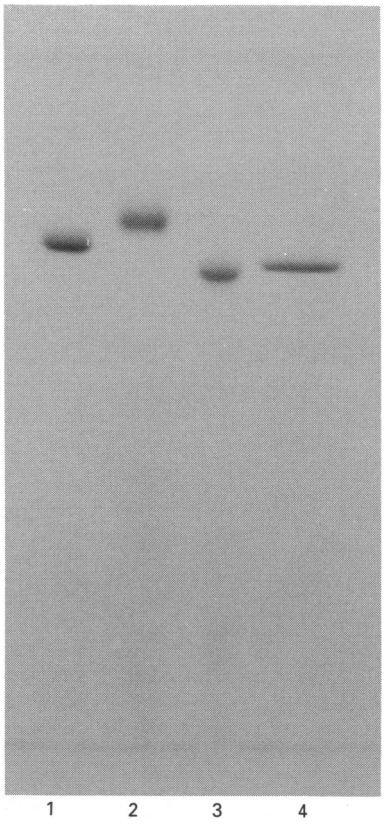

Fig. 5. SDS/PAGE analysis of human GST with activity for MS

Electrophoresis was performed in a resolving gel which contained 12% (w/v) polyacrylamide and 0.32% (w/v) *NN'*-methylenebisacrylamide. Human GST subunit markers B_1B_2 (M_r 26000), μ (M_r 26700) and π (M_r 24800) were loaded into tracks 1, 2 and 3 respectively. Purified human GST active with MS (2 μg of protein) was loaded into track 4.

A summary of the purification is shown in Table 1.

Purity and subunit analysis of human GST active with MS

The GST recovered from Mono Q (Fig. 4) was found to migrate as a single band during SDS/PAGE. By comparison with the Alpha-, Mu- and Pi-class GSTs, the enzyme that was active with MS was estimated to comprise subunits of M_r 25100 (Fig. 5); the M_r values of Alpha-, Mu- and Pi-class GSTs have been calculated as 26000, 26700 and 24800 respectively (Hussey *et al.*, 1986; Hayes, 1989).

The human GST was judged to be 95% pure when analysed by reversed-phase h.p.l.c. (Fig. 6). Human Alpha-class GST B_1 and B_2 subunits were applied to the μ-Bondapak C_{18} column as a means of calibration and were eluted at 50 and 56 min respectively. The GST with activity for MS was eluted from the reversed-phase column after the B_1 and B_2 subunits as a single symmetrical peak at 62 min.

Immunochemical identification of the MS-metabolizing GST as a Theta-class enzyme

Purified human cytosolic GSTs were probed with antisera raised against Alpha-, Mu- and Pi-class GSTs. The enzyme that catalysed the conjugation of MS with GSH was shown to be immunochemically distinct from other human enzymes of classes Alpha, Mu and Pi, in that no cross-reactivity was observed between this enzyme and antisera raised against either GST B_1B_1, GST μ or GST π.

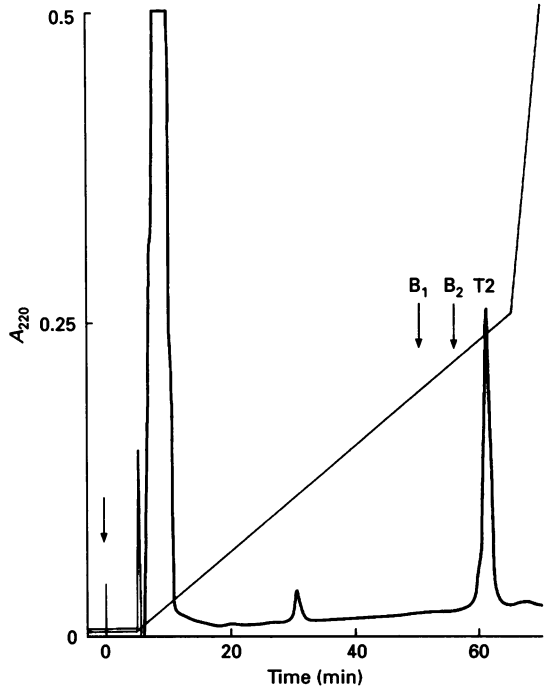

Fig. 6. Reversed-phase h.p.l.c. of human Theta-class GST

A portion (60 μg) of human hepatic GST T2–2*, purified by sequential DEAE-cellulose chromatography, hydroxyapatite chromatography, Matrex Gel Orange A chromatography and Mono Q f.p.l.c., was subjected to reversed-phase h.p.l.c. on a Waters μ-Bondapak C_{18} column (10 μm particle size; 0.39 cm × 30 cm). The column was developed by a linear 40–55 % (v/v) acetonitrile gradient in aq. 0.1 % (v/v) trifluoroacetic acid formed over 60 min, followed by a 55–70 % (v/v) acetonitrile gradient in aq. 0.1 % (v/v) trifluoroacetic acid formed over 5 min. The eluate was monitored continuously at 220 nm.

Table 2. Specific activities of human GST T2–2*

For experimental details see the text. Enzyme assays were performed at 37 °C. Results are expressed as means ± S.D. for three determinations. Abbreviation: ND, not detectable.

Substrate	Specific activity of GST T2–2* (μmol/min per mg)
CDNB	ND
MS	0.497 ± 0.019
EPNP	ND
p-Nitrobenzyl chloride	ND
Cumene hydroperoxide	6.9 ± 0.12

The MS-specific GST from human liver was also challenged with an antiserum raised against the original rat GST E preparation of Fjellstedt *et al.* (1973) and the fact that it displayed immunochemical cross-reactivity with anti-(GST E) IgG is consistent with its identification as a Theta-class transferase. The SDS/PAGE, Western Blot and h.p.l.c. data also suggest that this transferase is a homodimeric protein. As the Theta-class enzyme that catalyses the conjugation of MS with GSH is distinct from GST θ (see below) we have called it GST T2–2*, consistent with the proposed revised nomenclature (Mannervik *et al.*, 1992). (In the absence of complete amino acid sequence data, the T1 and T2 subunits are provisionally designated T1* and T2*.)

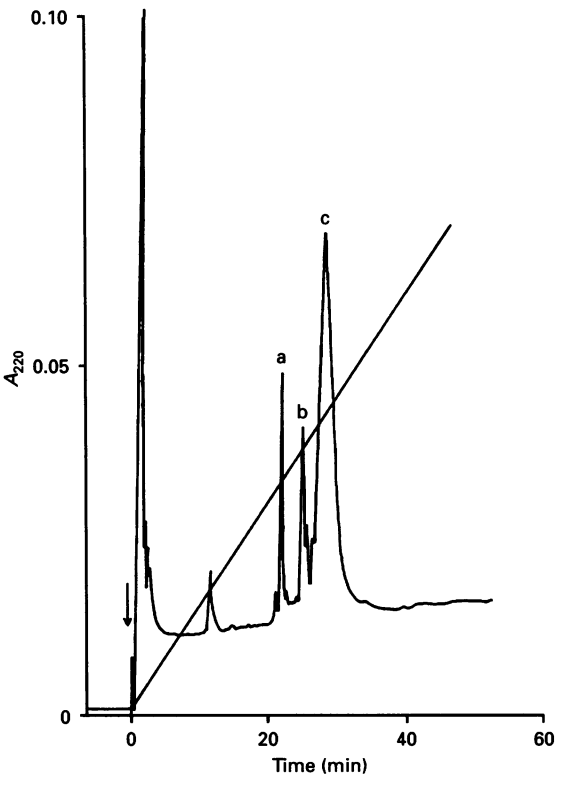

Fig. 7. Preparative peptide map of human GST T2–2*

A portion (60 μg) of purified GST subunit was digested with CNBr. The resulting fragments were dissolved in 0.2 ml of aq. 0.1 % (v/v) trifluoroacetic acid before chromatography on an Aquapore RP 300 column (7 μm particle size; 2.1 mm × 30 mm). The column was developed with a linear 0–70 % acetonitrile gradient in aq. 0.1 % (v/v) trifluoroacetic acid, formed over 45 min, as indicated by the straight line. The column eluate was monitored continuously at 220 nm.

Enzymic properties of human GST T2–2*

The specific activity of purified GST T2–2* towards a range of substrates is shown in Table 2. This enzyme was found to have no detectable activity towards CDNB and, unlike GST θ described by Meyer *et al.* (1991), it was not active with EPNP. GST T2–2* was also inactive with the substrate *p*-nitrobenzyl chloride. However, besides its transferase activity towards MS, the purified GST was found to have considerable glutathione peroxidase activity towards cumene hydroperoxide.

Protein sequence analysis of human Theta-class GST

The GST subunit T2* could not be sequenced directly when subjected to automated Edman degradation and was therefore concluded to possess a blocked *N*-terminus. After digestion of the GST T2* subunit with CNBr, the resulting peptides were resolved by reversed-phase h.p.l.c. (Fig. 7). Three of the CNBr-derived fragments, represented in Fig. 7 by protein-containing peaks a, b and c (which were eluted at 21, 24 and 28 min respectively), were collected and subjected to automated amino acid sequencing. The peptide present in peak a was found to possess a blocked *N*-terminus. Peak b was subjected to five cycles of Edman degradation, but unfortunately paucity of material would permit only a tentative identification of the amino acid sequence; the following residues were obtained: leucine, proline, isoleucine, alanine and leucine. The peptide fragment represented by protein peak c was subjected to 20 cycles of Edman degradation allowing identification of residues 1–21. This sequence is

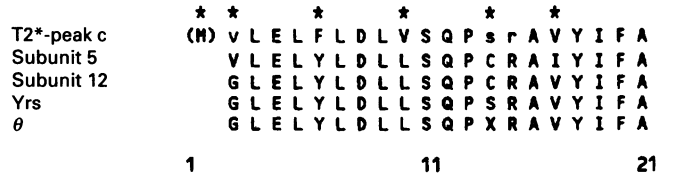

Fig. 8. Comparison of the *N*-terminal amino acid sequence of the T2* subunit with those of other Theta-class GSTs

The *N*-terminal amino acid sequence of subunit T2* was obtained after automated Edman degradation of a CNBr-derived peptide (peak c). This sequence is shown aligned for comparison with the *N*-terminal amino acid sequences of Theta-class GSTs 5–5, 12–12 and θ (Meyer *et al.*, 1991) and GST Yrs–Yrs (Hiratsuka *et al.*, 1990). The putative methionine residue is in parentheses. Where assignments were tentative the residues are shown in lower case. An asterisk (*) indicates amino acid residues that differ between the sequences.

shown in Fig. 8 aligned for comparison with the homologous *N*-terminal amino acid sequences of rat GST Yrs–Yrs (Hiratsuka *et al.*, 1990) as well as rat GST 5–5 and 12–12 and human GST θ (Meyer *et al.*, 1991). Five amino acid differences are apparent between the *N*-terminal sequence of the T2* subunit and those of the other Theta-class GSTs described in the literature. These changes occur at positions 2, 6, 10, 14 and 17; the amino acid residues are numbered according to the sequence of the T2* subunit. At position 2 the T2* subunit and subunit 5 both contain valine, whereas subunit 12, θ and Yrs all contain glycine. At position 6 the T2* subunit contains a phenylalanine residue, whereas GST 5–5, 12–12 and θ all contain tyrosine, and at position 10 GST T2* possesses valine whereas the other Theta-class GSTs contain leucine. The amino acid residue at position 14 of T2* subunit has been tentatively identified as serine; GST Yrs–Yrs also contains a serine at this position, whereas the other rat GST subunits, 5 and 12, both contain cysteine. At position 17 the T2* subunit, as well as GST θ and subunits 12 and Yrs, contain a valine residue, whereas subunit 5 contains isoleucine.

DISCUSSION

Certain sulphate esters, formed from the arylmethanols 7-hydroxymethyl-12-methylbenz[*a*]anthracene (HMBA), HCR or DHBA, by the actions of hydroxysteroid sulphotransferases (Okuda *et al.*, 1989) are potent carcinoigens and have been shown to react readily with purine bases in calf thymus DNA (Watabe *et al.*, 1987). It is believed that GSTs can provide protection against this class of compound, and Hiratsuka *et al.* (1990) have purified an enzyme from rat liver that can detoxify the sulphate esters of HMBA, HCR and DHBA. Surprisingly, this rat enzyme was found to possess higher specific activity towards the relatively harmless aralkyl sulphate ester MS than towards the sulphate esters of HMBA, HCR and DHBA.

During the present investigation we have purified a GST from human liver that catalyses the conjugation of MS with GSH. This transferase, isolated using the protocol we describe, appears to be a novel isoenzyme which has structural homology with the Theta-class GSTs described by Meyer *et al.* (1991). Other workers have isolated GSTs from rat liver cytosol which are active towards MS (Gillham, 1973; Hiratsuka *et al.*, 1990) but, to date, the existence of a human GST that can utilize MS as a substrate has not been documented.

Meyer *et al.* (1991) have isolated a human hepatic Theta-class transferase and called it GST θ. A comparison of the catalytic and structural properties of the GST purified during the present study with those of GST θ indicates that these two proteins represent distinct isoenzymes. The GST that is active with MS contains two subunits which co-migrate during SDS/PAGE (M_r value of approx. 25100) and are co-eluted from the μ-Bondapak C_{18} column. As this enzyme is a homodimer we have designated it GST T2–2* and suggest that GST θ be referred to as GST T1–1*; this is consistent with the principles of the proposed revised GST nomenclature (Mannervik *et al.*, 1992). GST T2–2* not only exhibits activity towards MS, but also has a high glutathione peroxidase activity towards cumene hydroperoxide. Of equal importance is the observation that GST T2–2* was found to have no detectable activity towards either EPNP or CDNB. By contrast, purified GST θ (T1–1*) was reported by Meyer *et al.* (1991) to have a low, but detectable, activity with EPNP and no detectable activity towards cumene hydroperoxide. Unfortunately, the activity of GST θ (T1–1*) towards MS was not documented.

GST T2–2* has a blocked *N*-terminus, and amino acid sequence analysis of the *N*-terminal portion of the T2* subunit was achieved by automated Edman degradation after CNBr cleavage of the protein. As was expected from the immunochemical data, the amino acid sequence of the *N*-terminal region of the GST T2* subunit was found to be closely similar to the published *N*-termini of other Theta-class GSTs (Hiratsuka *et al.*, 1990; Meyer *et al.*, 1991; Ogura *et al.*, 1991); no apparent sequence homology was noted between T2* and other human GST isoenzymes which belong to classes Alpha, Mu and Pi. Although T2* and GST θ have similar *N*-terminal amino acid sequences, three conservative amino acid substitutions are present in the first 20 residues. At positions 2, 6 and 10 the T2* subunit contains valine, phenylalanine and valine, whereas GST θ (T1–1*) contains glycine, tyrosine and leucine. It should also be noted that, whereas GST T2–2* has a blocked *N*-terminus, GST θ is amenable to direct sequence analysis (Meyer *et al.*, 1991), suggesting that an additional difference exists between T1* and T2* subunits at position 1.

The importance of GST in conferring resistance to alkylating agents and other chemotherapeutic drugs has resulted in these enzymes attracting significant attention (Hayes *et al.*, 1990). To date, the contribution of class-Theta GSTs to either clinical drug resistance or resistance in experimental models has not been investigated. Such studies will be assisted by knowledge of the multiplicity of class-Theta GSTs and characterization of individual isoenzymes. Our investigation suggests that the human GSTs T1–1* and T2–2* are equivalent to the rat GSTs 5–5 and 12–12 respectively. It will therefore be interesting to discover whether the human, like the rat, expresses the mitochondrial Theta-class GST (13–13) described by Harris *et al.* (1991). The precise role of this family of GSTs in protection against chemical insult awaits further study.

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