

Theta, a new class of glutathione transferases purified from rat and man

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Glutathione transferases (GSTs) of a novel class, which it is proposed to term Theta, were purified from rat and human liver. Two, named GST 5–5 and GST 12–12, were obtained from the rat, and one, named GST θ , was from the human. Unlike other mammalian GSTs they lack activity towards 1-chloro-2,4-dinitrobenzene and are not retained by GSH affinity matrices. Only GST 5–5 retains full activity during purification, and its activities towards the substrates 1,2-epoxy-3-(*p*-nitrophenoxy)propane, *p*-nitrobenzyl chloride, *p*-nitrophenethyl bromide, cumene hydroperoxide, dichloromethane and DNA hydroperoxide are 185, 86, 67, 42, 11 and 0.03 $\mu\text{mol}/\text{min}$ per mg of protein respectively. Earlier preparations of GST 5–5 or GST E were probably a mixture of GST 5–5 and GST 12–12, which was largely inactive, and may also have been contaminated by less than 1% with another GSH peroxidase of far greater activity. Partial analysis of primary structure shows that subunits 5, 12 and θ are related to each other, particularly at the *N*-terminus, where 25 of 27 residues are identical, but have little relationship to the Alpha, Mu and Pi classes of mammalian GSTs. They do, however, show some relatedness to subunit 1 of *Drosophila melanogaster* [Toung, Hsieh & Tu (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 31–35] and the dichloromethane dehalogenase of *Methylobacterium* DM4 [La Roche & Leisinger (1990) J. Bacteriol. **172**, 164–171].

INTRODUCTION

Rat GSH transferase (GST; EC 2.5.1.18) 5–5 differs from GSTs of the Alpha, Mu and Pi classes (Mannervik *et al.*, 1985) by its lack of activity towards the model substrate 1-chloro-2,4-dinitrobenzene (CDNB) (Fjellstedt *et al.*, 1973) and its failure to bind to GSH or *S*-hexyl-GSH affinity matrices (Meyer *et al.*, 1984). GST 5–5 is further distinguished by having relatively high activity towards 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP), *p*-nitrophenethyl bromide (PNPB), *p*-nitrobenzyl chloride (PNBC) and epoxyeicosatrienoic acids (Spearman *et al.*, 1985).

In the present study two GSTs, termed 5–5 and 12–12, have been isolated from rat liver, each having properties related to earlier preparations of GST 5–5 termed 'E'. The same purification has been applied to a sample of human liver yielding a related enzyme termed ' θ '. The three GSTs have been characterized structurally and enzymically as belonging to a distinct class, termed Theta.

EXPERIMENTAL

Tissues

Rat liver was obtained from either Wistar strain or Sprague–Dawley strain (200–400 g). A sample of male human liver (FH 47) was kindly supplied by Dr. P. Beaune of Necker–Enfants Malades, Paris, France.

Assay of enzyme activities

GST activities towards CDNB, EPNP, PNBC and PNPB were measured at 37 °C in accordance with Habig *et al.* (1974).

Dichloromethane dehalogenase activity was measured by incubating GST 5–5 at 37 °C in a final volume of 4.8 ml of 20 mM-Tris/HCl buffer, pH 7.4, containing 4 mM-GSH and saturated with dichloromethane (0.3%, v/v). The mixture, in a

5 ml glass tube, was kept sealed with Teflon tape. Samples (1 ml) were withdrawn at 5 min intervals and protein was precipitated with 5% (w/v) trichloroacetic acid. The supernatant was analysed for formaldehyde as described by Nash (1953).

DNA hydroperoxides were prepared by X-irradiation of a 1.5 mg/ml solution of herring testis DNA (type XIV; Sigma Chemical Co., Poole, Dorset, U.K.) as described previously (Tan *et al.*, 1988). GSH peroxidase activity was measured as described by Prohaska & Ganther (1976).

Determination of protein

Protein in crude fractions was assayed by using the bicinchonic acid reagent (Pierce and Warriner, Chester, U.K.) or in purer samples from the absorption spectrum in the region of 280 nm.

Assessment of enzyme purity

Purity of the enzymes was assessed by analytical reverse-phase h.p.l.c. (Meyer *et al.*, 1989) and SDS/PAGE (Laemmli, 1970) with 12.5% acrylamide with 2% cross-linker.

Purification of GSTs of the Theta class

All procedures were carried out at 0–4 °C unless otherwise stated. Rats of the Sprague–Dawley strain or Wistar strain were used with no different recovery of enzymes. Livers were perfused with 0.25 M-sucrose and stored if necessary at –70 °C. A homogenate was prepared (typically of 150 g of liver), by using a Waring blender, in 2 vol. (v/w) of buffer A (10 mM-sodium phosphate buffer, pH 7.0, containing 0.16 M-KCl, 25 μM -phenylmethanesulphonyl fluoride, 1 μg of leupeptin/ml, 2 mM-EDTA and 2 mM-dithiothreitol). Human liver was stored at –70 °C before use, and a homogenate was prepared as above but with 10 vol. (v/w) of buffer A. The soluble supernatant obtained by centrifugation at 105000 *g* for 1 h was passed through a GSH-agarose affinity column (0.5 ml per g of liver

Abbreviations used: GST, glutathione transferase; CDNB, 1-chloro-2,4-dinitrobenzene; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; PNBC, *p*-nitrobenzyl chloride; PNPB, *p*-nitrophenethyl bromide.

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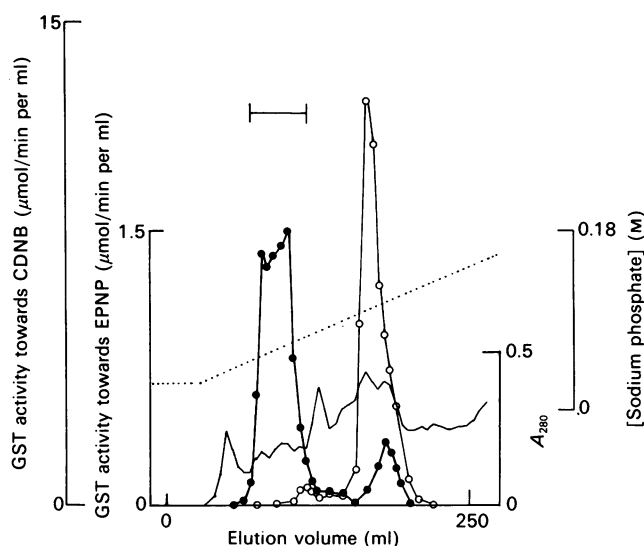


Fig. 1. Purification of GSTs 5-5 and 12-12: hydroxyapatite chromatography

The soluble fraction of rat liver (120 g) was subjected to GSH-agarose affinity chromatography. GSTs that were not bound by the affinity column were separated on a column of Matrex Gel Orange A. The GST 5-5/12-12-containing fractions, identified by their activity towards EPNP, were pooled, concentrated, dialysed against buffer C and fractionated on a 2.5 cm × 15 cm column of hydroxyapatite with a linear gradient of buffer C to buffer D all as described in the text. Fractions were assayed for A_{280} (—), for GST activity towards EPNP (●—●) and towards CDNB (○—○) and for phosphate concentration (.....). The barred line denotes active fractions pooled for further purification.

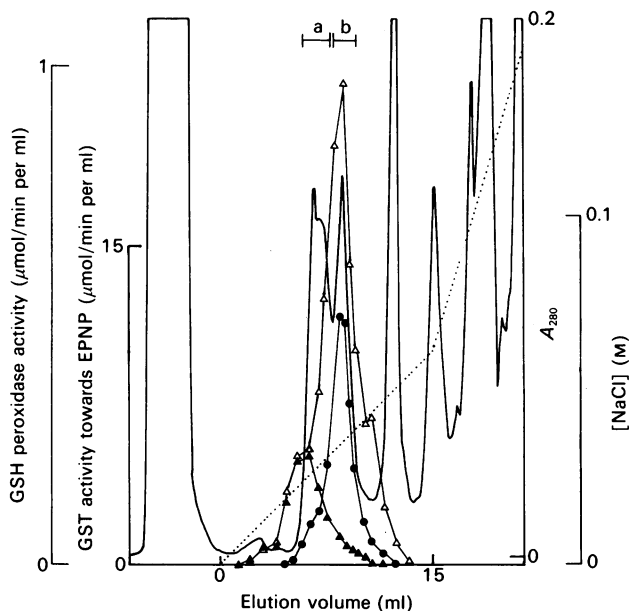


Fig. 2. Purification of GSTs 5-5 and 12-12: anion-exchange chromatography

Fractions from hydroxyapatite chromatography having GST activity towards EPNP were pooled, concentrated, transferred into buffer E and analysed by anion-exchange f.p.l.c. on a Mono Q column with a linear gradient from buffer E to buffer F at a flow rate of 0.35 ml/min, as described in the text. Elution was monitored for A_{280} (—), and fractions were assayed for GST activity towards EPNP (●—●) and GSH peroxidase activity towards cumene hydroperoxide (Δ — Δ) and towards DNA hydroperoxides (\blacktriangle — \blacktriangle). The NaCl gradient (.....) is indicated. Fractions a and b active towards EPNP were collected for further purification.

at approx. 2 ml/min). The protein, which was neither retained nor retarded by the affinity matrix, was collected and dialysed (three changes of 30 vol. over 48 h) against buffer B [10 mM-sodium phosphate buffer, pH 6.8, containing 2 mM-2-mercaptoethanol, 25 μ M-phenylmethanesulphonyl fluoride, 1 μ g of leupeptin/ml, 2 mM-EDTA and 10% (v/v) glycerol]. After centrifugation at 20000 g for 20 min, the sample was applied at 2 ml/min to a column (2.5 cm × 30 cm) of Matrex Gel Orange A (Amicon, Lexington, MS, U.S.A.) equilibrated in buffer B. Less than 10% of the protein was retained by the column. Elution was carried out with a gradient of 0–1 M-KCl in buffer B (450 ml). Fractions containing GSTs of the Theta class were identified as peaks of activity towards EPNP that did not correspond to peaks of activity towards CDNB. Active fractions were pooled, dialysed overnight against 30 vol. of buffer C [30 mM-sodium phosphate buffer, pH 6.8, containing 2 mM-2-mercaptoethanol, 2 mM-EDTA and 10% (v/v) glycerol] and applied to a column (2.5 cm × 16 cm) of hydroxyapatite HTP (Bio-Rad Laboratories, Richmond, CA, U.S.A.) equilibrated in buffer C. When necessary, the sample was first clarified by centrifugation. The column was developed with a 450 ml gradient of 30–180 mM-sodium phosphate in buffer C at approx. 0.6 ml/min. Active fractions were pooled, concentrated to approx. 4 ml by using Centriprep concentrators (Amicon) and transferred by rapid gel filtration on a PD-10 column (Pharmacia LKB, Uppsala, Sweden) into buffer D [30 mM-piperazine/HCl buffer, pH 9.55, containing 2-mercaptoethanol and 10% (v/v) glycerol]. The sample was applied at ambient temperature to a Mono Q anion-exchange f.p.l.c. column (HR 5/5; Pharmacia LKB), which was developed with a gradient of 0–0.15 M-NaCl in buffer D. The pH of collected fractions was reduced to approx. 8.0 by addition of 0.01 vol. of 3 M-sodium acetate buffer, pH 6.0, before they were placed on ice. Active fractions were rapidly transferred as in the previous step into the buffer E [0.1 M-sodium phosphate buffer, pH 6.8, containing 1.5 M-(NH₄)₂SO₄ (enzyme grade; BDH Chemicals, Poole, Dorset, U.K.), 5 mM-2-

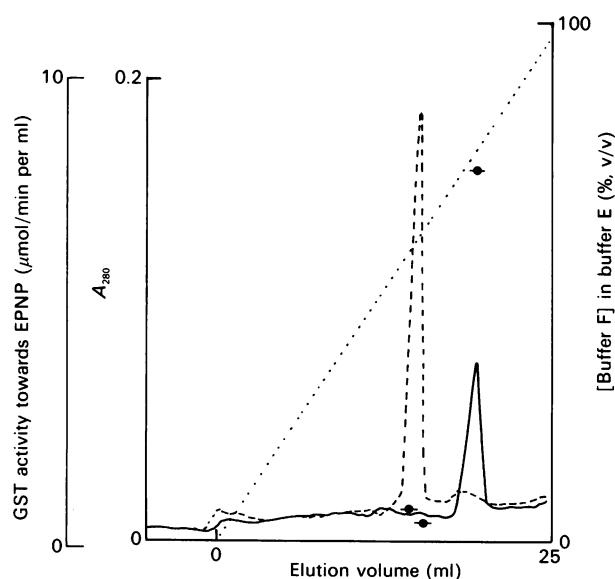


Fig. 3. Purification of GSTs 5-5 and 12-12: hydrophobic-interaction chromatography

Active fractions a and b (Fig. 2) were transferred into buffer E and separately subjected to hydrophobic-interaction chromatography on a PolyPROPYL A column with a gradient of buffer E to buffer F (.....) as described in the text. Elution was monitored for A_{280} : ----, pool a (GST 12-12); —, pool b GST (5-5). Peaks were assayed for GST activity towards EPNP (●).

mercaptoethanol, 2 mM-EDTA and 10% (v/v) glycerol] and applied to a hydrophobic-interaction PolyPROPYL A column (4.7 mm × 100 mm) (Poly LC, Columbia, MD, U.S.A.) operated at ambient temperature by an f.p.l.c. system (Pharmacia LKB). The column was eluted at 0.3 ml/min with a linear gradient from buffer E (see above) to buffer F [20 mM-sodium phosphate buffer, pH 6.8, containing 5 mM-2-mercaptoethanol, 2 mM-EDTA and 20% (v/v) glycerol].

The anion-exchange step could be replaced by chromatofocusing. In this case the sample was transferred into buffer G [25 mM-diethanolamine/HCl buffer, pH 9.5, containing 5 mM-2-mercaptoethanol and 10% (v/v) glycerol] and applied at 0.3 ml/min to a Mono P HR 5/20 f.p.l.c. column (Pharmacia LKB). The pH gradient was developed with buffer H [10% (v/v) Polybuffer/HCl, pH 6.5, containing 5 mM-2-mercaptoethanol and 10% (v/v) glycerol].

It is possible to accumulate material for a large-scale preparation with little loss of activity by freezing soluble supernatant dialysed against buffer B or the undialysed pool from Orange A chromatography.

Primary sequence analysis

Samples of GSTs 5-5, 12-12 and θ were desalted and concentrated by reverse-phase h.p.l.c. on a Brownlee Aquapore RP 300 (30 mm × 2.1 mm) C₈ column (Anachem, Luton, Beds., U.K.) with a solvent gradient of 5-65% (v/v) acetonitrile in water [both solvents containing 0.05% (v/v) trifluoroacetic acid] over 30 min at a flow rate of 0.13 ml/min. The retention times of subunits 5, 12 and θ were 23 min in each case. Approx. 10 μ g of each was subjected to automated Edman degradation in an Applied Biosystems gas-phase sequencer. In the case of the rat enzymes cysteine residues were modified before the sequencing by incubating approx. 50 μ g at 30 °C for 2 h with 4 mM-2-mercaptoethanol/6M-guanidinium chloride in the solvent from h.p.l.c. adjusted to approx. pH 7.5 with NH₄HCO₃. 4-Vinylpyridine (8 mM) was then added, and the samples were left overnight at room temperature. The modified proteins were repurified by reverse-phase h.p.l.c., being eluted at 21.5 min. A portion of the rat enzymes was subjected to CNBr cleavage in 70% (v/v) trifluoroacetic acid for 16 h. Cleavage products were separated by reverse-phase h.p.l.c. and subjected to Edman degradation as above.

A sample of GST θ (10 μ g) was subjected to final purification by reverse-phase h.p.l.c., and a portion was neutralized with NH₄HCO₃ and the acetonitrile removed at 35 °C under argon. This sample, which was shown to be antigenically similar to GST 5-5 by dot-blotting (Towbin *et al.*, 1979), was subjected to Edman degradation as described above.

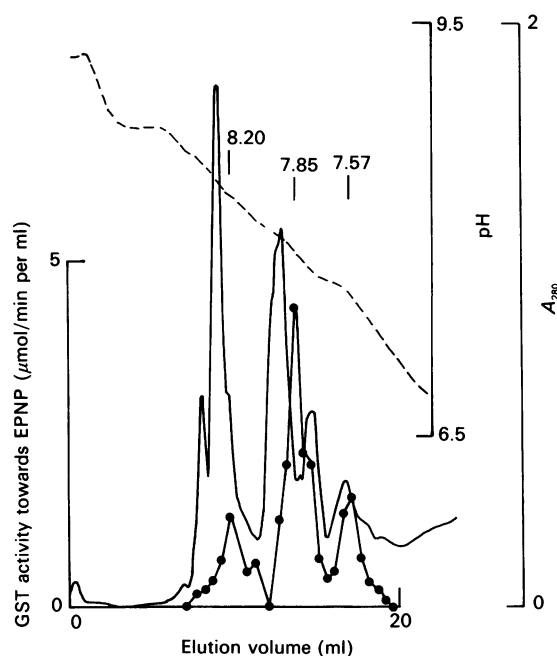


Fig. 4. Partial purification of GSTs 5-5 and 12-12 by chromatofocusing

Active fractions from the hydroxyapatite step from a separate preparation were concentrated, transferred into buffer G and subjected to f.p.l.c. chromatofocusing as described in the text. The A_{280} (—) and pH (---) were monitored and fractions were assayed for GST activity towards EPNP (●—●).

Immunochemistry

Polyclonal antiserum was raised in rabbits by three subcutaneous injections at 10-day intervals of 15 μ g of enzyme in water homogenized with 0.5 ml of Freund's incomplete adjuvant. Serum was collected 14 days after the last injection, and the IgG fraction was purified on a 1 ml column of Protein A-agarose (Sigma Chemical Co.) Antisera to other GSTs were obtained from Medlabs, Dublin, Ireland. A sample of antiserum to GST 'E' (5-5/12-12) purified by the procedure of Fjellstedt *et al.* (1973) was generously provided by Dr. W. B. Jakoby. Immunoreactivity was determined by Western blotting and dot-blotting.

RESULTS AND DISCUSSION

Purification of GSTs 5-5 and 12-12

Purification of GSTs 5-5 and 12-12 was achieved by sequential Orange A, hydroxyapatite, anion-exchange and hydrophobic

Table 1. GST activities of rat and human liver extracts not retained by GSH-agarose

Soluble extracts of rat liver (5 g) and human liver (13.5 g) were subjected to GSH-agarose affinity chromatography, and the GST activities were determined as described in the text.

Sample	Substrate...	GSH transferase activity (μ mol/min per g of liver)			
		CDNB	EPNP	PNBC	PNPB
Rat liver cytosol		120	5.0	7.8	0.8
GSH-agarose unbound		14 (12%)	1.8 (36%)	3.0 (38%)	0.8 (100%)
Human liver cytosol		139	1.8	2.3	0.5
GSH-agarose unbound		20 (14%)	1.9 (108%)	0.5 (22%)	0.5 (100%)

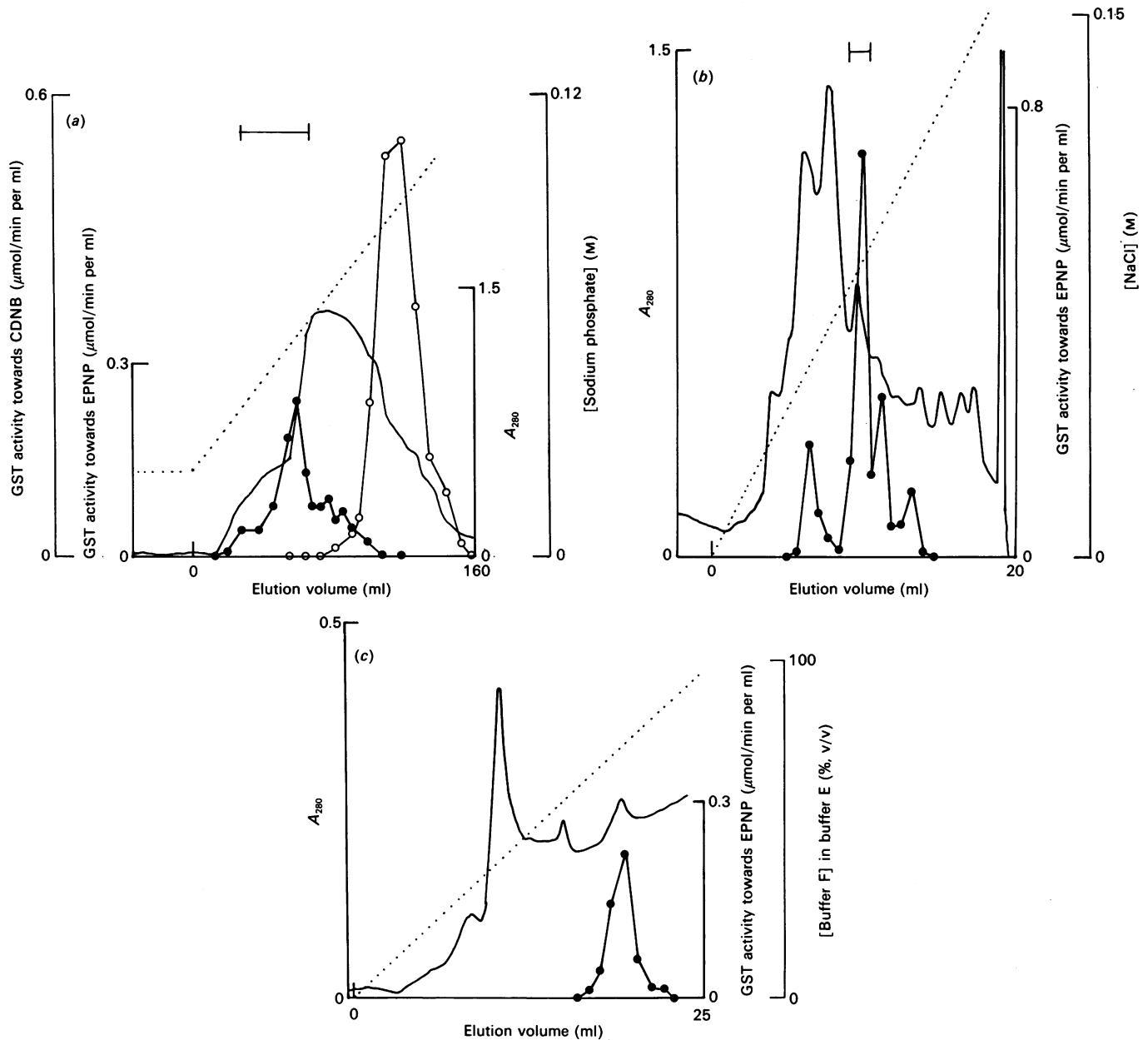


Fig. 5. Purification of GST θ

GST θ was purified from a 13.5 g sample of human liver by using the same protocol as that described in the text for rat liver. (a), (b) and (c) are the hydroxyapatite, anion-exchange and hydrophobic-interaction chromatography steps respectively, which correspond to the separations shown for the rat preparation in Figs. 2, 3 and 4. Line forms and symbols also correspond.

interaction chromatography. The bulk of other soluble GSTs was removed by prior passage through a column of GSH-agarose, a step that is convenient but not necessary. If the soluble supernatant was not dialysed for 48 h or if the livers were not perfused, a portion of GSTs 5-5 and 12-12 failed to bind to the Orange A matrix. In the latter case this was due to overloading with haemoglobin and albumin, which also bind to Orange A. This could be overcome by prior passage through columns of DEAE-cellulose and CM-cellulose, both equilibrated with 30 mM-Pipes/NaOH buffer, pH 7.2, followed by 16 h dialysis against buffer B. The Orange A chromatography step yielded a major peak of activity towards EPNP eluted at approx. 0.1 M-KCl with shoulders on both sides.

The next step of purification, hydroxyapatite chromatography, was carried out in a relatively short column since the essential

glycerol lowers the flow rate considerably (see Fig. 1). There was a partial separation of GSTs 5-5 and 12-12, the latter being eluted first; however, it was more convenient to pool them at this stage. For the highest purification, it is best to follow this with anion-exchange chromatography (Fig. 2), the high pH (9.55) being necessary to retain the GSTs. The partially purified GSTs 5-5 and 12-12 (fractions b and a respectively in Fig. 2) were then separately purified by hydrophobic-interaction chromatography on a PolyPROPYL A column (Fig. 3). A comparable column, 4.6 mm \times 100 mm Propyl cartridge from Brownlee (Anachem, Luton, Beds., U.K.), failed to separate the GSTs. GST 5-5 was usually frozen in small portions in the buffer from the final purification step and its activity was stable. During the last two steps the total activity fell considerably, mainly because of the instability of GST 12-12, which gave rise to a large peak of

Table 2. Activities of GST 5-5

GST 5-5 was purified and assayed for GST and GSH peroxidase activities as described in the text. Abbreviation: DMDH, dichloromethane dehalogenase of *Methylobacterium* (La Roche & Leisinger, 1990).

Substrate	Enzyme...	Specific activity ($\mu\text{mol}/\text{min}$ per mg of protein)				
		GST 5-5	GST 4-4	GST 3-3	GST 2-2	DMDH
EPNP		180	0.9*	0.2*	0.9*	-
PNBC		86	6.1	11†	0.09†	-
PNPB		65	< 0.01	0.1†	< 0.02	-
Cumene hydroperoxide		41	0.4*	0.1*	3*	-
Dichloromethane		11	< 0.1	< 0.1	< 0.1	1
DNA hydroperoxides		0.03	0.03*	0.02*	< 0.005	-
CDNB		< 0.5	20*	50*	38*	-

* Reviewed in Ketterer *et al.* (1988).

† Reviewed in Jakoby *et al.* (1976).

inactive GST 12-12 slightly separated from any GST 12-12 remaining active. It is not possible at present to determine accurately the activity of native GST 12-12, but its activity is estimated to be similar to that of GST 5-5.

Purified GSTs 5-5 and 12-12 were both at least 98% pure as judged by analytical reverse-phase h.p.l.c. Their retention times were similar (32 min) and were between those of subunits 6 and 11 (Meyer *et al.*, 1989). Both yielded a single band on SDS/PAGE co-migrating with subunits 3 and 4 (Yb) or very slightly faster (results not shown).

GSTs 5-5 and 12-12 can also be well separated by chromatofocusing of the pool from hydroxyapatite (Fig. 4). In this case one peak of GST 12-12 is obtained eluted earlier (pH 8.20) than two peaks (pH 7.85 and pH 7.57) both having activities of GST 5-5. This is the only method yielding useful amounts of active GST 12-12 free from other GSTs.

Purification of GST θ

The behaviour of GST activity from the soluble fraction of human liver upon GSH-agarose affinity chromatography is shown in Table 1. As with rat liver, considerable GST activity towards EPNP failed to bind to the affinity matrix, suggesting the presence of a GST similar to GSTs 5-5 and 12-12. Additionally, most of the activity towards PNBC and PNPB, also relatively specific for GST 5-5, is found in the non-retained fraction. GST θ was purified from this fraction by using a procedure virtually identical with that for GSTs 5-5 and 12-12. It was eluted slightly earlier than GSTs 5-5/12-12 from Orange A and hydroxyapatite and at the same time as GST 5-5 on anion-exchange and hydrophobic-interaction chromatography (Figs. 5a-5c). The activity fell rapidly in the latter stages, similarly to that of GST 12-12, being only 4 $\mu\text{mol}/\text{min}$ per mg of protein towards EPNP in the purified material.

N-Terminal sequence

Subunit 5	VLELYLD LLSQPCRAVY IFAKKNIPF QMHTVELRCG EHLCDXXAQV
Subunit 12	GLELYLD LLSQPCRAVY IFAKKNIPF QLRITVDLRCG QHLC.EFSOX
θ	GLELYLD LLSQPCRAVY IFAKKNIPF QLRITVDLRCG QHLXSDFAQX
<i>Methylobacterium</i>	VSPNPTNIHT GKTLRLVYH. PASQPCRSAH QEMYEIDVVF EEEVDISTD ITERQEFDRDK
<i>Drosophila</i>	MVDFEYVL PGSSPCRSVI MTAKAVGVEL NKKLLNLOAG EHLKHEFLKI
Prostaglandin D synthase	PNYKILYFN MRGRA.EIYY IFAYLXIK
Subunit 3	PMILGYWN VRGLT.HAIR LLELYTSSY EEKRYAMGDA PDYDR..SQW

CNBr-cleavage fragments

Subunit 5	KDGGFTLCEV VAILLYLAHK YKVPDHCYFQ XLQARARV
<i>Methylobacterium</i>	VDSEFTVWES VAIARYVNEK FDGAGNWFRG GTOERAQI
<i>Drosophila</i>	VDNGFALWES RAIQVYLVEK YGKTDLSLYPK CPKRAVI
Subunit 3	IDGSRKITQS NAIMRYLARK HHLGGET..E EERIRADI
Subunit 5	FPVFLGEQIR PE SPVGGCPVFE GRPRLAA
Subunit 12	VLALQRLQEDK FLRDRAFIAG QQV LVIARIPKKT LPVPPPEAXA VLRIARIPKT LPVPPPEAXA

Fig. 6. Primary structures of GST subunits 5, 12 and θ

Primary structure of GST subunits 5, 12 and θ are compared with those of the dichloromethane dehalogenase of *Methylobacterium* (La Roche & Leisinger, 1990), GST subunit 1 of *Drosophila melanogaster* (Toung *et al.*, 1990) and rat GST subunit 3 of the Mu family (Ding *et al.*, 1985). Note that evidence for heterogeneity was observed for subunit 12 since two of the CNBr-cleavage fragments have closely related sequences. Also, serine was observed in addition to cysteine at residue 13.

GST θ was 90% pure when analysed by reverse-phase h.p.l.c. and gave two bands upon SDS/PAGE, one between those of GST π and GST α and one of slightly higher mobility than GST π .

Yields

Since GST 12-12 and GST θ lose activity during the purification but do not obviously disappear in terms of protein, it is necessary to calculate yields in terms of protein rather than activity. The data indicate that GSTs 12-12, 5-5 and θ represent approx. 0.02%, 0.002% and 0.003% of the soluble liver protein respectively. In one experiment the activity due to GST 12-12 was lost early in the purification as a result of lengthy storage of the soluble fraction. The activity recovered in that experiment, which was all due to GST 5-5, was approx. 100% at each step, suggesting that the above yields based on protein are reasonably accurate.

Activities

The activities of GST 5-5 are given in Table 2. They are considerably higher than those measured previously, e.g. 6-fold for EPNP and approx. 20-fold for PNBC and PNPB (Fjellstedt *et al.*, 1973; Jakoby *et al.*, 1976; Meyer *et al.*, 1984). It seems likely, therefore, that the material purified earlier was a mixture of GST 5-5 and inactive GST 12-12. Furthermore, it can be deduced that 'active' GST 12-12 has a comparable activity towards EPNP but a somewhat lower activity towards PNPB and PNBC than GST 5-5. The specific activities of GST θ in its fully active form are also unknown but are likely, according to the data in Table 1, to be of the same order as those of GST 5-5. The activities of GST 5-5 towards EPNP, PNBC, PNPB, cumene hydroperoxide and dichloromethane are far higher than those of other rat GSTs.

We have previously attributed a DNA hydroperoxide GSH peroxidase activity to GST 5-5 of 0.5-3.5 $\mu\text{mol}/\text{min}$ per mg of protein (Tan *et al.*, 1988), much higher than that of other GSTs. It is now clear (see Fig. 2) that the activity was due to the presence of another GSH peroxidase of very high specific activity (possibly 1000 μmol per mg of protein). It contaminated earlier GST preparations at the level of approx. 0.3%. This GSH peroxidase co-purifies in every step of the purification except anion-exchange. If care is taken to remove this enzyme, the GSH peroxidase activity of GST 5-5 towards DNA hydroperoxides appears to be comparable with that of GST 4-4 (30 nmol/min per mg of protein).

Immunochemistry

When tested by dot-blotting, the antiserum to GST 'E' and the antiserum prepared against GST 5-5, purified as described above, gave similar results, namely a positive reaction against GST 5-5 > GST 12-12 > GST θ > GST 3-4 = GST 1-2 \gg GST 7-7. Several positive-reacting bands were observed when liver or testis soluble fractions were analysed by Western blot.

Other species

Both hamster and mouse liver soluble supernatant were found to contain a substantial portion of GST activity towards EPNP that was not bound by GSH-agarose and that was retained by the Orange A column. It is likely therefore that they also contain GSTs similar to GSTs 5-5, 12-12 and θ .

Protein sequence analysis

Partial amino acid sequences of subunits 5, 12 and θ are shown in Fig. 6 together with sequences of other GSTs with which there is some degree of similarity.

The N-termini of subunits 5, 12 and θ are very similar, with 29 of the first 40 (72.5%) residues identical. However, they show little similarity to GSTs of the Alpha, Mu or Pi classes (Mannervik *et al.*, 1985), e.g. four residues of the first 40 (10%) of subunit 3 (Mu class) identical with those of subunit 5 or subunit 12. The structural differences between subunits 5, 12 and θ and other classes of GSTs being so great, they belong in a separate class. We suggest that it is called 'Theta'.

Greater sequence similarity is found between subunits 5, 12 and θ and the recently described dichloromethane dehalogenase (GST) of *Methylobacterium DM4* (La Roche & Leisinger, 1990) and the major GST of *Drosophila melanogaster* (Toung *et al.*, 1990), i.e. 25% and 32.5% identities respectively of the first 40 residues and 35% and 46% identities respectively with subunit 5 in a highly conserved region obtained from a CNBr-cleavage fragment. The high activity of GST 5-5 towards dichloromethane (Table 2) suggests that the structural similarity to the *Methylobacterium* GST may be of functional significance. Perhaps the *Drosophila* and *Methylobacterium* enzymes should be associated with the Theta family.

Another interesting relationship is suggested by a sequence of five residues (16-20) in subunit 5 that is identical with residues 17-21 of the prostaglandin D synthase of rat spleen (Urade *et al.*, 1987).

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