Cytosolic glutathione transferases from rat liver

Primary structure of class Alpha glutathione transferase 8–8 and characterization of low-abundance class Mu glutathione transferases

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Six GSH transferases with neutral/acidic isoelectric points were purified from the cytosol fraction of rat liver. Four transferases are class Mu enzymes related to the previously characterized GSH transferases 3–3, 4–4 and 6–6, as judged by structural and enzymic properties. Two additional GSH transferases are distinguished by high specific activities with 4-hydroxyalk-2-enals, toxic products of lipid peroxidation. The most abundant of these two enzymes, GSH transferase 8–8, a class Alpha enzyme, has earlier been identified in rat lung and kidney. The amino acid sequence of subunit 8 was determined and showed a typical class Alpha GSH transferase structure including an N-acetylated N-terminal methionine residue.

INTRODUCTION

Glutathione transferases (EC 2.5.1.18) occur in multiple forms (Mannervik, 1985; Mannervik et al., 1985) and are of great importance in the biotransformation of xenobiotics. These enzymes catalyse conjugation of GSH with a variety of electrophilic compounds (Chasseaud, 1979), and may function as binding proteins for hydrophobic molecules (Smith & Litwack, 1980).

The cytosolic GSH transferases so far isolated and characterized are all dimeric proteins, and a nomenclature reflecting the subunit composition has been adopted (Jakoby et al., 1984). Eight rat GSH transferase subunits have been named according to this nomenclature (Mannervik & Danielson, 1988).

Studies of the GSH transferases in rat testis (Guthenberg et al., 1983), lung (Robertson et al., 1985), kidney (Guthenberg et al., 1985a), and small intestine (Tahir et al., 1988) have revealed marked differences in the distribution of the multiple forms. For instance, GSH transferase 7–7 is one of the major enzymes with basic pI values found in lung, kidney and small intestine. Testis contains only trace concentrations of transferase 7–7, and the concentration in normal liver is below the detection level of normal purification procedures (Ålin et al., 1985a). The basic GSH transferase 1–1, and therefore subunit 1, has not been detected in lung and testis. Furthermore, subunit 3, tentatively identified, occurs only in low concentration in kidney (Guthenberg et al., 1985a). The pattern of neutral/acidic GSH transferases also displays a tissue variation. In kidney only one peak of activity was found (Guthenberg et al., 1985a), whereas four forms were detected in the lung (Robertson et al., 1985). Transferase 6–6, which is the dominating form in testis, has been identified only in low concentrations in other organs. Transferase 8–8 has been found in rat kidney, lung and liver. This enzyme shows high specific activity against 4-hydroxyalk-2-enals and has been suggested to play an important role in detoxication of such activated alkenes (Jensson et al., 1986).

Although the basic GSH transferases from rat liver have been extensively studied (Habig et al., 1974; Askelöf et al., 1975; Beale et al., 1982; Mannervik & Jensson, 1982; Hayes & Clarkson, 1982; Hayes, 1983; Ålin et al., 1985a), much less is known about the properties of the near-neutral/acidic GSH transferases. In the present paper we describe the purification and characterization of several neutral/acidic GSH transferases from rat liver cytosol, including the important GSH transferase 8–8. We also report the primary structure of subunit 8, the first structure of a class Alpha transferase deduced by amino acid sequence analysis.

MATERIALS AND METHODS

Chemicals

Sepharose 6B, Sephadex G-25 and materials for f.p.l.c. were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden, and DEAE-cellulose (DE 52) was from Whatman Biochemicals, Maidstone, Kent, U.K. S-Hexylglutathione was synthesized and coupled to Sepharose 6B as previously described (Mannervik & Guthenberg, 1981). 4-Hydroxyalk-2-enals were generously provided by Dr. H. Esterbauer, Institut für Biochemie, Universität Graz, Graz, Austria. All other chemicals were standard commercial products.

Assays

Enzyme activity was determined spectrophotometrically at 30 °C by the methods cited: 1,2-dichloro-4-nitrobenzene (Booth et al., 1961); 1-chloro-2,4-dinitrobenzene, trans-4-phenylbut-3-en-2-one and ethacrynic acid (Habig et al., 1974); cumene hydroperoxide (Lawrence & Burk, 1976); 4-hydroxyalk-2-enals (Ålin et al., 1985b). Protein concentration was determined by the method of Lowry et al. (1951), after precipitation with ice-cold 10% (w/v) trichloroacetic acid, centrifugation (30 min), washing with trichloroacetic acid, and dissolution in 4 M-NaOH; bovine serum

albumin was used as standard. SDS/polyacrylamide-gel electrophoresis was performed essentially as described by Laemmli (1970).

Purification procedure

A hepatic cytosol fraction from male Sprague–Dawley rats was prepared by ultracentrifugation and a GSH transferase-enriched fraction was obtained by S-hexyl-glutathione–Sepharose 6B affinity chromatography (Ålin et al., 1985a). The material from the affinity step was desalted on a Sephadex G-25 column (9 cm × 30 cm) equilibrated with 10 mM-Tris/HCl buffer, pH 7.8, containing 1 mM-dithiothreitol (buffer A). It was then applied on a DEAE-cellulose column (2 cm × 10 cm) equilibrated with buffer A. After washing with buffer A until no activity of unbound basic GSH transferases was eluted, a linear concentration gradient (350 ml of buffer A and 350 ml of buffer A containing 0.1 M-NaCl) was used to elute the neutral/acidic GSH transferases.

The enzymes in peaks II and III from the DEAE-cellulose column (Fig. 1) were separately desalted on Sephadex G-25 in 25 mM-triethanolamine/acetate buffer, pH 8.0, containing 1 mM-dithioerythritol (buffer B), concentrated and subjected to chromatofocusing in the pH range 8 to 5.5 by using the Pharmacia f.p.l.c. system (Mono P, equilibrated with buffer B). A pH gradient was created by elution with buffer made up of 3 ml of Polybuffer 96 and 7 ml of Polybuffer 74 adjusted to pH 5.5 with acetic acid and diluted to 200 ml. The elution buffer contained 1 mM-dithioerythritol.

The active enzyme in two of the peaks from the chromatofocusing of the material in DEAE-cellulose peak II were separately chromatographed on the anion-exchanger Mono Q by using the Pharmacia f.p.l.c. system. The Mono Q column was equilibrated with 10 mM-Tris/HCl buffer, pH 8.0, containing 1 mM-dithioerythritol and eluted by a linear salt gradient (0–0.1 M-NaCl) in the same buffer.

Structural analysis

Fractions containing the three major acidic GSH transferases [A(2), A(3) and A(5)] were desalted on Sephadex G-50, equilibrated with 10 mM-NH$_4$HCO$_3$ and freeze-dried. The intact proteins were dissolved in 30% (v/v) acetic acid and subjected to sequencer degradation. GSH transferase 6–6 from testis (Guthenberg et al., 1985b) was treated in a corresponding manner.

A large amount (approx. 80 nmol) of the protein in peak A(5) (GSH transferase 8–8) was carboxymethylated with iodo[^{14}C]acetate followed by treatments, in separate samples, with CNBr and trypsin to generate peptides, which were purified by reverse-phase h.p.l.c. (Ålin et al., 1986). Peptides were analysed for amino acid composition and sequence. Redigestions to produce smaller fragments for analysis were performed with lysine-specific proteinase (Boehringer), or with trypsin after citraconylation to restrict cleavages to arginine residues (Cederlund et al., 1988). The mass of the N-terminal acetylated peptide was analysed by time-of-flight mass spectroscopy and the C-terminal peptide structure was determined by carboxypeptidase Y digestion (Hayashi et al., 1973).

For protein sequence analysis Beckman 890 D and Applied Biosystems 470 A instruments were used. Amino acid phenylthiohydantoin derivatives were identified by h.p.l.c. (Kaiser et al., 1988). Amino acid analyses were performed with a Beckman 121 M analyser after hydrolysis in evacuated tubes at 110 °C for 24 h with 6 M-HCl, containing 0.5% (w/v) phenol.

RESULTS

Enzyme purification

Tables 1 and 2 summarize the results of a purification of the neutral/acidic GSH transferases. In the DEAE-cellulose step the basic GSH transferases, which are not bound, are separated from the neutral/acidic enzymes, which are resolved into three peaks (Fig. 1). GSH transferase 4–4, previously isolated as a 'basic' enzyme (Ålin et al., 1985a), is eluted in peak I; analysis of the DEAE-cellulose flow-throw fraction by chromatofocusing (Ålin et al., 1985a) confirmed that this enzyme had been removed. Peaks II and III represent two groups of neutral/acidic GSH transferases, which can be distinguished by relatively high activity with 1,2-dichloro-4-nitrobenzene and ethacrynic acid respectively (results not shown). On the basis of activity with 1-chloro-2,4-dinitrobenzene as well as protein content, peaks II and III account for 3–4% of the total amount of GSH transferases bound to affinity matrix (Table 1).

The separation of GSH transferase in peak II by chromatofocusing in the pH range 8 to 5.5 demonstrated four active components designated A(1), A(2), A(3) and A(4) in order of decreasing pH in the gradient (Fig. 2a).

Table 1. Separation of basic and neutral/acidic rat liver GSH transferases

For experimental details see the text.

Fraction	Volume (ml)	Total activity (μmol/min)	Specific activity (μmol/min per mg)	Yield (%)
Supernatant	370	6290	0.72	(100)
Sephadex G-25	530	5300	0.83	84.3
Affinity chromatography	320	5120	16.0	81.4
DEAE-cellulose				
Flow-through (basic)	100	4000	20.0	63.6
Peak I ⎫ (neutral/	83	270	14.0	4.3
Peak II ⎬ acidic)	47	190	33.3	3.0
Peak III ⎭	28	60	10.3	1.0

Table 2. Chromatofocusing of neutral/acidic GSH transferases in DEAE-cellulose peaks II and III
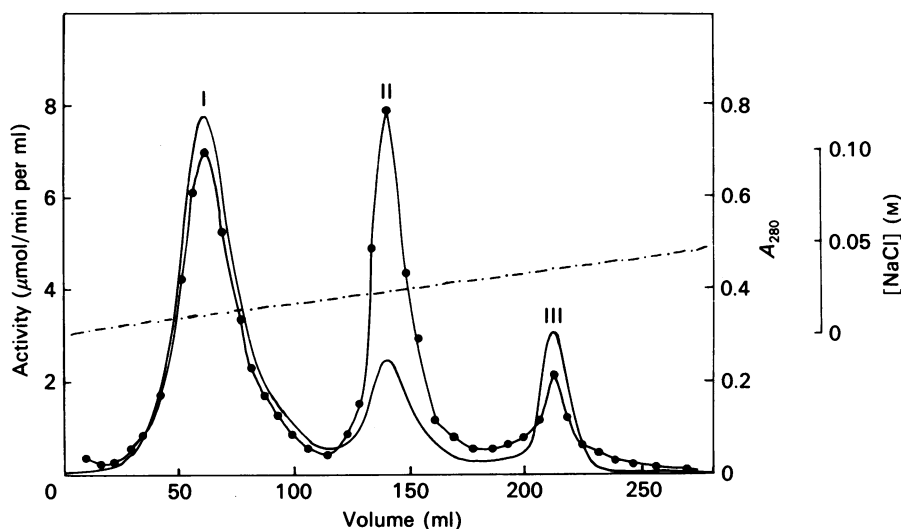
For experimental details see the text.

Fraction	Volume (ml)	Total activity (μmol/min)	Specific activity (μmol/min per mg)	Yield (%)
DEAE-cellulose peak II	47	190	33.3	(100)
Sephadex G-25	86	198	35.0	104
Mono P				
Peak A(1)	4.5	5.5	12.0	2.9
Peak A(2)	3.0	111	45.0	58.4
Peak A(3)	4.5	24.0	30.0	12.6
Peak A(4)	3.0	6.6	30.0	3.5
DEAE-cellulose peak III	28	60.0	10.3	(100)
Sephadex G-25	55	55.0	10.0	91.7
Mono P				
Peak A(5)	4.0	33.6	10.0	56.0
Peak A(6)	3.0	1.9	10.1	3.2

Fig. 1. Elution profile of DEAE-cellulose chromatography of near-neutral/acidic rat liver GSH transferases

Activity was measured with 1-chloro-2,4-dinitrobenzene as electrophilic substrate ($\bullet$). Protein concentration was monitored at 280 nm (——). Elution was performed with a concentration gradient of NaCl (–·–·–).

Complete resolution of components A(2) and A(3) was achieved by chromatography on Mono Q (Figs. 2b and 2c). As judged by SDS/polyacrylamide-gel electrophoresis, the GSH transferases in peaks A(1), A(2) and A(3) were homogeneous, whereas that in peak A(4) was not. Chromatofocusing of the material in peak III from the DEAE-cellulose column gave two activity peaks of apparently homogeneous GSH transferases, which were designated A(5) and A(6) (Fig. 3).

Molecular properties of the purified enzymes

The apparent M_r values of the neutral/acidic GSH transferases were estimated by SDS/polyacrylamide-gel electrophoresis; rat GSH transferase subunits 1, 2 and 3 were used as M_r markers (Jakoby et al., 1984). This analysis showed (Table 3) that the GSH transferases in peaks A(1) and A(3) were composed of subunits with an apparent M_r of 26 500, and that the GSH transferase in

peak A(2) contained non-identical subunits of M_r 26 000 and 26 500, approximately equally intensely stained. The apparent subunit M_r values of the components in peaks A(5) and A(6) were 24 500 and 26 500 respectively. These results suggest that the enzymes in peaks A(1), A(3), A(5) and A(6) are homodimers, whereas that in peak A(2) is a heterodimer. The two bands resolved by SDS/polyacrylamide-gel electrophoresis of the contents of peak A(4) have apparent M_r values of 26 500 and 26 000, the former being the major band.

Primary structure

Results of N-terminal degradations of the proteins in peaks A(2) and A(3) are shown in Table 4. The GSH transferase in peak A(5) did not yield any phenylthiohydantoin derivatives, suggesting a blocked α-amino group. The structures determined for the proteins in peaks A(2) and A(3) both contained a limited number of
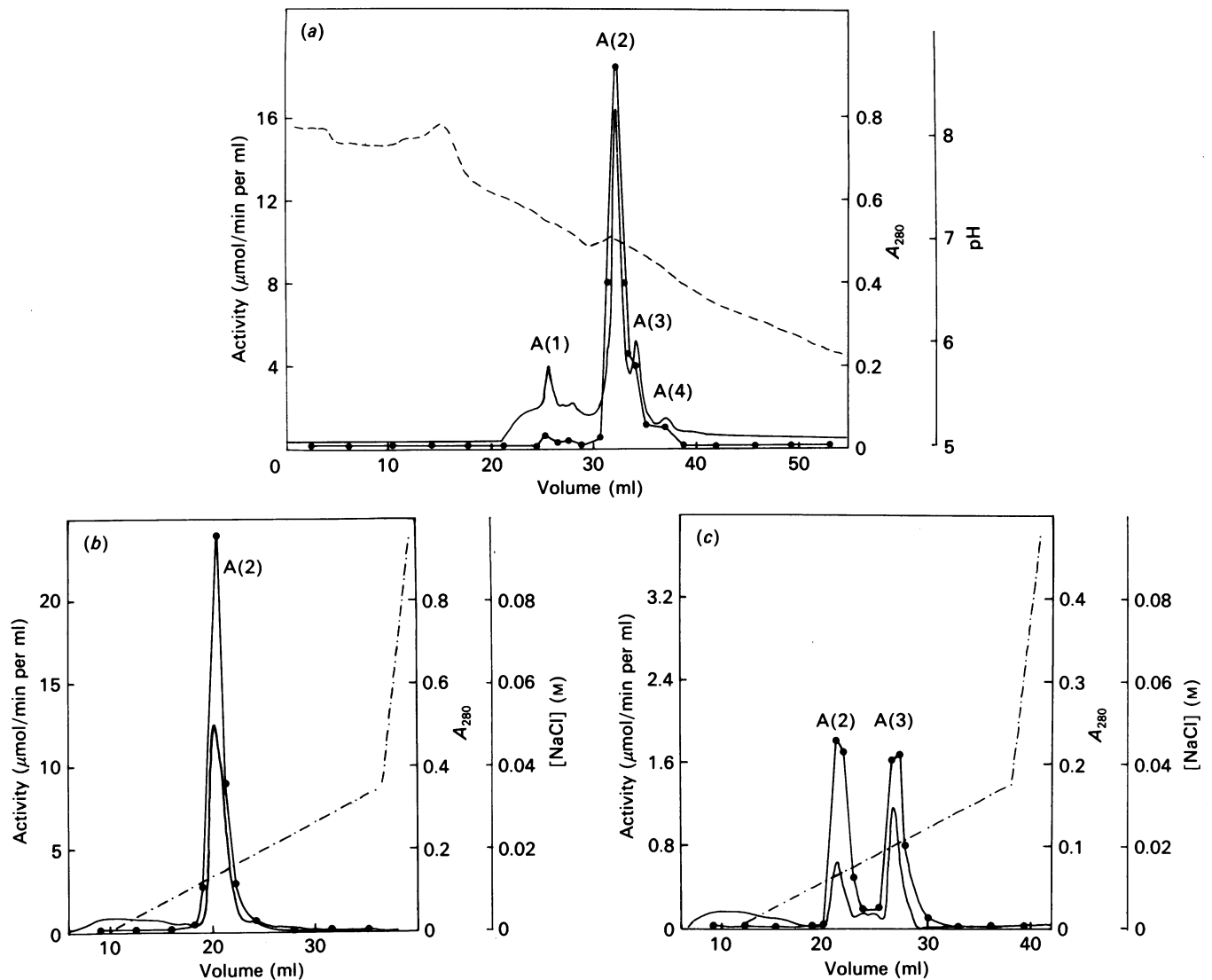
Fig. 2. Resolution of GSH transferases in DEAE-cellulose peak II

(a) Chromatofocusing on Mono P. pH (———) was monitored continuously in the effluent. (b) and (c) Ion-exchange chromatography on Mono Q of component A(2) (b) and component A(3) (c). Symbols used are explained in the legend to Fig. 1.
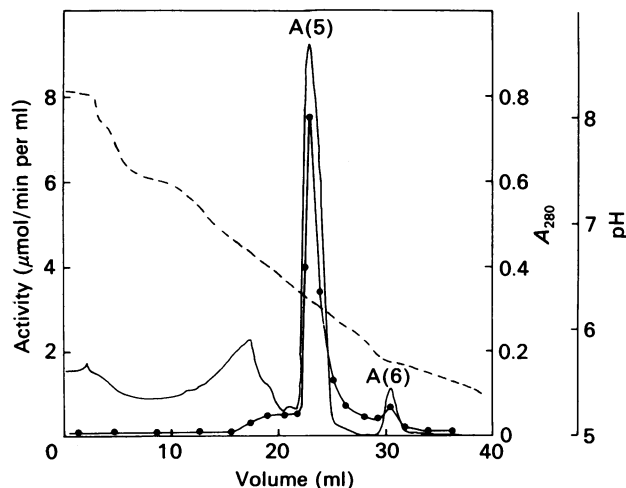

Fig. 3. Resolution of GSH transferases in DEAE-cellulose peak III

Symbols used are explained in the legends to Figs. 1 and 2.

positions with two amino acid residues in approximately equal concentration, indicating a heterodimeric structure of similar but non-identical subunits [in contrast with the results of SDS/polyacrylamide-gel electrophoresis for the protein in peak A(3), which did not reveal any difference between the subunits]. For comparison, GSH transferase 6–6 purified from rat testis (Guthenberg et al., 1985b) was analysed. Judged from the results (Table 4), this enzyme also is a heterodimer, a conclusion independently reached by Ishikawa et al. (1987) and Ostlund Farrants et al. (1987). Consequently, this enzyme has been renamed GSH transferase 6–9.

The amino acid sequence of the GSH transferase in peak A(5) is shown in Fig. 4. The low content of basic residues created some problems in generation of the peptides. Treatment with trypsin resulted in a large (44 residues) hydrophobic peptide (T43 in Fig. 4), recovered in low yield. However, the amount was sufficient for analysis. The blocked N-terminal amino acid residue was found to be an acetylated methionine residue, as shown by the amino acid composition of peptide T9 (cf. Fig. 4)

Table 3. Molecular and enzymic properties of neutral/acidic rat liver GSH transferases

For experimental details see the text. Values for activities with the various substrates are relative activities as compared with 1-chloro-2,4-dinitrobenzene (given as absolute values in μmol/min per mg in parentheses). Values for effects of the various inhibitors are I_{50} values in μM units. Abbreviation: N.D., not detectable in the assay used.

Property	Peak...	A(1)	A(2)	A(3)	A(4)	A(5)	A(6)
Apparent subunit M_r		26 500	26 000 26 500	26 5000	26 500 (26 000)	24 500	26 500
Eluted at pH		7.2	6.7	6.5	6.4	6.3	6.0
Substrate							
1-Chloro-2,4-dinitrobenzene		100 (12)	100 (45)	100 (25.7)	100 (30)	100 (10)	100 (10.1)
1,2-Dichloro-4-nitrobenzene		1.9	5.0	6.0	5.3	1.2	2.5
trans-4-Phenylbut-3-en-2-one		6.7	1.3	N.D.	1.2	1.0	10.1
Cumene hydroperoxide		N.D.	1.0	N.D.	N.D.	11.0	8.4
Ethacrynic acid		3.6	1.3	N.D.	1.5	70	25
4-Hydroxynon-2-enal		N.D.	N.D.	N.D.	N.D.	1700	3200
Inhibitor							
Cibacron Blue		0.05	0.2	0.05	0.2	0.2	1.0
Tributyltin acetate		1.0	100	50	100	> 100	1.0
Triethyltin bromide		50	> 100	0.5	100	> 100	50
Triphenyltin chloride		0.3	10	0.1	–	> 100	> 100

Table 4. Results of Edman degradation of acidic GSH transferases

Values within parentheses show nmol recovered in the analyses. For comparison, the N-terminal sequences of subunit Yb_3 ($= 6$) (Abramowitz & Listowsky, 1987), subunit 3 (Ding *et al.*, 1985) and subunit 4 (Alin *et al.*, 1986) are given.

| Position | GSH transferase | | | Subunit | | |
	A(2)	A(3)	6–9	Yb_3	3	4
1	Pro (1.8)	Xaa	Pro (0.5)	Pro	Pro	Pro
2	Met (+)	Met (0.7)	Met/Val (0.9/1.8)	Met	Met	Met
3	Thr (2.6)	Thr/Ile (0.4/0.6)	Thr (1.6)	Thr	Ile	Thr
4	Leu (6.8)	Leu (0.6)	Leu (2.6)	Leu	Leu	Leu
5	Gly (3.6)	Gly (0.3)	Gly (1.1)	Gly	Gly	Gly
6	Tyr (4.6)	Tyr (0.7)	Tyr (1.3)	Tyr	Tyr	Tyr
7	Trp (1.7)	Trp (0.4)	Trp (0.2)	Trp	Trp	Trp
8	Asp (4.3)	Asn/Asp (0.2/0.2)	Asp (1.3)	Asp	Asn	Asp
9	Ile (6.9)	Val/Ile (0.3/0.3)	Ile (1.6)	Ile	Val	Ile
10	Arg (2.3)	Arg (+)	Arg (0.5)	Arg	Arg	Arg
11	Gly (4.4)	Gly (0.3)	Gly (0.7)	Gly	Gly	Gly
12	Leu (5.8)	Leu (0.4)	Leu (1.5)	Leu	Leu	Leu
13	Ala (6.4)	Ala/Thr (0.3/0.2)	Ala (0.9)	Ala	Thr	Ala
14	His (2.0)	His (+)	His (0.6)	His	His	His
15	Ala (3.5)	Ala/Pro (0.2/0.1)	Ala (1.3)	Ala	Pro	Ala
16	Ile (4.2)	Ile (0.4)	Ile (1.1)	Ile	Ile	Ile
17	Arg (+)	Xaa	Arg (0.5)	Arg	Arg	Arg
18	Leu (3.7)	Leu (0.4)	Leu (1.7)	Leu	Leu	Leu
19	Phe/Leu (1.7/1.9)	Leu (0.5)	Leu (1.5)	Leu	Leu	Phe
20	Leu (3.8)	Leu (0.9)	Leu (1.5)	Leu	Leu	Leu
21	Glu (2.7)	Glu (0.4)	Glu (0.9)	Glu	Glu	Glu
22	Tyr (3.3)		Tyr (0.7)	Tyr	Tyr	Tyr
23	Thr (0.6)		Thr (0.6)	Thr	Thr	Thr
24	Asp (1.8)		Asp (0.3)	Asp	Asp	Asp
25	Thr (0.6)		(Ser) (+)	Ser	Ser	Thr

and its M_r (773) as determined by time-of-flight mass spectroscopy. The C-terminal segment was determined by redigestions with lysine- and arginine-specific enzymes to secure smaller overlapping segments (Fig. 4). The M_r of the subunit 8 structure thus deduced is 25 500, slightly higher than indicated by SDS/polyacrylamide-gel electrophoresis (24 500).

Substrate specificities

The substrate specificities of the six neutral/acidic GSH transferases are shown in Table 3. The GSH transferases in peaks A(2), A(3) and A(4) have comparatively high activities with 1,2-dichloro-4-nitrobenzene. In contrast, the GSH transferase in peak A(1)
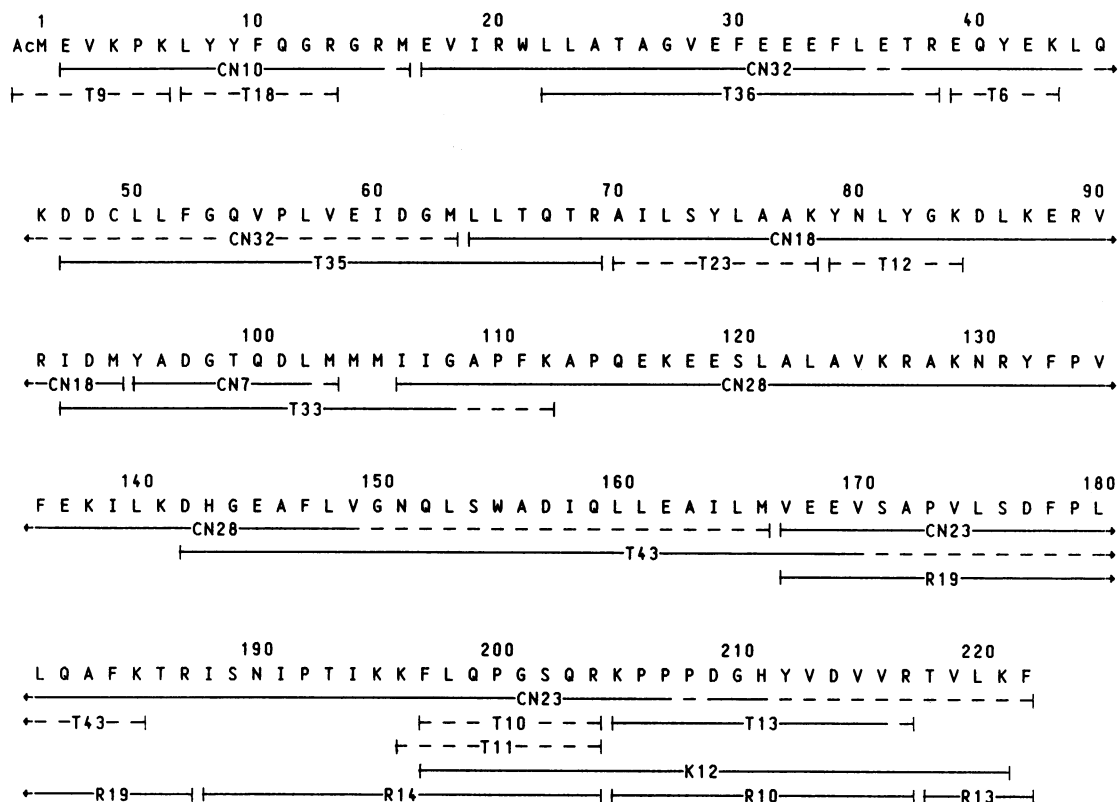
Fig. 4. Amino acid sequence of rat GSH transferase subunit 8 showing peptides used to determine the structure

Key: CN, CNBr-cleavage peptides; T, tryptic-digest peptides; R, peptides from arginine-specific cleavage by digestion with trypsin after substrate citraconylation. Peptide numbers refer to fractions from the h.p.l.c. separations. Continuous lines indicate regions analysed by sequencer degradations; dashed lines indicate regions analysed by amino acid composition only. At single positions (45, 46 and 189) and the overlap 16–17 the structure is tentative, since the corresponding positions were not unambiguously obtained as phenylthiohydantoin derivatives, but are compatible with the total composition and with the specificities of the enzymic cleavages observed.

displays an activity that is relatively high with *trans*-4-phenylbut-3-en-2-one and low with 1,2-dichloro-4-nitrobenzene. Ethacrynic acid and cumene hydroperoxide are good substrates for the GSH transferases in peaks A(5) and A(6), but the most conspicuous feature of these enzymes is the unusually high activity with 4-hydroxynon-2-enal (Table 3). 4-Hydroxyalk-2-enals are the best substrates found for these GSH transferases.

Sensitivities to inhibitors

The inhibitory effects of some substances on the enzymic activity are also shown in Table 3. Large differences exist in the sensitivities of the different enzymes. These features can be used for discrimination (Ålin et al., 1985a); for instance, the GSH transferases in peaks A(1) and A(3) are about 4 times more sensitive to Cibacron Blue than are those in peaks A(2) and A(4). Triethyltin bromide, on the other hand, gives a 100-fold higher I_{50} value with the enzyme in peak A(1) than with that in peak A(3) (Table 3). Tributyltin acetate is the inhibitor most clearly discriminating between the GSH transferases in peaks A(5) and A(6) (Table 3).

DISCUSSION

Characteristics of the purified enzymes

The affinity matrix used was originally developed for

binding and purification of glyoxalase I (Aronsson & Mannervik, 1977; Aronsson et al., 1979), but this enzyme is separated from the GSH transferases by the chromatography on DEAE-cellulose (Fig. 1). Glyoxalase I is eluted after the transferases, at an ionic strength of approx. 0.1 (results not shown; cf. Marmstål & Mannervik, 1979).

The separated neutral/acidic forms of GSH transferase from rat liver were characterized by several properties such as apparent subunit M_r, pH of elution upon chromatofocusing, substrate specificities and sensitivities to several inhibitors (Table 3), allowing comparisons with other rat GSH transferases (Ålin et al., 1985a; Mannervik, 1985). By use of such criteria, the enzyme in peak A(1) represents GSH transferase 4–4 that is tailing from peak I into peak II of the DEAE-cellulose chromatography (cf. Fig. 1).

The amino acid sequence data given in Table 4 show that peaks A(2) and A(3) contain class Mu GSH transferases (cf. Mannervik et al., 1985). The major acidic transferase from rat testis (Guthenberg et al., 1983, 1985b), GSH transferase 6–9 (previously designated '6–6'), showed obvious similarities with the other class Mu structures.

The SDS/polyacrylamide-gel-electrophoretic analysis demonstrated that the enzyme in peak A(2) is a heterodimer, and the data in Table 3 as well as the sequence information in Table 4 are consistent with a

combination of subunit 4 with one of the subunits of transferase 6–9. The apparent subunit M_r for GSH transferase 6–9 is 26000. One of the subunits appears to be identical with subunit Yb_3, recently analysed by cDNA cloning (Abramovitz & Listowsky, 1987). This entity has been designated as subunit 6. Consequently, it is concluded that peak A(2) contains the heterodimer GSH transferase 4–6; the lack of detectable serine (from subunit 6) in position 25 of the protein in A(2) (Table 4) is explainable by the lability of this residue in the Edman degradation. Furthermore, unpublished analyses (I.-M. Höidén, P. Ålin & B. Mannervik) indicate that the basic GSH transferase denoted '3–?' (Ålin et al., 1985a) is the homologous heterodimer GSH transferase 3–6.

Hayes & Chalmers (1983) and Hayes (1984) have reported the purification of GSH transferases designated P (Yb_1Yn) and S (Yb_2Yn) from rat liver. They concluded that the Yn subunit in GSH transferases P and S is the same as that in GSH transferase N, the major enzyme form in testis. The relative activities of GSH transferases P and S with 1,2-dichloro-4-nitrobenzene and trans-phenylbut-3-en-2-one suggest that these enzymes are identical with GSH transferases 3–? and the GSH transferase in peak A(2) respectively.

The nature of the enzyme in peak A(3) is obscure. The N-terminal amino acid sequence shows that it is composed of non-identical subunits. The apparent subunit M_r (26500) excludes the presence of any of the subunits of GSH transferase 6–9 (or Yb_3), and its pH of elution (Table 3) is different from that of GSH transferase 3–4. The data presented in Table 3 also demonstrate that the enzyme in peak A(3) is distinct from the other GSH transferases previously characterized in rat liver (cf. Tables 3 and 5 in Ålin et al., 1985a). Even if one of the subunits in peak A(3) might be subunit 3 or subunit 4, the only conclusion that can be drawn at present is that the enzyme is a heterodimeric class Mu GSH transferase not previously characterized.

The enzyme in peak A(4), although not subjected to sequence analysis, we believe to be an additional class Mu GSH transferase as judged by its apparent subunit M_r, substrate and inhibition characteristics. Thus it appears as if all four activity peaks, A(1)–A(4), resolved from DEAE-cellulose peak II contain class Mu GSH transferases.

The GSH transferase in peak A(5) is the predominant GSH transferase in the acidic pH region. It has an apparent subunit M_r of 24500, high relative activity with ethacrynic acid and extraordinarily high activity towards 4-hydroxyalk-2-enals (Table 3). This enzyme has been named rat GSH transferase 8–8 (Jensson et al., 1986), in accordance with the nomenclature recommended by Jakoby et al. (1984).

Hayes & Mantle (1986) and Hayes (1986) have described GSH transferase K in liver and GSH transferase J in kidney. According to their results the only difference between the enzymes is that GSH transferase K, in contrast with transferase J, does not bind to the S-hexylglutathione–Sepharose 6B affinity gel. In liver, lung (Robertson et al., 1985) and kidney (Guthenberg et al., 1985a), GSH transferase 8–8 is the predominating form in the neutral/acidic pH region; in all cases the enzyme binds to S-hexylglutathione. The apparent subunit M_r and the relative activity with ethacrynic acid for GSH transferase K are similar to those of GSH transferase 8–8 presented here. Peptide mapping and immunological

studies of GSH transferase K indicated a relationship to subunits 1 (Ya) and 2 (Yc), as established more extensively for GSH transferase 8–8 in the present investigation. We therefore conclude that GSH transferase 8–8 is identical with GSH transferase K (and J).

The GSH transferase in peak A(6) differs from GSH transferase 8–8 in peak A(5) in apparent subunit M_r, its high relative activity with trans-4-phenylbut-3-en-2-one and its higher sensitivity towards inhibition by tributyltin acetate (Table 3). This GSH transferase also shows high activity with 4-hydroxyalk-2-enals, but accounts only for a minor amount of the neutral/acidic GSH transferases found in the liver. The catalytic efficiency, $k_{cat.}/K_m$, of the enzyme in peak A(6) was found to be 900 $mM^{-1} \cdot s^{-1}$ with 4-hydroxynon-2-enal, which is second only to the value previously reported for GSH transferase 8–8, 7850 $mM^{-1} \cdot s^{-1}$ (Danielson et al., 1987).

Amino acid sequence comparisons

The primary structure of subunit 8 is clearly homologous to other class Alpha structures; positional identities with the amino acid residues in subunit 8 in the sequences shown in Fig. 5 range between 57 and 60%. These values are somewhat lower than those of any other pair of class Alpha structures (68–96%). Noteworthy are the number of charge alterations between the basic subunits (rat 1 and 2, human H1 and H2, mouse Ya) and the acidic rat subunit 8. Some of the alterations are compensated for by exchanges in the opposite direction, but the net result is a more acidic polypeptide of subunit 8, noted also from the isoelectric point of the native protein, pI 6.0. Differences in the number of basic residues are especially pronounced in the region between positions 140 and 182, where subunit 8 has four basic residues less than the other class Alpha proteins. This part of the structure corresponds conspicuously well to exon 6, deduced for rat subunit 1 and mouse subunit Ya (Telakowski-Hopkins et al., 1986; Daniel et al., 1987). Exon 6 codes for positions 139–182 in both these proteins. A speculative interpretation would be that this part is involved in binding of electrophilic substrates or in subunit interactions; GSH transferase 8–8 has a substrate specificity significantly different from those of the other proteins, and no heterodimeric enzymes containing subunit 8 have been found.

A common feature of most class Alpha GSH transferases is that they seem to be N-terminally blocked (Mannervik et al., 1985; Ålin et al., 1985c); exceptions are rat GSH transferase 2–2 and human skin GSH transferase 'pI 9.9' (Del Boccio et al., 1987). The N-terminus of subunit 8 was found to consist of an acetylmethionine structure, and constitutes the first identification of the chemical nature of a blocking group in any GSH transferase. As judged from homologies with the other class Alpha proteins, the blocked residue is the initiator amino acid. Furthermore, the assignment of the acetylated methionine as the initiator methionine residue is supported by the nature of residue 2 (glutamic acid; Fig. 4), since acidic residues in position 2 are generally associated with acetylation of N-terminal methionine residues (Flinta et al., 1986).

In summary, we have devised a simple and reproducible method for purification of neutral/acidic GSH transferases, determined the primary structure of GSH transferase 8–8, an enzyme of high potential importance in the protection of the cell against toxic products
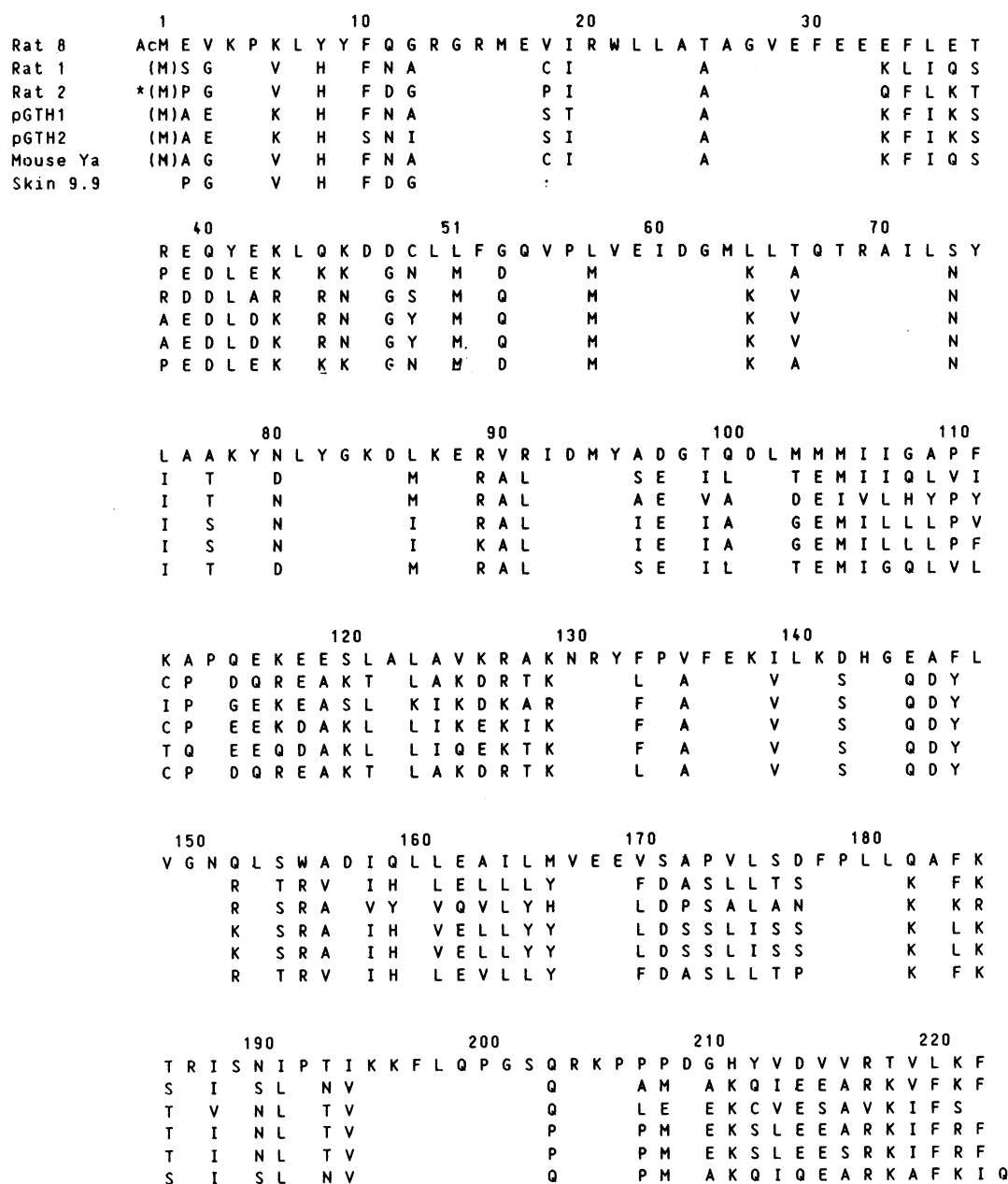
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              1                    10                   20                   30
Rat 8     AcM E V K P K L Y Y F Q G R G R M E V I R W L L A T A G V E F E E E F L E T
Rat 1     (M)S G   V   H   F N A           C I       A                   K L I Q S
Rat 2    *(M)P G   V   H   F D G           P I       A                   Q F L K T
pGTH1     (M)A E   K   H   F N A           S T       A                   K F I K S
pGTH2     (M)A E   K   H   S N I           S I       A                   K F I K S
Mouse Ya  (M)A G   V   H   F N A           C I       A                   K F I Q S
Skin 9.9     P G   V   H   F D G           :

              40                   51                   60                   70
          R E Q Y E K L Q K D D C L L F G Q V P L V E I D G M L L T Q T R A I L S Y
          P E D L E K   K K   G N   M D     M               K   A               N
          R D D L A R   R N   G S   M Q     M               K   V               N
          A E D L D K   R N   G Y   M Q     M               K   V               N
          A E D L D K   R N   G Y   M. Q    M               K   V               N
          P E D L E K   K K   G N   M D     M               K   A               N

              80                   90                   100                  110
          L A A K Y N L Y G K D L K E R V R I D M Y A D G T Q D L M M M I I G A P F
          I     T     D         M     R A L       S E   I L     T E M I I Q L V I
          I     T     N         M     R A L       A E   V A     D E I V L H Y P Y
          I     S     N         I     R A L       I E   I A     G E M I L L L P V
          I     S     N         I     K A L       I E   I A     G E M I L L L P F
          I     T     D         M     R A L       S E   I L     T E M I G Q L V L

              120                  130                  140
          K A P Q E K E E S L A L A V K R A K N R Y F P V F E K I L K D H G E A F L
          C P   D Q R E A K T   L A K D R T K       L   A       V     S     Q D Y
          I P   G E K E A S L   K I K D K A R       F   A       V     S     Q D Y
          C P   E E K D A K L   L I K E K I K       F   A       V     S     Q D Y
          T Q   E E Q D A K L   L I Q E K T K       F   A       V     S     Q D Y
          C P   D Q R E A K T   L A K D R T K       L   A       V     S     Q D Y

          150                  160                  170                  180
          V G N Q L S W A D I Q L L E A I L M V E E V S A P V L S D F P L L Q A F K
              R   T R V   I H   L E L L L Y     F D A S L L T S       K   F K
              R   S R A   V Y   V Q V L Y H     L D P S A L A N       K   K R
              K   S R A   I H   V E L L Y Y     L D S S L I S S       K   L K
              K   S R A   I H   V E L L Y Y     L D S S L I S S       K   L K
              R   T R V   I H   L E V L L Y     F D A S L L T P       K   F K

          190                  200                  210                  220
          T R I S N I P T I K K F L Q P G S Q R K P P P D G H Y V D V V R T V L K F
          S   I   S L   N V         Q       A M   A K Q I E E A R K V F K F
          T   V   N L   T V         Q       L E   E K C V E S A V K I F S
          T   I   N L   T V         P       P M   E K S L E E A R K I F R F
          T   I   N L   T V         P       P M   E K S L E E S R K I F R F
          S   I   S L   N V         Q       P M   A K Q I Q E A R K A F K I Q
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Fig. 5. Comparison of primary structures of class Alpha GSH transferases

of oxidative metabolism, and classified the proteins purified.

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