Isoenzymes of glutathione transferase in rat kidney cytosol

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Glutathione transferases from rat kidney cytosol were purified about 40-fold by chromatography on *S*-hexylglutathione linked to epoxy-activated Sepharose 6B. Further purification by fast protein liquid chromatography with chromatofocusing in the pH interval 10.6–7.6 resolved five major peaks of activity with 1-chloro-2,4-dinitrobenzene as the second substrate. Four of the peaks were identified with rat liver transferases 1–1, 1–2, 2–2 and 4–4 respectively. The criteria used for identification included physical properties, reactions with specific antibodies, substrate specificities and sensitivities to several inhibitors. The fourth major peak is a 'new' form of transferase, which has not been found in rat liver. This isoenzyme, glutathione transferase 7–7, has a lower apparent subunit M_r than any of the transferases isolated from rat liver cytosol, and does not react with antibodies raised against the liver enzymes. Glutathione transferases 3–3 and 3–4, which are abundant in liver, were only present in very small amounts. In a separate chromatofocusing separation in a lower pH interval, an additional peak was eluted at pH 6.3. This isoenzyme is characterized by its high activity with ethacrynic acid.

Conjugation with glutathione constitutes an important detoxication mechanism for many electrophilic substances, including mutagens and carcinogens. Many of these reactions are catalysed by glutathione transferases (Chasseaud, 1979). In addition to their catalytic function, these transferases display binding capacity for different classes of hydrophobic molecules (Smith & Litwack, 1980). The occurrence of glutathione transferases in the animal kingdom is widespread, and several isoenzymes have been found in many tissues (Mannervik, 1985). The isoenzymes of glutathione transferase have been most widely studied in rat liver. Six basic transferases in rat liver have been characterized as binary combinations of four protein subunits, L, B, A and C (Mannervik & Jensson, 1982). The nomenclature has more recently been changed so that subunits L, B, A and C are now named subunits 1, 2, 3 and 4 respectively (Jakoby *et al.*, 1984). An additional basic isoenzyme, related to transferase 3–3, as well as minor acidic forms of glutathione transferase, have also been detected in rat liver (Ålin *et al.*, 1985).

It is well known that organs show differential susceptibilities to toxic effects of foreign compounds. In view of the protective role of glutathione transferases and differences in the catalytic capacities of the isoenzymes, it is of importance to study the glutathione transferase composition in organs other than liver. Although several forms of glutathione transferase have been separated from rat kidney (Hales *et al.*, 1978; Oesch *et al.*, 1983; Sheehan & Mantle, 1984), the identities of the different isoenzymes have not been established.

The present paper describes the purification, separation and characterization of isoenzymes of glutathione transferase in rat kidney cytosol.

Materials and methods

Chemicals

Androst-5-ene-3,17-dione was generously provided by Dr. A. M. Benson and Dr. P. Talalay, The Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A. *S*-Hexylglutathione was synthesized and coupled to epoxy-activated Sepharose 6B as described previously (Mannervik & Guthenberg, 1981). Chromatography materials were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were standard commercial products.

Assays

Enzyme activity was determined at 30°C by monitoring changes in absorbance with a Beckman model A-25 spectrophotometer. The reaction with 1,2-dichloro-4-nitrobenzene was assayed by the method of Booth *et al.* (1961); those with 1-chloro-2,4-dinitrobenzene, *trans*-4-phenylbut-3-en-2-one and ethacrynic acid were assayed as described by Habig *et al.* (1974); that with androst-5-ene-3,17-dione was assayed as described by Benson & Talalay (1976) and the reaction with cumene hydroperoxide as described by Lawrence & Burk (1976). Protein concentration was determined by measurements of A_{280} and A_{260} (Kalckar, 1947). Sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis was performed essentially as described by Laemmli (1970).

Purification of glutathione transferases from rat kidney

Untreated male Sprague-Dawley rats (200–250 g) were killed by decapitation and the kidneys were removed. A homogenate (25%, w/v) was prepared in ice-cold 0.25 M-sucrose by use of a blender, and centrifuged at 16000 *g* for 10 min. The centrifugation and all subsequent operations up to the fast protein liquid chromatography were carried out at 4°C. The cytosol fraction was obtained by further centrifugation of the supernatant fraction at 105000 *g* for 60 min. The cytosol fraction was chromatographed on a column (9 cm × 33 cm) of Sephadex G-25 packed in 10 mM-Tris/HCl buffer, pH 7.8, containing 0.2 mM-dithioerythritol and 1 mM-EDTA (buffer A). The transferase-containing effluent was applied to an affinity column (4 cm × 17 cm) of *S*-hexylglutathione linked to epoxy-activated Sepharose 6B, equilibrated with the same buffer. The loaded affinity column was rinsed with 600 ml of 0.2 M-NaCl in buffer A, and the transferase was eluted with 300 ml of 5 mM-*S*-hexylglutathione in the NaCl-fortified buffer. The enzyme-containing fractions from the affinity gel were pooled, desalted on Sephadex G-25 (equilibrated with buffer A) and concentrated to approx. 6 ml by ultrafiltration on an Amicon PM 10 filter. Isoenzymes of glutathione transferase were separated by fast protein liquid chromatography on a column for Chromatofocusing (Mono P HR 5/20; Pharmacia Fine Chemicals, Uppsala, Sweden). A 2 ml portion of the sample was adjusted to pH 9 with 25 mM-triethylamine, filtered through a 0.22 µm Millipore filter and applied to the chromatofocusing column.

The column was equilibrated with 25 mM-triethylamine/HCl buffer, pH 10.6. The activity was eluted with Pharmalyte (pH 10.5–8) diluted

1:170 (approx. 50 ml), and subsequently with a mixture of 0.75 ml of Pharmalyte (pH 10.5–8) and 3.75 ml of Polybuffer 96 diluted to 150 ml with deionized water and adjusted to pH 7.6 with 1 M-HCl. The flow rate was 1 ml/min. The effluent was monitored spectrophotometrically at 280 nm, and protein-containing fractions were collected in 1 ml portions. Remaining activity was eluted with 2 M-NaCl. The effluent containing transferase activity was desalted on a Sephadex G-50 column, packed in 10 mM-triethanolamine/acetic acid buffer pH 8.0. The sample was applied to a Mono P column equilibrated with the same buffer. Elution was carried out with 3 ml of Polybuffer 96 and 7 ml of Polybuffer 74, diluted with deionized water, and adjusted to pH 5.0 with acetic acid to give a final volume of 200 ml. All solutions applied to the Mono P column were passed through a 0.22 µm filter.

The glutathione transferase eluted in peak IV after chromatofocusing was chromatographed on a Sephadex G-50 column (2 cm × 32 cm) packed in 10 mM-sodium phosphate buffer, pH 5.8, containing 0.2 mM-dithioerythritol (buffer B). The transferase-containing effluent was collected and applied to a CM-Sepharose column (1 cm × 12 cm) equilibrated with buffer B. The column was rinsed with the starting buffer, and the glutathione transferase activity was eluted with a linear concentration gradient made up from 150 ml of buffer B and 150 ml of buffer B containing 0.1 M-NaCl.

Results

The cytosol fraction of rat kidney was found to have a glutathione transferase activity with 1-chloro-2,4-dinitrobenzene of 0.3 µmol/min per mg of protein. This specific activity is about one-third of the activity in rat liver cytosol. The separation of glutathione transferase from most other proteins was achieved by use of affinity chromatography on *S*-hexylglutathione linked to Sepharose 6B. The purification data are summarized in Table 1.

Various isoenzymes of glutathione transferase were subsequently separated by chromatofocusing, with the fast protein liquid chromatography system. Chromatofocusing in the pH interval of 10.6–7.6 clearly resolved five major peaks of activity with the substrate 1-chloro-2,4-dinitrobenzene. The peaks were labelled I–V in the order of elution as the pH of the chromatographic gradient decreases. In addition to the five major peaks, very minor ones appeared between peaks III and IV. The elution profile is shown in Fig. 1. Peaks I, II, III and V were identified with the liver enzymes 1-1, 1-2, 2-2 and 4-4 respectively, by use of the position of the peaks in the elution profile,

Table 1. Purification by affinity chromatography of glutathione transferases from rat kidney
For experimental details see the text. Activity was measured at 30°C at pH6.5 with 1mM-1-chloro-2,4-dinitrobenzene and 1mM-glutathione as substrates. Protein was determined by the method of Kalckar (1947).

Fraction	Volume (ml)	Total activity (μmol/min)	Specific activity (μmol/min per mg of protein)	Yield (%)
Supernatant	450	223	0.293	(100)
Sephadex G-25	592	206	0.439	92
S-Hexylglutathione-Sepharose 6B + Sephadex G-25	280	136	17.3	61

Fig. 1. Separation of basic isoenzymes of glutathione transferase from rat kidney cytosol by chromatofocusing
The sample applied (2ml; 28mg of protein) had been purified by affinity chromatography. The elution profile was monitored as A_{280} (—); pH (----) was recorded in a flow-cell. The rectangles (shaded) show the volume and the activity pooled from each peak.

apparent M_r of the protein subunits, substrate specificity, reaction with specific antibodies and inhibition pattern (Tables 2–4). Analysis by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis showed that peak I contains a subunit with an apparent subunit M_r of 25000. Peak III contains a protein with an apparent subunit M_r of 28000. Peak II gives, in equal amounts, two bands with apparent subunit M_r values of 25000 and 28000. Peak IV showed a band with an apparent M_r of 24000 and traces of a band corresponding in size to that of subunit 3 (M_r 26500). Finally, the glutathione transferase eluted in peak V has an apparent subunit M_r of 26500 (Table 2). The Ouchterlony double-diffusion method with antibodies raised against glutathione transferases 1–1, 2–2, 3–3 and 4–4 revealed that peaks I and II reacted with antibodies to transferase 1–1 whereas peaks II and III reacted with antibodies to transferase 2–2. The glutathione transferase in peaks I, II and III gave no precipitin reaction with antibodies to transferases 3–3 and 4–4. Peak IV gave precipitates with antibodies to transferases 3–3 and 4–4 as well as to transferase 2–2, whereas

peak V was immunologically identical with transferase 4–4 (Table 2).
Table 3 gives ratios of catalytic activities with different substrates for glutathione transferases in peaks I–V. These ratios of activities were determined in order to identify the kidney transferases with the corresponding liver enzymes.
The I_{50} values for a set of inhibitors have been found to serve as important characteristics for the discrimination between different subunits of glutathione transferase (Yalçin *et al.*, 1983). Comparison of the I_{50} values found for the kidney isoenzymes with those for the rat liver isoenzymes support the identification of the basic rat kidney glutathione transferases (Table 4).
By combination of all the above results, peak I of the chromatofocusing was identified as glutathione transferase 1–1, peak II as transferase 1–2, peak III as transferase 2–2, and peak V as transferase 4–4. The isomerase activity (Benson & Talalay, 1976) confirmed the identification (results not shown). The major component eluted in peak IV at pH 8.7 did not correspond to any of the isoenzymes earlier isolated from rat liver. Trans-

Table 2. *Physical and immunochemical properties of basic glutathione transferases from rat kidney*
For experimental details see the text. The columns with arabic numerals within parentheses refer to values for isoenzymes isolated from rat liver (Alin *et al.*, 1985).
Abbreviation: f.p.l.c., fast protein liquid chromatography.

Property	I	(1-1)	II	(1-2)	III	(2-2)	(3-3)	IV	IV*	V	(4-4)
Apparent subunit M_r	25000	25000	25000 + 28000	25000 + 28000	28000	28000	26500	24000 + minor 26500	24000	26500	26500
pH in f.p.l.c. peak fraction	← Above 9 →										
Precipitin reaction with antiserum to transferase											
1-1	+	+	+	+	-	-	-	-	-	-	-
2-2	-	-	+	+	+	+	-	+	-	-	-
3-3	-	-	-	-	-	-	+	+	-	-	-
4-4	-	-	-	-	-	-	-	+	-	+	+

* After further purification by ion-exchange chromatography on CM-Sepharose.

Table 3. *Relative substrate specificities of renal glutathione transferases eluted after chromatofocusing in the pH interval 10.6-7.6*
For experimental details see the text. Activities were measured at 30°C as indicated in the Materials and methods section. Activities within parentheses are those previously determined for isoenzymes isolated from rat liver (Alin *et al.*, 1985).

Substrate	Relative activity (% of activity obtained with 1-chloro-2,4-dinitrobenzene)										
	Peak after Chromato-focusing (isoenzyme) ...	I	(1-1)	II	(1-2)	III	(2-2)	(3-3)	IV*	V	(4-4)
1-Chloro-2,4-dinitrobenzene		100	(100)	100	(100)	100	(100)	(100)	100	100	(100)
1,2-Dichloro-4-nitrobenzene			(50)†		(25)†		(17)†	(58)†	24‡		(17)†
<i>trans</i> -4-Phenylbut-3-en-2-one		0.08	(0.08)	0.08	(0.16)	0.3	(0.24)	(9.1)	0.2	0.4	(1)
Ethacrynic acid		0.01	(0.008)	0.01	(0.016)	0.01	(0.02)	(0.09)	0.9	9	(7)
Cumene hydroperoxide		0.1	(0.16)	3	(2)	6	(7.3)	(0.14)	16	5	(4)
		9	(6)	19	(21)	31	(46)	(0.6)	0.2	5	(4)

* After further purification by ion-exchange chromatography on CM-Sepharose.
† The absolute specific activities determined with 1-chloro-2,4-dinitrobenzene as substrate are cited for the isoenzymes isolated from liver (Alin *et al.*, 1985). The presence of ampholytes prevented accurate determination of protein concentration in fractions I, II, III and V; thus specific activities were not calculated.
‡ Absolute specific activity.

Table 4. Sensitivities to selected inhibitors of basic glutathione transferases from rat kidney. For experimental details see the text. The I_{50} value is the concentration of inhibitor giving 50% inhibition of the enzyme activity assayed at pH 6.5 at 30°C with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM-glutathione as substrates. The I_{50} values within parentheses are those earlier determined for isoenzymes isolated from rat liver (Ålin *et al.*, 1985).

Inhibitor	Peak after chromato- focusing (isoenzyme) ...	I_{50} (μM)								
		I	(1-1)	II	(1-2)	III	(2-2)	(3-3)	IV*	V
Cibacron Blue	0.5	(0.6)	2	(4)	20	(20)	(0.25)	0.3	0.1	(0.1)
Tributyltin acetate	1	(2)	1	(1)	0.7	(0.5)	(1)	4	1	(2)
Triethyltin bromide	200	(350)	100	(100)	1	(3)	(1)	100	80	(70)
Triphenyltin chloride	0.5	(0.5)	1	(1)	20	(30)	(0.1)	10	0.5	(0.2)
Bromosulphophthalein	2	(2)	10	(10)	200	(200)	(10)	20	0.5	(0.5)
Haematin	0.05	(0.05)	0.1	(0.2)	3	(2)	(1)	4	1	(1)
S-(<i>p</i> -Bromobenzyl)glutathione	50	(50)	10	(10)	5	(2)	(25)	1	5	(6)

* After further purification by ion-exchange chromatography on CM-Sepharose.

ferase 3-3 was tentatively identified in the minor peaks between peaks III and IV. By identification of subunits 3 and 4 in transferases 3-3 and 4-4 the presence of transferase 3-4 could be postulated. Indeed, peak IV was found to be contaminated with a protein having an apparent subunit M_r of 26500. This contamination can be noted as a shoulder on the protein concentration profile of peak IV (Fig. 1). The subunit size (M_r 26500), the immunoprecipitation with antibodies to transferases 3-3 and 4-4 and the activity with various substrates suggests the presence of transferase 3-4 as a minor component in peak IV.

The 'new' transferase in peak IV was further purified to homogeneity by ion-exchange chromatography on CM-Sepharose. The characteristics of this purified glutathione transferase are shown in Tables 2-4. The most conspicuous property is the apparent subunit M_r , which is lower (24000) than that of any of the basic glutathione transferases isolated from rat liver cytosol (Ålin *et al.*, 1985). The lack of reactivity with antibodies raised against transferases 1-1, 2-2, 3-3 and 4-4 should also be noted.

The subunits of the isoenzyme appear to be identical, since a unique *N*-terminal sequence of amino acids was obtained by automated sequencer degradation (B. Mannervik, P. Ålin, C. Guthenberg, H. Jansson, M. K. Tahir, M. Warholm & H. Jörnvall, unpublished work). Furthermore, inhibition studies in which residual activity is plotted versus inhibitor concentration have given the curve shape typical of that for a homodimeric enzyme (M. K. Tahir & B. Mannervik, unpublished work). Such kinetic studies have proven valuable in distinguishing heterodimers from homodimers.

Discussion

The renal cytosolic glutathione transferase activity measured with 1-chloro-2,4-dinitrobenzene as the electrophilic substrate was considerably lower than that found for the liver and testis, but higher than the activity in most other organs. Approx. 75% of the cytosolic activity from kidney was retained on the *S*-hexylglutathione affinity gel, whereas 25% passed unabsorbed through the column. The corresponding value of unabsorbed activity found for liver and testis is only 3-5% and that for lung is 17% (Robertson *et al.*, 1985). Although it is known that most of the studied isoenzymes of glutathione transferase are bound to the affinity gel used, transferase 5-5, earlier referred to as transferase E, is not retained on the gel. Therefore the present findings may suggest that a significant percentage of the renal cytosolic activity could be ascribed to transferase 5-5, even

though the specific activity with 1-chloro-2,4-dinitrobenzene is comparatively low (Meyer *et al.*, 1984).

After chromatofocusing in the pH interval 10.6–7.6 of the fraction eluted from the affinity gel with *S*-hexylglutathione, five major peaks of activity against 1-chloro-2,4-dinitrobenzene were found. Four of the peaks were identified with the liver isoenzymes 1–1, 1–2, 2–2 and 4–4. In earlier studies, it was claimed that transferases 3–3 and 3–4 (at the time called transferases A and C respectively) are not present in rat kidney (Hales *et al.*, 1978; Oesch *et al.*, 1983). However, the present investigation suggests small, but significant, amounts of both transferases 3–3 and 3–4. The fifth major peak does not correspond to any of the glutathione transferase isoenzymes isolated from normal rat liver. The most characteristic property of this isoenzyme is the low apparent subunit M_r . A high relative activity with ethacrynic acid should also be noted. A very similar or identical transferase has been isolated from rat lung (Robertson *et al.*, 1985).

In summary, kidney differs from liver in the pattern of basic cytosolic glutathione transferases by the small amount of subunit 3 and by the presence of a 'new' isoenzyme. The consulting group for nomenclature of glutathione transferases has suggested that the new enzyme eluted at pH 8.7 (peak IV) should be named rat glutathione transferase 7–7, consistent with earlier designations (Jakoby *et al.*, 1984). The same enzyme form was earlier identified in rat lung and tentatively referred to as transferase 'pH 8.7' (Robertson *et al.*, 1985). Independently, Ketterer and co-workers have isolated this isoenzyme from rat kidney, testis and primary hepatocellular carcinoma (B. Ketterer & D. J. Meyer, personal communication). The additional basic forms isolated from kidney were identified with hepatic isoenzymes that have already been named (Jakoby *et al.*, 1984).

In the purification, further elution of the chromatofocusing column with 2M-NaCl released approx. 5% of the activity that had been applied to the column. This fraction of activity was used for chromatofocusing in the pH interval 8–5, and only one major peak of activity was found. This isoenzyme was eluted at pH 6.3 and was characterized by a high ratio of activity between ethacrynic acid and 1-chloro-2,4-dinitrobenzene (approx. 50%) and by an apparent subunit M_r of 24 500. This percentage of near-neutral or acidic forms of glutathione transferase is very similar to that found for rat liver. A much higher percentage of near-neutral or acidic isoenzymes has been detected for rat testes (Guthenberg *et al.*, 1983; Dierickx & De Beer, 1981), rat lung (Robertson *et al.*, 1985) and rat heart (Ishikawa & Sies, 1984).

Expression of a previously uncharacterized cytosolic rat glutathione transferase in heart, kidney, lung, spleen and testis has been demonstrated by translation of mRNA *in vitro* (Tu *et al.*, 1983). The only properties known for this isoenzyme are a low apparent subunit M_r and its absence from normal liver. It is not possible to identify this isoenzyme definitely with any of the glutathione transferases separated from rat kidney in the present study. However, the new isoenzyme 7–7 has low apparent subunit M_r , and may be identical with the enzyme demonstrated by Tu *et al.* (1983). Isoenzymes of glutathione transferase with low apparent subunit M_r , and which are not present in rat liver, have also been found in rat placenta and pre-neoplastic hepatocyte nodules (Sato *et al.*, 1984; Kitahara *et al.*, 1984). It may be of significance that rat kidney and liver, which both contain transferase subunit 1, only have small amounts of near-neutral or acidic forms. In contrast, testes and lung, in which subunit 1 is lacking, show a much higher proportion of transferase activity in near-neutral or acidic isoenzymes.

Note added in proof (received 29 July 1985)

We have recently shown that antibodies against rat glutathione transferase P purified from hyperplastic nodule-bearing rat liver (generously provided by Dr. K. Sato, Hirosaki University School of Medicine, Japan) gave an identity reaction with isoenzyme 7–7 from rat kidney. Thus glutathione transferase 7–7 appears to be identical with the previously described transferase P in rat placenta and hyperplastic liver nodules (Sato *et al.*, 1984; Kitahara *et al.*, 1984).

References

- Ålin, P., Jansson, H., Guthenberg, C., Danielsson, U. H., Tahir, M. K. & Mannervik, B. (1985) *Anal. Biochem.* **146**, 313–320
- Benson, A. M. & Talalay, P. (1976) *Biochem. Biophys. Res. Commun.* **69**, 1073–1079
- Booth, J., Boyland, E. & Sims, P. (1961) *Biochem. J.* **79**, 516–524
- Chasseaud, L. F. (1979) *Adv. Cancer Res.* **29**, 175–274
- Dierickx, P. J. & De Beer, J. O. (1981) *Biochemistry Int.* **3**, 565–571
- Guthenberg, C., Åstrand, I.-M., Ålin, P. & Mannervik, B. (1983) *Acta Chem. Scand. B* **37**, 261–262
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139
- Hales, B. F., Jaeger, V. & Neims, A. H. (1978) *Biochem. J.* **175**, 937–943
- Ishikawa, T. & Sies, H. (1984) *FEBS Lett.* **169**, 156–160

- Jakoby, W. B., Ketterer, B. & Mannervik, B. (1984) *Biochem. Pharmacol.* **33**, 2539–2540
- Kalckar, H. M. (1947) *J. Biol. Chem.* **167**, 461–475
- Kitahara, A., Satoh, K., Nishimura, K., Ishikawa, T., Ruike, K., Sato, K., Tsuda, H. & Ito, N. (1984) *Cancer Res.* **44**, 2698–2703
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lawrence, R. A. & Burk, R. F. (1976) *Biochem. Biophys. Res. Commun.* **71**, 952–958
- Mannervik, B. (1985) *Adv. Enzymol. Relat. Areas Mol. Biol.* **57**, 357–417
- Mannervik, B. & Guthenberg, C. (1981) *Methods Enzymol.* **77**, 231–235
- Mannervik, B. & Jensson, H. (1982) *J. Biol. Chem.* **257**, 9909–9912
- Meyer, D. J., Christodoulides, L. G., Tan, K. H. & Ketterer, B. (1984) *FEBS Lett.* **173**, 327–330
- Oesch, F., Milbert, U., Friedberg, T. & Wolf, C. R. (1983) in *Extrahepatic Drug Metabolism and Chemical Carcinogenesis* (Rydström, J., Montelius, J. & Bengtsson, M., eds.), pp. 163–170, Elsevier, Amsterdam
- Robertson, I. G. C., Jensson, H., Guthenberg, C., Tahir, M. K., Jernström, B. & Mannervik, B. (1985) *Biochem. Biophys. Res. Commun.* **127**, 80–86
- Sato, K., Kitahara, A., Satoh, K., Ishikawa, T., Tatematsu, M. & Ito, N. (1984) *Gann* **75**, 199–202
- Sheehan, D. & Mantle, T. J. (1984) *Biochem. J.* **218**, 893–897
- Smith, G. J. & Litwack, G. (1980) *Rev. Biochem. Toxicol.* **2**, 1–47
- Tu, C.-P.D., Weiss, M. J., Li, N.-Q. & Reddy, C. C. (1983) *J. Biol. Chem.* **258**, 4659–4662
- Yalçın, S., Jensson, H. & Mannervik, B. (1983) *Biochem. Biophys. Res. Commun.* **114**, 829–834