Acidic glutathione S-transferases of rat testis

Thomas D. BOYER* and William C. KENNEY

Liver Studies Unit, Veterans Administration Medical Center, San Francisco, CA 94121, and Department of Medicine and Liver Center, University of California, San Francisco, CA 94143, U.S.A.

(Received 28 January 1985/1 April 1985; accepted 25 April 1985)

In most organs of the rat the predominant forms of glutathione S-transferase have alkaline (>7.0) pI values. In contrast, in the cytosol from rat testes almost 50% of the transferase activity is due to isoenzymes with acidic (<7.0) pI values. We have purified three acidic forms of glutathione S-transferase from rat testis cytosol. One form accounted for more than 90% of the enzymic activity in the acidic fraction. This major form was a homodimer of a new subunit, termed Yt. This subunit had an electrophoretic mobility that was different from the subunits that form the alkaline transferases. In addition, functional and immunological studies were consistent with the unique nature of the Yt subunit. The two minor acidic enzymes of rat testis appeared to be heterodimers of the Yt subunit and a subunit with an electrophoretic mobility identical with that of the Yb subunit present in some alkaline enzymes.

The GSH S-transferases (EC 2.5.1.18) are an important family of enzymes that catalyse the reaction of GSH with numerous electrophiles. The enzymes are dimeric proteins (M_r 45000–50000) and are present in a variety of tissues (Jakoby, 1978). Most of the GSH S-transferases previously characterized in the rat have alkaline isoelectric points (pI >7.0). In rat liver the alkaline isoenzymes account for over 90% of this organ's transferase activity, and six alkaline isoenzymes have been identified. These six isoenzymes are *in vivo* hybrids of four different subunits, termed Ya, Yb₁, Yb₂ and Yc (Kitahara & Sato, 1981; Mannervik & Jonsson, 1982; Boyer *et al.*, 1983; Beale *et al.*, 1983; Frey *et al.*, 1983). [A new nomenclature has been proposed by Jakoby *et al.* (1984) for the GSH S-transferases of the rat. The subunits described in the present paper, in the new nomenclature, are: Ya = 1 (M_r 25000); Yc = 2 (M_r 28000); Yb₁ = 3 (M_r 26500); Yb₂ = 4 (M_r 26500); Yt = 6 (M_r 26000).] Similar alkaline isoenzymes have been shown to be present in the rat testis (Bass *et al.*, 1977; Scully & Mantle, 1981; Eidne & Kirsch, 1982). In addition, rat testis contains acidic GSH S-transferase isoenzymes, which ac-

count for up to 50% of the transferase activity present in this organ (Dierickx & DeBeer, 1981).

In the present investigation we have purified three acidic forms of GSH S-transferase from rat testis. One enzyme form accounted for more than 90% of the transferase activity in the acidic fraction of rat testis cytosol, and we have characterized it as to subunit structure, substrate specificities and immunological cross-reactivity with the alkaline GSH S-transferases.

Methods

Preparation of enzymes

Testes were obtained from 250–300 g Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA, U.S.A.) and were kept frozen at -80°C until used. The testes were thawed and homogenized in 5 vol. of 0.25 M-sucrose/10 mM-Tris/HCl buffer, pH 8.0 (at 4°C), containing 1 mM-EDTA. This and all subsequent steps were performed at 4°C . Mitochondria and microsomal fraction were removed by centrifugation at 10000g for 20 min and then at 100000g for 1 h respectively. The postmicrosomal supernatant was applied to a DEAE-cellulose (Whatman DE-52) column (5 cm diam. $\times$ 3.8 cm) that had been equilibrated with 10 mM-Tris/HCl buffer, pH 8.0 (at 4°C). The column was washed with the above buffer until no transferase activity was present in the eluate (alkaline transferase fraction). The column was washed with an

Abbreviations used: GSH, reduced glutathione, NaDodSO₄, sodium dodecyl sulphate.

* To whom all correspondence should be addressed, at: Veterans Administration Medical Center (151-K), 4150 Clement Street, San Francisco, CA 94121, U.S.A.

additional 250 ml of 20 mM-Tris/HCl buffer, pH 8.0 (at 4°C), before the adherent transferase activity was eluted with the above buffer containing 150 mM-NaCl (acidic transferase fraction). The latter fraction was applied to and eluted from an S-hexylglutathione-agarose affinity column as described previously (Guthenberg & Mannervik, 1979). The resultant purified transferases were concentrated, with stirring, over an Amicon PM-10 membrane. The concentrated transferases were applied to a second DEAE-cellulose column (1.2 cm × 38 cm) equilibrated with 10 mM-Tris/HCl buffer, pH 8.0 (at 4°C). The column was washed with 170 ml of the above buffer and then developed with a linear salt gradient of 0–150 mM-NaCl in a total volume of 700 ml of buffer. Fractions of 4 ml volume were collected and assayed for transferase activity. The peaks of enzyme activity were pooled, concentrated and dialysed against 10 mM-potassium phosphate buffer, pH 6.7, containing 30% (v/v) glycerol and 1 mM-EDTA. The purified enzymes were frozen at –80°C until used.

Spectrophotometric assay of enzyme activity

GSH S-transferase and GSH peroxidase activities were determined as described previously (Habig *et al.*, 1974; Lawrence & Burk, 1976). All activities were corrected for non-enzymic rates, and are expressed as $\mu\text{mol/min per mg}$ of protein. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Binding studies

Binding studies were performed at 25°C with the use of a Perkin-Elmer MPF-44B fluorescence spectrophotometer, as described previously (Boyer *et al.*, 1983). The decrease in fluorescence that followed the addition of ligand (bilirubin or Indocyanine Green) was monitored at 340 nm with excitation at 285 nm. Corrections were made for absorption of incident and fluorescent light. The K_D values were determined from a double-reciprocal plot of change in fluorescence versus the concentration of free ligand.

Chromatofocusing

The enzyme solution was equilibrated with the chromatofocusing buffer (25 mM-imidazole/acetic acid, pH 7.4 at 4°C) on a Sephadex G-50 column (1.2 cm × 41 cm). The enzyme-containing fractions were pooled and concentrated to 8 ml, with stirring, over an Amicon PM-10 membrane. Chromatofocusing was performed on a PBE 94 column (0.5 cm × 23.5 cm) (Pharmacia) equilibrated with 25 mM-imidazole buffer, pH 7.4. After application of the enzyme, the column was developed with 300 ml of a 1:8 dilution of

Polybuffer 74, pH 4.0 at 4°C (Pharmacia). Fractions (1 ml) were collected and assayed for enzymic activity. The fractions containing enzyme activity were pooled and subjected to a S-hexylglutathione-agarose affinity column to remove the Ampholines.

NaDodSO₄/polyacrylamide-gel electrophoresis and electrofocusing

The GSH S-transferases were subjected to NaDodSO₄/polyacrylamide-gel electrophoresis as described previously (Maizel, 1971). Protein standards were bovine serum albumin (M_r 66000), ovalbumin (M_r 45000), glyceraldehyde-3-phosphate dehydrogenase (M_r 36000), carbonic anhydrase (M_r 29000), trypsinogen (M_r 24000), soyabean trypsin inhibitor (M_r 20100) and α -lactalbumin (M_r 14200). The protein subunits also were cross-linked with dimethyl pimelimidate in the following manner. Purified transferase (12 μg) was kept at 30°C for 4.5 h in 70 μl of 100 mM-sodium pyrophosphate buffer, pH 8.5, containing 10 mM-dimethyl pimelimidate. The cross-linked protein was then subjected to NaDodSO₄/polyacrylamide-gel electrophoresis. Electrofocusing and detection of transferase activity were performed in polyacrylamide gels as described previously (Kenney & Boyer, 1981).

Amino acid analysis

Before amino acid analysis, the protein was freed of glycerol, buffer and salts by dialysis or by precipitation with 10% (w/v) trichloroacetic acid. When the latter procedure was used, the precipitate was washed with 1% (w/v) trichloroacetic acid followed by diethyl ether to remove residual acid. The residual diethyl ether was removed by evaporation. Acid hydrolysis of the protein (approx. 5 μg) was performed at 100°C *in vacuo* in 200 μl of 6 M-HCl containing 5% (w/v) phenol for 25 h, 49 h or 72 h. Performic acid oxidation was performed according to the procedure of Moore (1963), except that the performic acid reagent was removed *in vacuo*. The amino acid composition was determined as described previously (Spackman *et al.*, 1958), except as modified for single-column analysis, on a Beckman 119 automated amino acid analyser with a System AA integrator. Tryptophan content was determined by a fluorimetric technique (Pagot, 1976) with the use of a Perkin-Elmer MPF-44B fluorescence spectrophotometer.

Preparation of antibody

A 100 μg portion of purified rat testis acidic GSH S-transferase was mixed with complete Freund's adjuvant and injected at multiple sites intradermally into male New Zealand White rabbits (Nitabel Rabbitry, Hayward, CA, U.S.A.).

At 3 and 5 weeks later, the rabbits were injected intramuscularly with 50 µg of the same protein again mixed with complete Freund's adjuvant. Then 1 week after the second booster injection serum was collected and used for Ouchterlony immunodiffusion studies. Antisera directed against alkaline transferase YaYc (gift from Dr. Neil Kaplowitz, University of California, Los Angeles, CA, U.S.A.) also were used.

Hybridization

We attempted to create alkaline forms of the GSH S-transferases from acidic enzymes by denaturing the latter in 6M-guanidinium chloride or 8M-urea followed by renaturation. Alkaline hybrids were sought by anion-exchange chromatography or isoelectric focusing as described previously (Boyer *et al.*, 1983).

Results

Enzyme preparation

Approx. 40% of the GSH S-transferase activity present in the postmicrosomal supernatant of rat testes adhered to the first DEAE-cellulose column (Table 1). In contrast, under identical conditions, less than 10% of the transferase activity present in rat liver cytosol adhered to a similar DEAE-cellulose column (Habig *et al.*, 1974). The specific activity of the transferases present in the acidic fraction obtained from the first DEAE-cellulose column was increased about 30-fold on elution from the S-hexylglutathione-agarose affinity column (Table 1) and, on the basis of NaDodSO₄/polyacrylamide-gel electrophoresis, the fraction was essentially free of contamination by other cytosolic proteins. The preparation, however, contained several transferase subunits of slightly different *M_r* values. To ascertain whether the acidic fraction, as eluted from the S-hexylglutathione-agarose affinity column, contained several forms of GSH S-transferase, the preparation was applied to a second DEAE-cellulose

column and eluted with a linear salt gradient as described in the Methods section. One large peak (fractions 105–135) and two smaller peaks (fractions 73–83 and 87–97) of transferase activity were obtained, with 98% of the activity present in peak III (Fig. 1). This fraction is referred to below as acidic transferase fraction III.

Molecular characteristics

The acidic fraction, as eluted from the affinity column, contained two major subunits (*M_r* 27 000 and 26 000) and two minor subunits (*M_r* 24 000 and

Fig. 1. DEAE-cellulose chromatography of acidic fraction of rat testes

After elution from the S-hexylglutathione-agarose affinity column, the acidic fraction of rat testes was applied to a second DEAE-cellulose column and then eluted with a linear salt gradient (0–150 mM-NaCl). Fractions 73–83 (I), 87–97 (II) and 105–135 (III) were pooled separately. Individual assay mixtures contained 0.1 M-potassium phosphate buffer, pH 6.5, 0.5 mM-1-chloro-2,4-dinitrobenzene and 1 mM-GSH.

Table 1. Purification of acidic GSH S-transferases from rat testis

For full experimental details see the text. The transferase activity that failed to adhere to the first DEAE-cellulose column contained the alkaline enzymes. The enzyme assay mixtures contained 0.5 mM-1-chloro-2,4-dinitrobenzene, 1 mM-GSH and 0.1 M-potassium phosphate buffer, pH 6.5.

Step	Volume (ml)	Protein (mg)	Total activity (µmol/min)	Specific activity (µmol/min per mg)
100 000g supernatant	355	2386	2996	1.26
1st DEAE-cellulose chromatography: acidic fraction	50	764	1273	1.67
Affinity chromatography	38	29	859	29.7
2nd DEAE-cellulose chromatography:				
Fractions 73–83 (I)	3.6	0.19	3.8	19.7
Fractions 87–97 (II)	3.6	0.27	4.4	16.2
Fractions 105–135 (III)	31.0	8.9	579.0	65.1

Fig. 2. *NaDodSO₄/polyacrylamide-gel electrophoresis of rat acidic GSH S-transferases*

The channels contain: 1, standards; 2, 100000g supernatant; 3, alkaline fraction; 4, acidic fraction; 5, post-affinity-chromatography fraction; 6, transferase I; 7, transferase II; 8, transferase III; 9, transferase III after third DEAE-cellulose chromatography; 10, transferase III after chromatofocusing column. The electrophoretic mobilities of the Ya, Yb, and Yc subunits of the alkaline enzymes are shown (with arrows).

29000). Three of the subunits (M_r 24000, 27000 and 29000) had mobilities that, respectively, were identical with those of the Ya, Yb and Yc subunits present in alkaline rat liver and testis transferases (Fig. 2). The subunit with M_r value of 26000 (referred to below as Yt), however, was absent from the alkaline enzymes of rat liver and testes. This Yt subunit was the predominant subunit in acidic transferase fraction III (Fig. 2). The two minor acidic fractions (I and II; Fig. 1) both contained three subunits: equal amounts of a 27000- M_r subunit and the Yt subunit in addition to a small amount of 29000- M_r subunit.

Acidic transferase fraction III also contained a small amount of a subunit with an electrophoretic mobility identical with that of Ya (M_r 24000). To ascertain whether fraction III was contaminated with an alkaline enzyme, it was again chromatographed on a DEAE-cellulose column (salt gradient). The ratio of Yt to the 24000- M_r subunit was unchanged by this treatment (Fig. 2). To investigate this association further, fraction III was subjected to chromatofocusing as described in the Methods section. A single peak of enzymic activity

Fig. 3. *Chromatofocusing of acidic GSH S-transferase III*
After elution from a third DEAE-cellulose column, transferase III was subjected to chromatofocusing as described in the Methods section. Assay conditions are indicated in Fig. 1 legend. ●, Enzyme activity; □, pH.

(pI 5.9) was obtained (Fig. 3), and the ratio of Yt to the 24000- M_r subunit was unaltered (Fig. 2).

The dimeric nature of the acidic transferase in fraction III was studied by cross-linking the

subunits with dimethyl pimelimidate. The M_r value of the cross-linked subunits was 50000, as determined by NaDodSO₄/polyacrylamide-gel electrophoresis.

Isoelectric focusing

Rat testis cytosol contained several forms of GSH S-transferase (Fig. 4). Two peaks of activity had pI values less than 7.0 (pI6.7 and 5.7 respectively). The pI values of the three acidic transferases in the three fractions obtained from the second DEAE-cellulose column (Fig. 1) were: I, pI6.8; II, pI5.4; III, pI5.8 (Fig. 5). The last-mentioned value is similar to the pI value (5.9) for transferase III obtained with chromatofocusing.

Amino acid composition

The amino acid composition of transferase III (Table 2) was similar to those reported previously for alkaline transferases (especially those with Yb subunits) purified from rat liver (Jakoby, 1978).

Fig. 4. Isoelectric focusing of 100000g supernatant fraction of rat testis

The gels were stained for activity by placing them in 10ml of 0.25M-phosphate buffer, pH6.5, containing 5mM-GSH and 1mM-1-chloro-2,4-dinitrobenzene. The gel was scanned at 5, 10 and 20min for the appearance of product S-(2,4-dinitrophenyl)glutathione. Enzyme applied to the gel contained 0.048 activity unit ($\mu\text{mol/min}$). The pH range was 3–11.

The acidic transferase III purified from testes did have, however, a somewhat higher content of serine.

Substrate specificities and binding values

Substrate specificities were determined for each of the three acidic transferases. The three acidic

Fig. 5. Isoelectric focusing of purified acidic GSH S-transferases I (a), II (b) and III (c)

Conditions were the same as for Fig. 4 except that the pH range was 5–8. Enzyme applied to the gel contained 0.01–0.07 activity unit.

enzymes had similar activities with each substrate, except for the significantly greater activity of transferase III when 1-chloro-2,4-dinitrobenzene and ethacrynic acid were used as substrates (Table 3). The substrate specificities of the acidic transferases were quite different from those obtained with the alkaline enzymes purified from rat liver (Boyer *et al.*, 1983).

The affinities of acidic transferase III for bilirubin and Indocyanine Green were determined as described in the Methods section. The K_D values for bilirubin and Indocyanine Green were respectively $5.9\mu\text{M}$ and $0.57\mu\text{M}$. These K_D values are

similar to values obtained with the alkaline transferases (Boyer *et al.*, 1983).

Antibody studies

Antibodies were raised in rabbits against purified acidic transferase III obtained after its elution from the chromatofocusing column (Fig. 2). The antisera obtained did not form precipitin reactions with purified alkaline rat liver transferases YaYa, YaYc, YcYc or Yb₁Yb₂ or the alkaline transferase fraction from rat testis. A weak precipitin reaction was formed between fraction III and the anti-YaYc sera.

The soluble fractions of rat liver, kidney, spleen and lung were prepared as described for the testes. By using the antisera raised against acidic transferase III, kidney, liver and lung, but not spleen, were shown to contain an isoenzyme(s) of GSH *S*-transferase that formed a line of identity with acidic transferase III.

Hybridization

We attempted to form alkaline hybrids from acidic transferase III to determine whether the Ya subunit associated with this enzyme fraction had the same charge as the Ya subunit present in the alkaline transferases of rat liver. The enzyme was denatured with either 6M-guanidinium chloride or 8M-urea as described in the Methods section. After renaturation, the enzyme was subjected to isoelectric focusing in polyacrylamide gels. No alkaline ($pI > 7.0$) hybrids were formed. When similar studies were performed with alkaline transferases, hybrids were readily identified (Boyer *et al.*, 1983).

Discussion

Several investigators have studied the alkaline forms ($pI > 7.0$) of GSH *S*-transferase present in the cytosol of rat testis (Bass *et al.*, 1977; Scully &

Table 2. Amino acid composition of acidic GSH *S*-transferase III

Amino acid	Amino acid composition (residues/1000 residues)
Asp	102
Thr*	48
Ser*	113
Glu	106
Pro	49
Gly	60
Ala	50
Cyst†	21
Val	28
Met	26
Ile‡	41
Leu	104
Tyr	41
Phe	47
Trp§	10
His	22
Lys	78
Arg	54

* Extrapolated value to zero time.

† Determined as cysteic acid.

‡ Value obtained from 72h hydrolysis.

§ Determined via fluorimetric analysis.

Table 3. Specific activities of GSH *S*-transferases

The conditions of the assays were identical with those described previously (Boyer *et al.*, 1983). Abbreviation: N.D., no activity detected.

Substrate	Enzyme activity ($\mu\text{mol}/\text{min per mg}$)				
	73-83* (I)	87-97* (II)	105-135* (III)	YaYc†	Yb ₁ Yb ₂ †
1-Chloro-2,4-dinitrobenzene	22.0	18.0	61.0	24.4	31.1
Ethacrynic acid	0.15	0.17	1.14	0.51	0.19
1,2-Dichloro-4-nitrobenzene	0.55	0.66	0.37	0.08	3.28
<i>p</i> -Nitrobenzyl chloride	4.28	1.72	2.40	0.54	8.00
Bromosulphophthalein	N.D.	0.002	0.027	0.021	0.45
<i>trans</i> -4-Phenylbut-3-en-2-one	0.20	0.05	0.10	0.005	0.89
Cumene hydroperoxide	1.30	0.73	0.25	4.10	0.20

* Fraction numbers given to enzymes in Fig. 1.

† Data taken from Boyer *et al.* (1983).

Mantle, 1981; Eidne & Kirsch, 1982). In the current investigation we have examined the acidic forms of the rat testis GSH S-transferase. We isolated three forms of GSH S-transferase that have pI values of less than 7.0 and that account for at least 40% of the transferase activity present in the cytosol of the rat testis. The major acidic enzyme (III) appeared to be a dimer of a subunit (Yt) that differed (on the basis of electrophoretic mobility, pI, substrate specificities and immunological studies) from the Ya, Yb and Yc subunits that form the alkaline GSH S-transferases. Transferase III appears to be identical with the major acidic enzyme purified by Guthenberg *et al.* (1983a,b) in that both enzymes have the same pI values, high activities with 1-chloro-2,4-dinitrobenzene and similar electrophoretic mobilities (Jakoby *et al.*, 1984). Also, the subunit purified by Guthenberg *et al.* (1983a) did not form a precipitin reaction with antisera raised against Yb, Yb₁, YaYa or YcYc. Yt therefore appears to be a unique subunit that is not related to the three subunits that form the alkaline GSH S-transferases.

We found that liver, kidney and lung contained a form(s) of GSH S-transferase that reacted with an antibody directed against acidic transferase III. On the basis of NaDodSO₄/polyacrylamide-gel electrophoresis, kidney and lung, but not liver or spleen, also contained identifiable amounts of Yt (results not shown). We believe, therefore, that kidney and lung have Yt-containing enzymes that are similar to those identified in the testis. Although liver cytosol does contain a form of GSH S-transferase that also cross-reacts with the antisera, we were unable to identify the Yt subunit in the liver. Fraction III did contain a second subunit, and the antisera may have contained additional antibodies directed against this latter subunit. This may explain the observed cross-reaction with liver cytosol. Interestingly, Tu *et al.* (1983), using translation *in vitro* of polyadenylated RNA obtained from rat testes, generated a subunit that was similar in electrophoretic mobility to Yt. The Yt subunit was not formed, however, when RNA from rat liver, spleen, kidney or lung was used. The inability of these investigators to produce Yt may indicate that it is expressed poorly in tissues other than the testis.

In addition to the Yt subunit, fraction III also contained a small amount of a subunit with an M_r value (24000) identical with that of the Ya subunit that is present in the alkaline GSH S-transferases (Fig. 2). We believe that the 24000- M_r subunits in the alkaline and acidic enzymes are similar, as antisera raised against alkaline transferase YaYc formed a precipitin reaction with fraction III. Our failure to form alkaline hybrids from fraction III, however, suggests that these two subunits are not

identical. Singh *et al.* (1984) identified two distinct subunits in rat lung with M_r values equal to that of Ya. One of the subunits formed an alkaline enzyme (pI9.4), whereas the other formed an acidic enzyme (pI4.8). It is possible, but unproven, that the Ya-like subunit in fraction III has an acidic pI, and hence alkaline hybrids would not be expected to form. The presence of the Ya-like subunit in fraction III was not due to contamination by alkaline YaYa or YaYc enzymes, as fraction III, when subjected to isoelectric focusing in polyacrylamide gels, was free of any transferase activity with a pI greater than 7.0, and the Ya subunit could not be separated from fraction III by chromatofocusing (Figs. 2 and 3). We conclude, therefore, that the 24000- M_r subunit in fraction III was associated with the Yt subunit forming a heterodimer in addition to the predominant YtYt homodimer.

The enzymes in fractions I and II that focused at the acidic pI values (Fig. 4) contained equal amounts of a 26500- M_r subunit and Yt, and may therefore contain heterodimers. The presence of the 29000- M_r subunit (Fig. 2) in these two fractions appeared to be due to contamination by an alkaline enzyme. Although the M_r values of the subunits of the enzymes in fractions I and II were similar, their different pI values (Fig. 5) suggest that the subunits are not identical. Further studies are required before the nature of the enzymes in fractions I and II is fully understood.

Note added in proof (received 19 June 1985)

Recently GSH S-transferase heterodimers of the Yb subunits and a Yt-like subunit have been purified from rat liver (Hayes, 1984). The presence of a small amount of Yt in rat liver cytosol may explain the observed cross-reaction of liver cytosol with the antisera directed against YtYt.

We thank Mr. Neil Saley and Ms. Joan Okasako for their skilled technical assistance and Ms. Blanche Mays for typing the manuscript. This work was supported in part by grants from the Veterans Administration, the National Institutes of Health (Grant no. GM31555) and the Liver Center, University of California, San Francisco.

References

- Bass, N. M., Kirsch, R. E., Tuft, S. A., Marks, I. & Saunders, S. J. (1977) *Biochim. Biophys. Acta* **492**, 163–175
- Beale, D., Meyer, D. J., Taylor, J. B. & Ketterer, B. (1983) *Eur. J. Biochem.* **137**, 125–129
- Boyer, T. D., Kenney, W. C. & Zakim, D. (1983) *Biochem. Pharmacol.* **32**, 1843–1850

- Dierickx, P. J. & DeBeer, J. O. (1981) *Biochem. Int.* **3**, 565–571
- Eidne, K. A. & Kirsch, R. E. (1982) *Biochem. J.* **203**, 193–199
- Frey, A. B., Friedberg, T., Oesch, F. & Kreibich, G. (1983) *J. Biol. Chem.* **258**, 11321–11325
- Guthenberg, C. & Mannervik, B. (1979) *Biochem. Biophys. Res. Commun.* **86**, 1304–1310
- Guthenberg, C., Alin, P., Astrand, I.-M., Yalcin, S. & Mannervik, B. (1983a) in *Extrahepatic Drug Metabolism and Chemical Carcinogenesis* (Rydstrom, J., Montelius, J. & Bengtsson, M., eds.), pp. 171–176, Elsevier, Amsterdam
- Guthenberg, C., Astrand, I.-M., Alin, P. & Mannervik, B. (1983b) *Acta Chem. Scand. Ser. B* **37**, 261–262
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139
- Hayes, J. D. (1984) *Biochem. J.* **224**, 839–852
- Jakoby, W. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* **46**, 383–414
- Jakoby, W. B., Ketterer, B. & Mannervik, B. (1984) *Biochem. Pharmacol.* **33**, 2539–2540
- Kenney, W. C. & Boyer, T. D. (1981) *Anal. Biochem.* **116**, 344–348
- Kitahara, A. & Sato, K. (1981) *Biochem. Biophys. Res. Commun.* **103**, 943–950
- Lawrence, R. A. & Burk, R. (1976) *Biochem. Biophys. Res. Commun.* **71**, 952–958
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maizel, J. V. (1971) *Med. Virol.* **5**, 179–246
- Mannervik, B. & Jenson, H. (1982) *J. Biol. Chem.* **257**, 9909–9912
- Moore, S. (1963) *J. Biol. Chem.* **238**, 235–237
- Pagot, P. (1976) *Eur. J. Biochem.* **63**, 263–269
- Scully, N. C. & Mantle, T. J. (1981) *Biochem. J.* **193**, 367–370
- Singh, S. V., Partridge, C. A. & Awasthi, Y. C. (1984) *Biochem. J.* **221**, 609–615
- Spackman, D. H., Stein, W. H. & Moore, S. (1958) *Anal. Chem.* **30**, 1190–1206
- Tu, C.-P., Weiss, M. J., Li, N.-Q. & Reddy, C. C. (1983) *J. Biol. Chem.* **258**, 4659–4662