Multiple Inhibition of Glutathione S-Transferase A from Rat Liver by Glutathione Derivatives: Kinetic Analysis Supporting a Steady-State Random Sequential Mechanism

KINETIC ANALYSIS SUPPORTING A STEADY-STATE RANDOM SEQUENTIAL MECHANISM

By INGA JAKOBSON,*† MARGARETA WARHOLM*† and BENGT MANNERVIK*

*Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, and

†Unit of Occupational Toxicology, National Board of Occupational Safety and Health, S-100 26 Stockholm 34, Sweden

(Received 7 July 1978)

Glutathione derivatives inhibit glutathione S-transferase A [cf. *Biochem. J.* (1975) **147**, 513–522]. The steady-state kinetics of this inhibition have been investigated in detail by using S-octylglutathione, glutathione disulphide and S-(2-chloro-4-nitrophenyl)glutathione; the last compound is a product of the enzyme-catalysed reaction. Interpreted in terms of generalized denotations of inhibition patterns, the compounds were found to be competitive with the substrate glutathione. Double-inhibition experiments involving simultaneous use of two inhibitors indicated exclusive binding of the inhibitors to the enzyme. The discrimination between alternative rate equations has been based on the results of weighted non-linear regression analysis. The experimental error was determined by replicate measurements and was found to increase with velocity. The established error structure was used as a basis for weighting in the regression and to construct confidence levels for the judgement of goodness-of-fit of rate equations fitted to experimental data. The results obtained support a steady-state random model for the mechanism of action of glutathione S-transferase A and exclude a number of simple kinetic models.

Glutathione S-transferases play an important role in the biotransformation and detoxification of electrophilic xenobiotics (Chasseaud, 1976). Physical characteristics of highly purified preparations of these enzymes have been described (Fjellstedt *et al.*, 1973; Habig *et al.*, 1974; Askelöf *et al.*, 1975; Jakoby *et al.*, 1976). The kinetic properties of glutathione S-transferases have also been studied in different laboratories, but early studies were limited by the use of small ranges of substrate concentrations and impure enzyme preparations. Studies in our laboratory on glutathione S-transferase A from rat liver support a random sequential mechanism for the reaction between 3,4-dichloro-1-nitrobenzene and GSH (Jakobson *et al.*, 1976; Jakobson *et al.*, 1977). An alternative reaction scheme involving a hybrid ping-pong-sequential mechanism (Pabst *et al.*, 1974) for this reaction has previously been rejected on the basis of the asymptotic properties of the corresponding rate equation (Mannervik & Askelöf, 1975). The inhibition studies presented in the present paper lend further support to the random sequential mechanism. Some of the data have previously been presented (Jakobson *et al.*, 1976).

Materials and Methods

Chemicals

3,4-Dichloro-1-nitrobenzene, glutathione (GSH), glutathione disulphide (GSSG), S-methylglutathione and S-n-octylglutathione were obtained as described by Askelöf *et al.* (1975). S-(2-Chloro-4-nitrophenyl)-glutathione was prepared enzymically (Jakobson *et al.*, 1977).

Enzyme preparation and assay

The enzyme was prepared and assayed as described earlier (Askelöf *et al.*, 1975; Jakobson *et al.*, 1977). The inhibitors, like GSH (Mannervik & Askelöf, 1975; Jakobson *et al.*, 1977), were always pre-incubated with the enzyme and the reaction was started by addition of 3,4-dichloro-1-nitrobenzene.

Analysis of kinetic data

Weighted non-linear regression analysis of initial-velocity data was performed as earlier described (Askelöf *et al.*, 1976; Jakobson *et al.*, 1977). A weighting factor of the inverted velocity raised to the power two was used; this weighting procedure

was based on an analysis of the error structure involving replicate velocity determinations over the range studied (cf. Askelöf *et al.*, 1976). In the analysis of inhibition data the concept of generalized inhibition patterns for non-Michaelis kinetics was used (Mannervik, 1978). For Michaelis kinetics the generalized inhibition types are equivalent with the common definitions; for example, generalized competitive inhibition implies that excess substrate will eliminate the inhibition, and generalized non-competitive inhibition means that the inhibition is abolished neither at high nor low substrate concentrations.

Results

Inhibition by single GSH derivatives

Glutathione *S*-transferase A, assayed with 3,4-dichloro-1-nitrobenzene and GSH as substrates, was inhibited by GSSG. The inhibition was studied in the concentration range 0.20–20 mM-GSSG at two combinations of fixed substrate concentrations, 0.47 mM-GSH and 0.20 mM-3,4-dichloro-1-nitrobenzene, and 0.047 mM-GSH and 0.10 mM-3,4-dichloro-1-nitrobenzene. A Dixon plot of the results is shown in Fig. 1. The graphs displayed slight but significant

Fig. 1. Effect of GSSG on initial velocity at two different combinations of substrate concentrations
○, 0.047 mM-GSH and 0.10 mM-3,4-dichloro-1-nitrobenzene; □, 0.47 mM-GSH and 0.20 mM-3,4-dichloro-1-nitrobenzene.

curvature. Similar results were obtained with *S*-methylglutathione used as an inhibitor. *S*-Octylglutathione exerted a much stronger inhibition than GSSG and *S*-methylglutathione, in agreement with earlier observations (Askelöf *et al.*, 1975). A Dixon plot of experiments with this compound, in the concentration range 1–200 μM and at about the same substrate concentrations as used in the experiments with GSSG and *S*-methylglutathione, appeared almost linear. However, a v versus $v \cdot [I]$ plot, which like the Eadie-Augustinsson-Hofstee plot (Mannervik, 1975) more clearly displays non-linearities, shows that the graphs are non-linear (Fig. 2).

Double inhibition by GSH derivatives

The kinetics were investigated with two inhibitors varied in the same experiment. The resulting Dixon plot gave lines with slight curvature when plotted against either GSSG or *S*-octylglutathione concentration (Fig. 3a and 3b). The inhibition of the enzyme was also performed with GSSG and *S*-(2-chloro-4-nitrophenyl)glutathione used at the same time. A Dixon plot of the results displayed with GSSG as the independent variable showed a set of apparently parallel curves with a slight deviation from linearity, but when the *S*-(2-chloro-4-nitrophenyl)glutathione concentration was used as the independent variable in the plot the curve shapes appeared parabolic. Fig. 4 shows the effect of simultaneous use of *S*-octylglutathione and *S*-(2-chloro-4-nitrophenyl)glutathione as inhibitors. As in the previous experiment in combination with GSSG (data not shown), the product demonstrated more curvature than any of the other inhibitors (Fig. 4b).

Fig. 2. Effect of *S*-octylglutathione on initial velocity at two different combinations of substrate concentrations
○, 0.047 mM-GSH and 0.10 mM-3,4-dichloro-1-nitrobenzene; □, 0.47 mM-GSH and 0.20 mM-3,4-dichloro-1-nitrobenzene. In a Dixon plot such as Fig. 1 non-linearities and inflexion points were less easily discerned.

Fig. 3. Double-inhibition experiment involving GSSG and S-octylglutathione as inhibitors

The substrate concentrations were 0.90 mM-GSH and 0.50 mM-3,4-dichloro-1-nitrobenzene. (a) The fixed inhibitor (S-octylglutathione) concentrations were: $\circ$, zero; $\bullet$, 20 μM ; $\square$, 50 μM ; $\blacksquare$, 100 μM . (b) The fixed inhibitor (GSSG) concentrations were: $\circ$, zero; $\bullet$, 5 mm; $\square$, 10 mm; $\blacksquare$, 20 mm. In the plots some of the curves have been omitted for clarity. The equation giving the best fit of the complete data set was:

$$v = \frac{K_1 + K_2 I + K_3 J}{K_4 + K_5 I + K_6 J + K_7 I^2 + K_8 J^2 + IJ}$$

in which I and J denote the concentrations of GSSG and S-octylglutathione respectively. The parameter values ($\pm$ s.e.) obtained were: $K_1 = 3.40$ (± 1.92); $K_2 = 0.227$ (± 0.020); $K_3 = 0.0$; $K_4 = 0.236$ (± 0.131); $K_5 = 0.0448$ (± 0.0183); $K_6 = 10.1$ (± 5.9); $K_7 = 0.00342$ (± 0.00017); $K_8 = 14.1$ (± 8.1). Proper units of the constants are obtained by considering that velocity is expressed in $\mu\text{M}/\text{min}$ and inhibitor concentrations in mm. A corresponding residual plot based on the regression is presented in Fig. 7.

Fig. 4. Double-inhibition experiment involving S-octylglutathione and S-(2-chloro-4-nitrophenyl)glutathione as inhibitors

The fixed substrate concentrations were 0.97 mM-GSH and 0.50 mM-3,4-dichloro-1-nitrobenzene. (a) The fixed inhibitor (S-(2-chloro-4-nitrophenyl)glutathione) concentrations were: $\circ$, zero; $\bullet$, 20 μM ; $\triangle$, 50 μM ; $\square$, 100 μM ; $\blacksquare$, 150 μM . (b) The fixed inhibitor (S-octylglutathione) concentrations were: $\circ$, zero; $\bullet$, 20 μM ; $\triangle$, 50 μM ; $\square$, 75 μM ; $\blacksquare$, 100 μM . For clarity of presentation, some of the curves have been omitted from the plots.

Fig. 5. Effect of *S*-octylglutathione and GSH on initial velocity

Concentrations of *S*-octylglutathione were: ○, zero; ▲, 5 μM; □, 10 μM; ●, 20 μM; △, 50 μM; ■, 100 μM; ▽, 200 μM. The concentration of 3,4-dichloro-1-nitrobenzene was 0.50 mM; the concentration of GSH varied between 0.017 and 8.6 mM. The rate equation giving the best fit of the data set was:

$$v = \frac{K_1 G + K_2 G^2 + K_3 G I}{K_4 + G + K_5 G^2 + K_6 I + K_7 I^2 + K_8 G I}$$

in which *G* and *I* denote the concentrations of GSH and *S*-octylglutathione respectively. The parameter values (±s.e.) obtained were: $K_1 = 15.1 (\pm 2.2)$; $K_2 = 40.9 (\pm 12.5)$; $K_3 = 1058 (\pm 652)$; $K_4 = 0.0295 (\pm 0.0078)$; $K_5 = 1.15 (\pm 0.37)$; $K_6 = 30.9 (\pm 6.5)$; $K_7 = 1142 (\pm 730)$; $K_8 = 69.1 (\pm 18.5)$. Proper units of the constants are obtained by considering that velocity is expressed in μM/min and reactant concentrations in mM.

Effect on inhibition of varying the concentration of the substrates

The concentration of each substrate was varied at a fixed concentration of the second substrate and several fixed concentrations of *S*-octylglutathione. When GSH was varied (0.017–8.6 mM) at 0.50 mM-3,4-dichloro-1-nitrobenzene concentration (Fig. 5) the inhibition was of the (generalized) competitive type (Mannervik, 1978), the inhibitory effect being extinguished at high GSH concentrations. However, *S*-octylglutathione displayed (generalized) non-competitive inhibition when the concentration of 3,4-dichloro-1-nitrobenzene was varied (0.01–1.0 mM) at 4.6 mM-GSH concentration (Fig. 6). Qualitatively, the same inhibition patterns have previously been found when *S*-(2-chloro-4-nitrophenyl)glutathione was used as inhibitor (Jakobson *et al.*, 1977).

Non-linear regression analysis

Regression analysis of inhibition data in which a single inhibitor concentration varied at constant substrate concentrations (cf. Figs. 1 and 2) indicated that the denominator of the rate equation required fourth-degree terms in inhibitor concentration. The numerator had to include at least up to second-degree terms (Table 1). The action of inhibitors versus the substrates was also analysed by non-linear regression. It was found that the effect was essentially competitive with GSH in the generalized sense of competitive inhibition (Mannervik, 1978). The simplest equation that could satisfactorily describe the data was:

$$v = \frac{A + A^2 + AI}{K + A + A^2 + I + I^2 + AI}$$

Fig. 6. Effect on initial velocity of *S*-octylglutathione and 3,4-dichloro-1-nitrobenzene. The concentrations of *S*-octylglutathione were: ○, zero; △, 5 μM; □, 10 μM; ●, 20 μM; ▲, 50 μM; ■, 100 μM; ▼, 200 μM. The concentration of GSH was 4.6mM; the concentration of 3,4-dichloro-1-nitrobenzene varied between 0.01 and 1.0mM.

Table 1. Fitting by weighted non-linear regression of alternative rate equations to initial-velocity data obtained by varying a single inhibitory GSH derivative

Data sets 1 and 3 are plotted in Figs. 1 and 2 respectively. The inhibitor concentrations were varied in the ranges indicated, and the substrate concentrations were fixed at 0.05mM-GSH and 0.10mM-3,4-dichloro-1-nitrobenzene. For simplicity of presentation, the rate equations are written without coefficients; in the regression analysis the coefficients were used as parameters. *K* denotes a constant term and *I* the variable inhibitor concentration (cf. Jakobson *et al.*, 1977).

Rate equation	No. of parameters	Residual sum of squares (arbitrary units)		
		Data set 1 GSSG (0.2–20mM) <i>n</i> = 26	Data set 2 <i>S</i> -Methylglutathione (0.2–20mM) <i>n</i> = 28	Data set 3 <i>S</i> -Octylglutathione (1–200 μM) <i>n</i> = 34
$v = \frac{K+I}{K+I+I^2}$	4	0.06008	0.20968	0.12166
$v = \frac{K+I+I^2}{K+I+I^2+I^3}$	6	0.03528	0.15958	0.08507
$v = \frac{K+I+I^2}{K+I+I^2+I^3+I^4}$	7	0.03528	0.11567	0.06882
$v = \frac{K+I+I^2+I^3}{K+I+I^2+I^3+I^4}$	8	0.02446	0.11567	0.06868

where *A* and *I* denote substrate and inhibitor concentrations respectively and *K* is a constant term (the coefficients are omitted for simplicity). The inhibition appeared (generalized) non-competitive with 3,4-dichloro-1-nitrobenzene (cf. Jakobson *et al.*, 1977).

These inhibition patterns were established with *S*-octylglutathione and with *S*-(2-chloro-4-nitrophenyl)glutathione. The analysis of the double-inhibition experiments was based on a simple steady-state random reaction

scheme (Scheme 1), in which it was assumed that the inhibitory glutathione derivatives could bind to the free enzyme and to the binary enzyme-3,4-dichloro-1-nitrobenzene complex by use of the vacant GSH-binding subsite in the active centre of the enzyme. If the binary enzyme-inhibitor complex can bind 3,4-dichloro-1-nitrobenzene, and if only a single inhibitor molecule can be bound at a time to the enzyme, the rate equation will have the following form, with respect to two inhibitors (concentrations I and J):

$$v = \frac{K + I + J}{K + I + J + I^2 + J^2 + IJ}$$

Scheme 1. Reaction scheme of a simple random sequential mechanism used to interpret the double-inhibition experiments

The enzyme and the substrates GSH and 3,4-dichloro-1-nitrobenzene are denoted E, G and D respectively. The inhibitors are symbolized by I and J.

Fig. 7. Residual plot of the data in Fig 3 after fitting by weighted non-linear regression analysis

The straight lines diverging from the origin correspond to the 95% confidence limits computed from separate experiments, in which the structure of the experimental error was investigated by replicates (cf. Askelöf *et al.*, 1976). The estimated experimental error was multiplied by the appropriate t -statistic to construct the confidence limits.

Table 2. Fitting by weighted non-linear regression of alternative rate equations to initial-velocity data obtained by simultaneous use of two inhibitors

Data sets 4 and 6 are plotted in Figs. 3 and 4 respectively. One inhibitor concentration was varied at several constant concentrations of the second inhibitor. The substrate concentrations were fixed at 1 mM-GSH and 0.5 mM-3,4-dichloro-1-nitrobenzene. In the rate equations K denotes a constant term, whereas I and J denote the concentrations of the two inhibitors respectively (see also legend to Table 1).

Rate equation	No. of parameters	Residual sum of squares (arbitrary units)		
		Data set 4 GSSG + <i>S</i> -octyl- glutathione $n = 97$	Data set 5 GSSG + <i>S</i> -(2-chloro-4- nitrophenyl)glutathione $n = 56$	Data set 6 <i>S</i> -(2-Chloro-4-nitro- phenyl)glutathione + <i>S</i> - octylglutathione $n = 56$
$v = \frac{K}{K + I + J}$	3	0.1777	1.6910	0.4603
$v = \frac{K}{K + I + J + IJ}$	4	0.1189	1.5333	0.3248
$v = \frac{K + I + J}{K + I + J + IJ + I^2 + J^2}$	8	0.0858	0.1368	0.1739

This rate law was the best of several alternatives tested when all data sets were considered. However, one of the parameter values given in the legend to Fig. 3 is zero, showing that with this particular data set the model is over-determined. In Table 2 a comparison is made of the results of fitting the above rate equation and two simple alternatives of the various models that we have considered.

The fit of the models selected as the 'best' rate equations were generally good and fulfilled the goodness-of-fit criteria previously assembled (Bartfai & Mannervik, 1972; Mannervik & Bartfai, 1973). The experimental error was determined by replicates (cf. Askelöf *et al.*, 1976) and allowed 95% confidence levels to be constructed for residual plots. Fig. 7 shows a residual plot obtained after fitting the above equation to the double-inhibition data presented in Fig. 3. The diverging confidence limits define an error proportional to velocity (i.e. constant relative error), which was the best approximation of the experimental variance. As expected for a good model, almost all residual values in Fig. 7 fall within the confidence limits.

Discussion

The kinetics of glutathione S-transferase A, as studied with 3,4-dichloro-1-nitrobenzene and GSH, have been interpreted in terms of a steady-state random mechanism (Jakobson *et al.*, 1976; Jakobson *et al.*, 1977). An alternative hybrid reaction scheme (Pabst *et al.*, 1974) composed of a ping-pong and a sequential branch, similar to the one previously put forward for glutathione reductase (Mannervik, 1973), has been excluded, because the experimental data are not consistent with the asymptotic properties of the corresponding rate law (Mannervik & Askelöf, 1975). The inhibition studies of the present investigation support the proposal of a random mechanism.

The S-substituted GSH derivatives tested are competitive inhibitors with GSH in the sense of generalized inhibition (Mannervik, 1978). Because of the structural similarity, it is reasonable to assume that GSH and the derivatives compete for the same site on the enzyme. This assumption has support from the double-inhibition experiments (Figs. 3 and 4), which also indicate exclusive binding, i.e. two different inhibitor molecules cannot be bound at the same time to the enzyme.

A steady-state random mechanism involving random addition of substrates can explain the experimental data presented here and elsewhere (Pabst *et al.*, 1974; Askelöf *et al.*, 1975; Jakobson *et al.*, 1977). Basic findings to be taken into account are: (i) simple hyperbolic binding of S-(2-chloro-4-nitrophenyl)glutathione (Jakobson *et al.*, 1977) (indicating no co-operativity between subunits); (ii) mutually competitive behaviour between GSH and

GSH derivatives (cf. Figs. 3, 4 and 5 in the present paper, and Fig. 5 in Jakobson *et al.*, 1977); (iii) non-linear inhibition by GSH derivatives (Figs. 1–4). These findings lead to the conclusion that one subunit contains a single binding site for GSH and inhibitory GSH derivatives and that higher-degree dependence of velocity on reactant concentrations results from alternative branches in the reaction scheme. The simplest assumption is that GSH and GSH derivatives bind only to free enzyme and the enzyme–3,4-dichloro-1-nitrobenzene complex (see Scheme 1).

None of the simple classical mechanisms of two-substrate reactions explains the higher-degree dependence of substrate concentration observed in the absence of inhibitors (Pabst *et al.*, 1974; Askelöf *et al.*, 1975; Jakobson *et al.*, 1977). It is therefore sufficient to discuss the two alternative branching mechanisms previously advocated by Jakoby and co-workers (Pabst *et al.*, 1974) and by Mannervik and co-workers (Mannervik & Askelöf, 1975; Jakobson *et al.*, 1976; Jakobson *et al.*, 1977). The first of these is a hybrid between a ping-pong mechanism in which 3,4-dichloro-1-nitrobenzene is added as the first substrate and an ordered sequential mechanism in which GSH is added first (Pabst *et al.*, 1974). As formulated, it includes only one enzyme form (E), which can bind the product S-(2-chloro-4-nitrophenyl)glutathione. Thus only linear inhibition by the product can be obtained. The original scheme may be expanded by the assumption that the product can bind to the modified enzyme (E') and/or the enzyme–3,4-dichloro-1-nitrobenzene complex in the ping-pong branch and thereby increase the degree of the rate law with respect to product concentration. However, at high concentrations of GSH only the sequential branch operates and in this branch free enzyme alone is available for binding of product. In contrast, our product inhibition data show non-linear inhibition in the range of GSH concentrations in which the sequential branch should predominate >0.25 mM, provided that the 3,4-dichloro-1-nitrobenzene concentration ≤ 1 mM (Pabst *et al.*, 1974)]. Thus the hybrid model is inconsistent with the experiments even in an expanded form. Other variants of the hybrid model, in which the order of addition of substrates and release of products have been permuted, have similarly been excluded.

It remains to consider the steady-state random mechanism. This model is consistent with all the basic findings (i)–(iii) given above. The corresponding rate laws with two independent variables (e.g. one substrate and one inhibitor, or two inhibitors) considered explicitly have been fitted to the data. The fits were generally very satisfactory according to the goodness-of-fit criteria (Bartfai & Mannervik, 1972; Mannervik & Bartfai, 1973), but it is clear that the model is a simplification, since it has been shown by regression analysis that the denominator of the

rate equation should contain fourth-degree terms in inhibitor concentration (Table 1; Jakobson *et al.*, 1977).

In the simple classical steady-state random mechanism a product is expected to be non-competitive with both substrates, whereas, in the present case, *S*-(2-chloro-4-nitrophenyl)glutathione appears to be competitive versus GSH. This finding is explained by the assumption that GSH can bind to each enzyme form that can bind a GSH derivative. Such an explanation seems likely from the structural point of view (see below).

The inhibition data also give information about the active site of the enzyme, if the competitive nature of the inhibition is accepted as evidence that GSH and the GSH derivatives used bind to the same site, i.e. a subsite of the active centre of the enzyme. It has previously been shown that increasing the carbon-chain length of *S*-alkyl derivatives of GSH increases the inhibitory properties of the compound (Askelöf *et al.*, 1975). Thus it appears that the active centre can be divided topographically into at least two subsites: one site binding the peptide part of GSH and GSH derivatives and one adjacent site binding a hydrophobic *S*-substituent of GSH. The latter site is probably also the binding site of the second substrate (3,4-dichloro-1-nitrobenzene). The hydrophobic subsite must be relatively large to accommodate the variety of bulky molecules which are substrates of the enzyme. Thus it is possible that a hydrophobic substituent of a glutathione derivative and a hydrophobic substrate (3,4-dichloro-1-nitrobenzene) can be bound simultaneously to the hydrophobic subsite. If such a binding is not operative it is still possible that a GSH derivative and 3,4-dichloro-1-nitrobenzene are bound simultaneously to the active centre if the GSH derivative has a second binding mode, which makes use only of the peptide-binding subsite. In both events a non-competitive inhibition pattern would be predicted for the action of GSH derivatives versus 3,4-dichloro-1-nitrobenzene.

In the context of the non-linear regression analysis it is shown that confidence levels which are functions of the velocity can be constructed on the basis of replicates. In a residual plot the residuals of a

satisfactory mathematical model of the kinetics will have the expected distribution within the confidence levels (Fig. 7). Obviously, a rigorous quantitative analysis necessitates the establishment of the structure of the experimental variance, not only to obtain proper weights for weighted regression analysis (Askelöf *et al.*, 1976), but also to judge the goodness-of-fit of a model subjected to analysis.

We thank Mr. Claes Guthenberg for helpful collaboration in the preparation of enzyme. This work was supported by grants (to B. M.) from the Swedish Natural Science Research Council.

References

- Askelöf, P., Guthenberg, C., Jakobson, I. & Mannervik, B. (1975) *Biochem. J.* **147**, 513–522
- Askelöf, P., Korsfeldt, M. & Mannervik, B. (1976) *Eur. J. Biochem.* **69**, 61–67
- Bartfai, T. & Mannervik, B. (1972) *FEBS Lett.* **26**, 252–256
- Chasseaud, L. F. (1976) in *Glutathione: Metabolism and Function* (Arias, I. M. & Jakoby, W. B., eds.), pp. 77–114, Raven Press, New York
- Fjellstedt, T. A., Allen, R. H., Duncan, B. K. & Jakoby, W. B. (1973) *J. Biol. Chem.* **248**, 3702–3707
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139
- Jakobson, I., Askelöf, P., Korsfeldt, M. & Mannervik, B. (1976) *Abstr. Commun. Int. Congr. Biochem.* 10th p. 162
- Jakobson, I., Askelöf, P., Warholm, M. & Mannervik, B. (1977) *Eur. J. Biochem.* **77**, 253–262
- Jakoby, W. B., Habig, W. H., Keen, J. H., Ketley, J. N. & Pabst, M. J. (1976) in *Glutathione: Metabolism and Function* (Arias, I. M. & Jakoby, W. B., eds.), pp. 189–202, Raven Press, New York
- Mannervik, B. (1973) *Biochem. Biophys. Res. Commun.* **53**, 1151–1158
- Mannervik, B. (1975) *Anal. Biochem.* **63**, 12–16
- Mannervik, B. (1978) *FEBS Lett.* **93**, 225–227
- Mannervik, B. & Askelöf, P. (1975) *FEBS Lett.* **56**, 218–221
- Mannervik, B. & Bartfai, T. (1973) *Acta Biol. Med. Germ.* **31**, 203–215
- Pabst, M. J., Habig, W. H. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7140–7148