Inhibition by inorganic anions of glutathione S-transferases from insect and mammalian sources

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Glutathione S-transferases 1-1, 3-3, 3-4 and 4-4 from rat liver and the major glutathione S-transferase from the wax moth (Galleria mellonella) are all inhibited by several simple inorganic anions. For each of 3-3, 3-4 and the insect enzyme, the order of inhibitory potency was \( \text{ClO}_4^- > \text{SCN}^- > \Gamma > \text{NO}_2^- > \text{Br}^- \). A more limited range of anions was tested on the isoenzymes 1-1 and 4-4, but the same trend was apparent. Values for \( K_i \) ranged from about 200 mM for \( \text{Cl}^- \) to 6 mM for \( \text{SCN}^- \) in the case of the insect enzyme and from 50 mM for \( \text{Br}^- \) to 0.3 mM for \( \text{SCN}^- \) for the rat isofoms 3-3. Acetate, \( F^- \), \( \text{SO}_4^{2-} \) and \( \text{PO}_4^{3-} \) were not found to have significant inhibitory properties. The mode of inhibition was characterized as non-competitive in the case of the insect enzyme and rat transferase 1-1, whereas the mode of inhibition was partially non-competitive in the case of the rat isoforms 3-3, 3-4 and 4-4.

INTRODUCTION

Glutathione S-transferases (GSTs) from the insects Galleria mellonella (wax moth) (Chang et al., 1981) and Wiseana cervinata (porina moth) (Clark & Drake, 1984) have been shown to be inhibited by \( \text{Cl}^- \). This is one of the products of the conjugation of GSH and 1-chloro-2,4-dinitrobenzene (CDNB) that is frequently studied as a model for the many such reactions catalysed by these enzymes. The kinetics of the GSTs are frequently complex, and it has been difficult to determine whether the inhibition observed with these insect enzymes was due to simple product inhibition or not. We have undertaken the examination of anions as possible inhibitors in an attempt to elucidate the mode of inhibition. It has also been reported that GST A from rat liver was unaffected by \( \text{Cl}^- \) (Pabst et al., 1974).

We have included in our present study enzymes purified from both an insect source (Galleria mellonella) and rat liver with a view to establishing whether or not there is a species difference in this regard.

MATERIALS AND METHODS

Glutathione, bromosulphphthalein (BSP), Coomassie Brilliant Blue R, Tris, glycine, acrylamide and methylenebis-acrylamide were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. CDNB, NaF, \( \text{Br}^- \), \( \Gamma^- \), \( \text{NO}_2^- \), \( \text{ClO}_4^- \) and \( \text{SCN}^- \) were purchased from BDH Chemicals Ltd., Poole, Dorset, U.K. NaCl was obtained from E. Merck, Darmstadt, Germany. Sepharose 4-B, QAE (quaternary amine)-Sephadex A-25, Polybuffer exchangers PBE 118 and 94, Pharmalyte 3-10, Polybuffers 8-10.5 and 9-6 were from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Sepharose 4-B was activated with epichlorohydrin using the procedure described by Porath & Fornstedt (1970). BSP-GSH conjugate was synthesized and covalently linked to epichlorohydrin-activated Sepharose 4B as described by Clark & Dauterman (1982). Glutathione was linked to epichlorohydrin-activated Sepharose 4B (Porath & Fornstedt, 1970) using the conditions employed by Simons & Vander Jagt (1977) to link GSH to a bisoxirane-activated Sepharose.

Animals

Galleria mellonella larvae were reared on dark brood honey-comb. They were collected just before pupation and stored at \( -20 \leq 0 \) °C until required. Male rats (Sprague-Dawley) of 200-400 g body weight were killed by cervical dislocation, and their livers were removed and frozen immediately. They were stored at \( -20 \leq 0 \) °C until required.

Enzyme preparation

The GST from Galleria mellonella was prepared by affinity chromatography on BSP-GSH conjugate immobilized on Sepharose 4-B activated with epichlorohydrin (Clark et al., 1977; Clark & Dauterman, 1982). The technique employed was that described by Clark & Dauterman (1982). BSP (2.5 mM) was used as the eluting agent and was removed from the eluate by passage through a small column (5 cm \( \times \) 1.5 cm) of QA-Sephadex A-25.

The multiple GSTs from rat liver were purified by adsorption onto GSH immobilized on epichlorohydrin-activated Sepharose 4-B and elution from it with 10 mM-GSH, pH 9.6. This was followed by the separation of individual isofoms by chromatofocusing. In some cases the chromatofocusing step was repeated to eliminate cross-contamination between adjacent peaks. The detail of the method is given elsewhere (Clark et al., 1990).

The GST activity was measured during preparative procedures using CDNB as substrate, employing the conditions adopted by Habis et al. (1974), namely 1 mM-CDNB, 1 mM-GSH at pH 6.5 and 25 °C.

Electrophoresis

PAGE was carried out in the absence of SDS by the method of Davis (1964), and in the presence of SDS by the method of Laemmli (1970). Isoelectrofocusing in polyacrylamide gels was carried out in a Bio-Rad MiniProtein II Minigel apparatus, in either the presence or absence of 8 mM-urea, using the procedures described by Robertson et al. (1987). After the run, ampholytes were removed by soaking the gels in 1% (w/v) trichloroacetic acid for 10 min before staining. Gels were stained to 0.05% (w/v) Coomassie Brilliant Blue R250 dissolved in methanol/acetic acid/water (91:18:91, by vol.) for 60 min at room temperature. Gels were destained overnight in the solvent mixture used to dissolve the stain.

Kinetic analysis

Kinetic experiments were carried out in 0.1 M-sodium phos-
phate buffer, pH 6.5 at 30 °C. In experiments using the GST from *G. mellonella*, CDNB was added to reaction mixtures from a stock solution of 60 mM-CDNB in ethanol. However, in the case of the rat liver enzymes, in order to avoid more satisfactorily the effects of organic solvents on the enzymes (Aitio & Bend, 1979), CDNB was dissolved directly in the buffer by prolonged stirring to give a stock solution of final concentration 1 mM. This was then diluted as required in subsequent experiments. GSH was added to the reaction mixture (final volume 3 ml) in a volume of 50 µl from a stock solution to give the required final concentrations.

The effects of inorganic ions were studied by adding these to the reaction mixture, either dissolved in the CDNB-containing buffer to the required concentration, or in volumes of 5–50 µl from concentrated (0.06–6 M) stock solutions. In either case the pH of the salt-containing solution was adjusted back to pH 6.5 by addition of 2 M-NaOH solution. In preliminary experiments, IC$_{50}$ (median inhibitory concentration) values were determined by adding inhibitor to the standard reaction mixture containing both substrates at a concentration of 1 mm, at pH 6.5 and 25 °C. In experiments with the enzyme from *G. mellonella*, six concentrations of the varied substrate were employed at three inhibitor concentrations. With the enzymes from rat liver, experiments were generally as described above, except that four inhibitor concentrations were used. Experiments on the rat liver isozyme 3-3, designed to determine the most appropriate rate equation to use for fitting, employed ten concentrations of the varied substrate at six inhibitor concentrations.

In view of the potential reactivity of some of the anions tested, in particular SCN$^-$ and ClO$_4^-$, it was thought necessary to test the various enzymes for stability in the presence of these ions. This was done as follows: the test enzyme was made up into a solution, in 0.1 M-sodium phosphate buffer, pH 6.5, containing 1 mM-GSH, at a concentration such that 20–50 µl gave an increase in absorbance of approx. 0.05 units/min when added to the standard assay system. ClO$_4^-$ or SCN$^-$ was added to this stock enzyme solution to a final concentration of 2 mM. These mixtures were then incubated at 30 °C for up to 15 min. Aliquots were removed for assay at intervals. The assay was carried out using the standard assay system containing 2 mm-ClO$_4^-$ or SCN$^-$, so that the enzyme was exposed to the same concentration of these anions during both the preincubation and the assay. Controls, to which no additions of SCN$^-$ or ClO$_4^-$ had been made, were incubated and sampled as described above.

Rate measurements were corrected for the spontaneous reaction where necessary. In most instances the corrections were negligible. Corrected, but otherwise untransformed, rate data were fitted to rate equations using a non-linear regression program (KINETICS 301) on Apple IIe and Commodore PC-10 microcomputers. Both unweighted and weighted ($w = 1/v^2$ and $1/v^4$) regression procedures were tested in determining which rate equations best described the data. A weighting of $1/v^2$ gave generally the smallest sum of squared residuals and the lowest s.d. values on the regression parameters, and this weighting function was therefore the one used.

**RESULTS**

**Enzyme preparation**

The use of affinity chromatography on immobilized BSP-GSH conjugate is well established for the preparation of the major GSTs from *G. mellonella*. In the present instance, the procedure produced an enzyme preparation exhibiting only a single protein zone on SDS/PAGE.

For the preparation of isozymes from rat liver, affinity chromatography on GSH immobilized on epichlorohydrin-activated Sepharose was employed (Clark et al., 1990). This was followed by chromatofocusing, repeated if necessary to obtain homogeneous enzyme preparations. Unless otherwise stipulated, the enzyme preparations 1-1, 1-2, 3-3, 3-4 and 4-4 were homogeneous when examined by SDS/PAGE, PAGE and isoelectrofocusing in polyacrylamide gel.

**Inhibition studies**

Preliminary experiments with the GST from *G. mellonella* established that almost all inorganic anions tested did have a detectable inhibitory effect on the activity of the enzyme. IC$_{50}$ values ranged from 200 mm for Cl$^-$ to 5 mm for SCN$^-$. Some of the ions examined (S$^-$, NO$_3^-$, dithionite and arsenate) appeared to react with GSH, as judged by loss of reactivity with Ellman's reagent. These were not examined any further. CN$^-$, which appeared to be a good inhibitor, was not studied further in the present series of experiments, since at the pH used in this work it would be only partially ionized. Anions that were not shown to react with GSH were tested with the enzyme from *G. mellonella*, and approximate IC$_{50}$ values were determined.

These were as follows: Cl$^-$, 240 mm; I$^-$, 190 mm; Br$^-$, 85 mm; SCN$^-$, 85 mm; NO$_3^-$, 35 mm; and ClO$_4^-$, 20 mm. Arsenite (IC$_{50}$ 300 mm), molybdate (20 mm), SO$_4^{2-}$ (10 mm) and tungstate (6 mm) were also found to be inhibitory, but were not studied.

**Fig. 1. Inhibition by NaSCN of the major GST from *G. mellonella***

Reaction rates were measured with various concentrations of (a) CDNB and (b) GSH. When CDNB was the varied substrate, the GSH concentration was maintained at 1 mm, and when GSH was varied, the concentration of CDNB was held at 0.9 mm. NaSCN concentrations are as shown on the graph.
Table 1. Inhibition of GSTs from rat liver and from larvae of _G. mellonella_ by inorganic anions

Values for the inhibition constants (_K_1) and, in the case of partial non-competitive inhibitions, the _β_ values, were determined as described in the Materials and methods section. Results shown in this Table were derived from the analysis of sets of 24 rate measurements.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Varied substrate</th>
<th>GST...</th>
<th>Galleria</th>
<th>1-1</th>
<th>3-3</th>
<th>3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>_K_1 (mM)</td>
<td>_K_2 (mM)</td>
<td><em>β</em></td>
<td>_K_1 (mM)</td>
<td><em>β</em></td>
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<tr>
<td>Cl^-</td>
<td>GSH</td>
<td>154 ± 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>CDNB</td>
<td>177 ± 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Br^-</td>
<td>GSH</td>
<td>82 ± 3</td>
<td>-</td>
<td>-</td>
<td>49 ± 13</td>
<td>0.5 ± 0.05</td>
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<tr>
<td></td>
<td>CDNB</td>
<td>82 ± 4</td>
<td>213 ± 57</td>
<td>-</td>
<td>42 ± 1</td>
<td>0.5 ± 0.06</td>
</tr>
<tr>
<td>NO_3^-</td>
<td>GSH</td>
<td>33 ± 2</td>
<td>-</td>
<td>-</td>
<td>21 ± 4</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>CDNB</td>
<td>46 ± 5</td>
<td>-</td>
<td>-</td>
<td>19 ± 5</td>
<td>0.26 ± 0.04</td>
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<tr>
<td>F^-</td>
<td>GSH</td>
<td>18 ± 1</td>
<td>-</td>
<td>-</td>
<td>3 ± 0.6</td>
<td>0.3 ± 0.04</td>
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<tr>
<td></td>
<td>CDNB</td>
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<td>ClO_4^-</td>
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<td>-</td>
<td>-</td>
<td>2 ± 0.3</td>
<td>0.19 ± 0.03</td>
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<tr>
<td></td>
<td>CDNB</td>
<td>6 ± 0.1</td>
<td>12.6 ± 5</td>
<td>-</td>
<td>1.2 ± 0.2</td>
<td>0.25 ± 0.03</td>
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<tr>
<td>SCN^-</td>
<td>GSH</td>
<td>6 ± 0.4</td>
<td>-</td>
<td>-</td>
<td>0.8 ± 0.2</td>
<td>0.11 ± 0.07</td>
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<tr>
<td></td>
<td>CDNB</td>
<td>6 ± 0.4</td>
<td>24 ± 4</td>
<td>-</td>
<td>0.35 ± 0.0</td>
<td>0.17 ± 0.03</td>
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</table>

Table 2. Kinetic data relating to the inhibition of rat liver GST 3-3 by NaSCN

_V_ max is measured in units of _µmol/min_; _V_ as _min^-1·ml_; _K_ m and _K_ as _mM_; and _β_ is dimensionless. Best-fit regression parameters are tabulated as mean ± S.D. The experiment involved the examination of 60 triplicated rate measurements. A weighted non-linear regression procedure, as described in the Materials and methods section, was employed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Varied substrate</th>
<th>GSH</th>
<th>CDNB</th>
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<tr>
<td><em>V</em> max</td>
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<td>0.43 ± 0.02</td>
<td>0.51 ± 0.03</td>
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<tr>
<td><em>K</em> m</td>
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<td>0.054 ± 0.012</td>
</tr>
<tr>
<td><em>K</em> i</td>
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<td>0.36 ± 0.03</td>
<td>0.20 ± 0.021</td>
</tr>
<tr>
<td><em>β</em></td>
<td></td>
<td>0.11 ± 0.012</td>
<td>0.12 ± 0.012</td>
</tr>
<tr>
<td><em>v</em></td>
<td></td>
<td>0.14 ± 0.011</td>
<td>0.15 ± 0.014</td>
</tr>
</tbody>
</table>

Mean of squared residuals ... 0.007 0.0104

In order to establish whether the inhibition was due to a Mass Action effect of high concentrations of one product (or product analogue; i.e. the anion) on the reverse reaction, the conjugate S-(2,4-dinitrophenyl)-L-GSH (0.1 mM) was incubated with concentrations of the _K_ m. _SCN^-_ and _ClO_4^-_ in concentrations as high as 100 mM and _Cl^-_, _Br^-_ and _NO_3^-_ concentrations up to 300 mM, both in the presence and in the absence of the enzyme. There was no detectable decrease in the absorbance of the solutions at 344 nm over periods of up to 2 h. It was concluded that there was no evidence for a Mass Action effect in the mechanism of inhibition.

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More detailed kinetic studies were carried out using the purified _Galleria_ enzyme. The mechanism of action was determined to be classically non-competitive, with respect to both substrates, for all the anions tested. Typical Hanes (1982) plots for the experiments using _SCN^-_ as inhibitor are shown in Fig. 1. Inhibition constants are shown in Table 1.

Very similar results were obtained with GSTs prepared from rat liver. The kinetics were found to be generally more complex and more difficult to analyse. Inhibition experiments using _SCN^-_ with isoenzyme 3-3 were performed to determine the best
in which CDNB was the variable substrate are shown in Fig. 2. The data are presented as Eadie–Hofstee plots, which demonstrate clearly non-linearity with respect to substrate concentration and by Hunter & Downs (1945) plots. Derived from eqn. (1) the latter plotting method corresponds to eqn. (2):

$$\frac{v - v_i}{v} = \frac{(s + K_m)(i + K_i) + V(\beta + K_i)}{V(1 - \beta)}$$

This equation is linear with respect to $s$, and the gradient and the vertical intercept are both functions of $i$. This is the situation seen in Fig. 2(a). The existence of multiple lines in these graphs is diagnostic of a partial mode of inhibition, and the linearity in response to variation of substrate concentration is indicative of a non-competitive mode of inhibition (see Webb, 1963). The plotting patterns obtained when GSH was the variable substrate were qualitatively identical with those shown in Fig. 2.

It may be noticed that there is some bias in the distribution of residuals in individual curves in Figs. 2(a) and 2(b), and this suggests that even eqn. (1) does not provide a complete fit to the experimental data, particularly with regard to the effects of varying substrate concentration. However, it should be noted that the two plotting methods used tend to distort the distribution of residuals, especially for the most inhibited rate measurements and particularly in Fig. 2(a). Although this reservation must be borne in mind, this model was found to be the most appropriate for all the experiments in which the inhibition of isoenzymes 3-3, 3-4 and 4-4 was studied. Results are shown in Table 1. Reasonably good agreement is seen between the values obtained for $K_i$ and $\beta$ for the inhibition of 3-3 by SCN$^-$ in the different experiments represented in Tables 1 and 2. The values obtained in Table 1 are characterized by larger coefficients of variation than those in Table 2. This reflects the differing sizes of the data sets analysed.

Limited amounts of material permitted only few experiments on isoenzymes 1-1 and 4-4. The inhibition with Br$^-$ and SCN$^-$, in the case of isoenzyme 4-4, was found to be qualitatively and quantitatively similar to that seen with isoenzyme 3-3. Values of $1.35 \pm 0.31$ and $116 \pm 15$ mm were obtained for the $K_i$ for SCN$^-$ and Br$^-$ respectively, and the corresponding $\beta$ values were $0.45 \pm 0.11$ and $0.15 \pm 0.08$. These experiments were carried out at 30 °C.

In the case of isoenzyme 1-1, the mode of inhibition was difficult to characterize. Values for $K_i$ were similar to those found with the Galleria enzyme and are displayed in Table 1. The mode of inhibition was better characterized as non-competitive rather than as partial non-competitive.

It is evident from Table 1 that, although the potency of the inorganic anions as inhibitors varies quite markedly from enzyme to enzyme, the order of inhibitory power is consistent. It seems likely that what is being observed is a phenomenon that is fundamental to the mechanism of these enzymes, regardless of source.

**DISCUSSION**

**Kinetics of inhibition**

It is known that the principal GST from *G. mellonella* demonstrates non-Michaelian kinetics with respect to substrate concentration (Chang et al., 1981). The range of substrate concentrations used in the present work was chosen to avoid the non-linearities previously observed. Given this limitation, the mechanism of inhibition by the inorganic ions studied appears to be one of simple non-competitive inhibition.

In contrast, with the rat liver class Mu enzymes studied here, saturation with both substrates and also with the inhibitory ions examined are kinetically complex phenomena. That substrate-saturation isotherms for these enzymes were complex has been known for a long time (Palnt et al., 1974; Askelof et al., 1975). Our results resemble those reported by Askelof et al. (1975), in that Eadie–Hofstee plots for both substrates show a marked discontinuity. We have found that our data fit more closely to eqn. (1) than to the 2:2 type of function employed by Askelof et al. (1975) in terms of substrate saturation.

The nature of the inhibition of the Mu class enzymes appears clear, however: for numerous equations, involving various degrees of complexity with respect to substrate-concentration terms, the mode of inhibition was found in each case to be best represented as partially non-competitive. The possible significance of this is discussed below.

**Mechanism of inhibition and mechanism of catalysis**

It is now widely accepted that the catalytic mechanism of the GSTs proceeds by nucleophilic attack of the GSH thiolate anion on the electrophilic substrate. Experimental evidence to support this proposal was first produced by Keen et al. (1976). This

![Diagram](image_url)
Inhibition of glutathione S-transferases

Scheme 1. Proposed mechanism of action of GSTs

Essential features of this mechanism are a general base at site 1 and a positively charged group at site 2. The base at site 1 assists the deprotonation of GSH and promotes its nucleophilic attack on the substrate. The positively charged group at site 2 aids the departure of the negatively charged leaving group. Inhibition would occur if an inorganic anion formed an ion-pair with the group in site 2. Since this group is proposed to be in a hydrophobic environment, this ion-pair formation would occur most readily with anions that can easily shed their hydration sphere and penetrate into this region.

group demonstrated a parallel dependence of both uncatalysed and enzyme-catalysed reactions on the electron-withdrawing power of substituents in a range of analogous substrates having the same leaving group. The same finding, for different compounds, was reported by Chen et al. (1988). This latter group also suggested that attack by the nucleophilic GSH to form an intermediate complex was the rate-limiting step. This was based on the observation that substitution of fluoride for chloride as the leaving group in otherwise identical substrate molecules led to an enhanced rate of product formation catalysed by rat liver GST 4-4. It was argued that if departure of the leaving group were rate-limiting, then breakage of the strong C–F bond would make conjugation of the F- analogue the slower reaction.

On the supposition that the reaction did proceed in this fashion, it was suggested that an enzyme-bound $\sigma$- or Meisenheimer complex was a likely intermediate (Chen et al., 1988; Clark & Sinclair, 1988). Strong supporting evidence that this was indeed the case was provided by Graminski et al. (1989), who showed the enzyme-assisted formation of an enzyme-bound Meisenheimer complex between GSH and the substrate analogue 1,3,5-trinitrobenzene by rat liver transferases 3-3 and 4-4.

The nucleophilic attack by GSH is thought to be promoted by the enzyme increasing the local concentration of the active nucleophilic G$^-$ anion, that is, by decreasing the $pK_a$ of the thiol group (Mannervik & Danielson, 1988). This might be achieved by the participation of a general base, possibly an imidazole nitrogen (Graminski et al., 1989), abstracting the thiol proton. In the case of the rat liver GST 4-4 it has been proposed that almost all of the rate enhancement seen is due to increased ionization of the GSH thiol group (Chen et al., 1988). Given the low catalytic efficiency reported for this enzyme ($K_{cat}/k_{cat} = 1 M$; Chen et al., 1988), it has been argued that it is unlikely that there is any enhancement of the intrinsic nucleophilicity of the thiolate anion.

However, other GSTs may show a much greater rate enhancement than that seen with GST 4-4, and in these cases it is necessary to invoke the participation of additional catalytic mechanisms. Mannervik & Danielson (1988) have proposed the involvement of a general acid as a source of protons for neutralizing the negatively charged intermediates formed during the conjugation of epoxides, hydroperoxides or 4-hydroxyalkenals. Their suggestion was that a carboxylic acid group could act as a general acid. Douglas (1988) proposed a mechanism involving a protonated base acting as a general acid. Either mechanism might give additional rate enhancement by assisting the departure of negatively charged leaving groups. Our data, however, favour the mechanism proposed by Douglas (1988).

The order of efficacy as inhibitors of the anions studied in the present paper resembles closely their order of activity with respect to a number of different phenomena. These include their effect on the salting-out of proteins, the order of the nucleophilicities of the ions in aqueous solution, their behaviour with respect to reverse-osmosis membranes or their partition into organic solvents (e.g. Gibson & Weatherburn, 1972). Arnett et al. (1977) proposed that all these phenomena derive directly from the ease of hydration or dehydoration of the anions and may be related quantitatively to their enthalpies of hydration. The inhibition of the GSTs by these anions appears to be the same type of phenomenon. There is a strong relationship between the $K_i$ values reported here and the enthalpies of hydration determined by Arnett et al. (1977) (see Fig. 3). We suggest that the order of inhibitory potency of these anions is determined by their ability to penetrate into a hydrophobic region of the catalytic environment.
site, a process that would be limited by the ease with which the ions may lose some or all of their hydration sphere.

In this case the mechanism of inhibition would most likely involve formation of an ion-pair with a positively charged group actively involved in the catalytic process. The positively charged general acid group proposed by Douglas (1988), assisting the departure of the leaving group from the electrophilic substrate, is a possible candidate for participation in ion-pair formation. If the rate enhancement produced by this group were additive to that produced by reduction of the $p_K$ of the thiol group of GSH, then its neutralization by an anionic inhibitor would lead to only a partial reduction in the catalytic rate, as has been observed here with rat liver transferases 3-3, 3-4 and 4-4. The greater the contribution of the cation-assisted component of the rate enhancement then the more closely the inhibition will resemble classical non-competitive inhibition. Such reasoning may explain the apparent qualitative differences in the mode of inhibition seen here between the transferases from rat liver and the higher-specific-activity enzyme from G. mellonella. The mechanism proposed is shown diagrammatically in Scheme 1.

A final speculation may be made concerning the possible toxicological significance of these findings. There is a toxic syndrome associated with the chronic consumption of foodstuffs such as cassava or sorghum, both of which contain cyanogenic glycosides. The syndrome is thought to be due largely to direct effects of CN$^-$. Some of the toxic effects, however, are thought to be due to SCN$^-$, produced from the detoxication of CN$^-$. An example is a goitre, which may be caused by SCN$^-$ acting as a competitor for I$^-$ in thyroid uptake (Westley, 1980). SCN$^-$ is not well excreted by the kidneys, and may normally exist in some tissues at quite high concentrations. The normal salivary concentration, for instance, is reported to be in the millimolar range (Long, 1961). If this ion accumulated in body tissues owing to sustained detoxication of CN$^-$, then the action of some or all of the GSTs might be affected. Given that the $K_a$ of SCN$^-$ with respect to GST 3-3 is less than 1 mM, there would not need to be a massive accumulation of the ion to have quite marked effects on the activity of this isoenzyme. The GSTs are known to mediate a variety of important metabolic processes (see, e.g., Huber & Keppler, 1988), so that a continued and severe depression of the activity of these enzymes might itself constitute a toxic insult. In addition, as the GSTs are important detoxication enzymes, the sustained depression of their activity might increase the susceptibility of the organism to other toxic substances of both exogenous and endogenous origin.

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