Inactivation of mouse liver glutathione S-transferase YfYf (Pi class) by ethacrynic acid and 5,5'-dithiobis-(2-nitrobenzoic acid)

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Mouse liver glutathione S-transferase YfYf (Pi class) reacts with [^{14}C]ethacrynic acid to form a covalent adduct with a stoichiometry of 1 mol per mol of subunit. Proteolytic digestion of the enzyme–[^{14}C]ethacrynic acid adduct with V8 protease produced an 11 kDa fragment containing radioactivity. Sequencing revealed this to be an N-terminal peptide (minus the first 15 residues, terminating at Glu-112) which contains only one cysteine residue (Cys-47). This is tentatively identified as the site of ethacrynic attachment. Kinetic studies reveal that glutathione S-conjugates protect against inactivation by ethacrynic acid, but

the level of protection is not consistent with their potency as product inhibitors. A model is proposed in which glutathione S-conjugates and ethacrynic acid compete for the free enzyme, and a second molecule of ethacrynic acid reacts covalently with the enzyme–ethacrynic acid complex. The native protein contains one thiol reactive with 5,5'-dithiobis-(2-nitrobenzoic acid) at neutral pH. The resultant mixed disulphide, like the ethacrynic acid adduct, is inactive, but treatment with cyanide (which incorporates on a mol for mol basis) restores activity to 35% of that of the native enzyme.

INTRODUCTION

Cytosolic glutathione S-transferase (GST) activity is associated with a large number of related enzymes which can be grouped on structural grounds into four families, Alpha, Mu, Theta and Pi (Mannervik et al., 1985; Meyer et al., 1991). These exist as homo- and hetero-dimers which appear to follow the basic rule that quaternary structures involve subunits within a family but not between families (Hayes and Mantle, 1986). The Yf subunit [subunit 7 and Yp in the alternative nomenclatures of Jakoby et al. (1984) and Sato et al. (1984) respectively] is the only definitively described Pi family subunit in the rat and has commanded considerable attention as an immunocytochemical marker for preneoplasia [see Tsuchida and Sato (1992) for a recent review]. The molecular mechanisms responsible for the enhanced expression of this subunit in preneoplasia have been delineated in a careful set of studies by Muramatsu and co-workers who have defined a novel palindromic TRE enhancer and a silencer in the 5'-upstream region of the rat gene (Okuda et al., 1987, 1989; Imagawa et al., 1991; Sakai et al., 1992). Intriguingly, over-expression of the Yf subunit may promote neoplasia since male mice, in which the Yf structural gene is upregulated by testosterone (Hatayama et al., 1986), have been reported to be more prone to hepatocarcinogenesis than females (Smith et al., 1973; Kemp et al., 1989).

The possible role of this protein in promoting neoplasia, coupled with an apparent constraint on the evolution of this form, is consistent with the hypothesis that GST YfYf may play some regulatory role. Ethacrynic acid is a good substrate for the Pi-class GST and we have presented evidence that it also binds to an allosteric site on the mouse liver enzyme (Phillips and Mantle, 1991). Ethacrynic acid is known to bind covalently to GST, and in a preliminary study Yamada and Kaplowitz (1980) presented evidence that *in vivo* GST 3–4 is the major acceptor. Ploemen et al. (1990) presented evidence that the inhibition of GST 3–4 by ethacrynic acid was reversible by dialysis and that similar results were obtained with the rat Pi-class enzyme. In

preliminary studies with the mouse Pi-class enzyme GST YfYf, we have demonstrated a non-saturable time-dependent inactivation by ethacrynic acid (McCusker et al., 1990). We now report the stoichiometry of covalent ethacrynic acid binding, a tentative identification of the residue involved and a detailed examination of the kinetics of protection by S-(2,4-dinitrophenyl)glutathione (DNPG). We also describe various covalent modifications of the enzyme, tentatively identified as occurring at Cys-47, which modulate enzyme activity.

MATERIALS AND METHODS

Preparation of mouse liver GST YfYf

This was prepared as previously described (Phillips and Mantle, 1991) and stored at $-20\text{ }^{\circ}\text{C}$ in 40 mM sodium phosphate, pH 6.7.

Enzyme assays

GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was measured at $30\text{ }^{\circ}\text{C}$ as described by Habig et al. (1974) except that A_{400} rather than A_{340} was monitored (see Phillips and Mantle, 1991).

Inactivation studies with ethacrynic acid

The enzyme ($15\text{ }\mu\text{M}$) was incubated with ethacrynic acid ($0.2\text{--}2\text{ mM}$) for various periods of time at $30\text{ }^{\circ}\text{C}$ when portions ($10\text{ }\mu\text{l}$) were diluted into assay buffer (2 ml) and the CDNB conjugation reaction was monitored at 400 nm. In some experiments, DNPG or S-methylglutathione was included during the preincubation period with ethacrynic acid.

Reaction of the enzyme with [^{14}C]ethacrynic acid

The enzyme ($80\text{ }\mu\text{M}$) was incubated with 1 mM [^{14}C]ethacrynic acid ($3\text{ }\mu\text{Ci}\cdot\mu\text{mol}^{-1}$) in 0.1 M sodium phosphate, pH 6.5, and, at various times, samples were removed and residual activity was

measured. The incorporation of radioactivity was determined by diluting a sample with an equal volume of 25% (w/v) ice-cold trichloroacetic acid. The trichloroacetic acid-treated sample was left on ice for 20 min and the precipitated protein collected by centrifugation. The pellet was washed twice with 1 ml of ice-cold acetone and resuspended in 0.2% (w/v) SDS (50 μ l). A portion was taken for protein determination using the method of Markwell et al. (1978) and a further portion counted for radioactivity using liquid-scintillation counting.

Peptide mapping, electrophoresis and autoradiography

Samples of the modified enzyme (45 μ g) were incubated with the glutamate-specific endopeptidase from the V8 strain of *Staphylococcus aureus* (V8 protease) (0.45 μ g) in a solution containing 0.125 M Tris (pH 6.7), 0.5% (w/v) SDS and 10% (v/v) glycerol at 37 °C, and the reaction was terminated, at the desired time, by boiling in a solution containing 10% (v/v) 2-mercaptoethanol and 2% (w/v) SDS. SDS/PAGE was performed on 20% (w/v) acrylamide gels by the method of Laemmli (1970). After electrophoresis, gels were dried on a Bio-Rad 1125B gel drier, and autoradiography was conducted using Kodak X-ray film (ortho G) for 1 month.

Amino acid sequencing

Peptide bands of interest were blotted from SDS/polyacrylamide gels on to Immobilon membranes as described by Hsieh et al. (1988), and sequenced directly using an Applied Biosystems 477A as described previously (Phillips and Mantle, 1991).

Modification of the enzyme with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and displacement of the 2-nitro-5-thiobenzoate (NTB) anion by cyanide

The stock solution of DTNB (2 mM, in 0.1 M sodium phosphate, pH 7.5) was kept on ice. Incubations of protein (9.6 μ M subunit) with DTNB (17 μ M) were carried out in 0.1 M sodium borate (pH 10, 9.5, 9, 8.5, 8), 0.1 M sodium phosphate (pH 8, 7.5, 7, 6.5, 6) and 0.1 M sodium acetate (pH 6, 5.5, 5, 4.5, 4). The release of the NTB anion over time was monitored at 412 nm (ϵ 14.15 mM⁻¹·cm⁻¹). Blank rates were monitored in the absence of protein. In a separate series of experiments the enzyme (64 μ M) was mixed with a 15-fold molar excess of DTNB (in 0.05 M Tris/HCl, pH 8) and then immediately gel-filtered on Sephadex G-25. The enzyme-NTB mixed disulphide (34 μ M) was then mixed with a 50-fold molar excess of K¹⁴CN (18.8 μ Ci $\cdot$ μ mol⁻¹), and the displacement of NTB monitored at 412 nm. Excess cyanide was then removed on Sephadex G-25. Incorporation of [¹⁴C]cyanide was measured after protein precipitation with trichloroacetic acid, followed by acetone washing and liquid-scintillation counting. Assessment of total thiol content was carried out by incubation of the denatured protein (18 μ M) with DTNB (15-fold molar excess) in 6 M guanidinium chloride (in 0.05 M Tris/HCl, pH 8).

RESULTS

Kinetics of inactivation of enzyme activity by ethacrynic acid

Ethacrynic acid was found to inactivate GST YfYf in a time-dependent manner. No loss in enzyme activity was seen over the

same time period in the absence of ethacrynic acid. Figure 1 shows a series of time courses for the loss of enzyme activity at a range of concentrations of ethacrynic acid. When the apparent first-order rate constant is plotted against the concentration of ethacrynic acid, there is a linear relationship up to a final concentration for ethacrynic acid of 2 mM giving a simple bimolecular rate constant of 0.2 mM⁻¹·min⁻¹.

Covalent modification of the enzyme with ethacrynic acid

In order to determine the stoichiometry and covalent nature of the reaction, the enzyme was incubated with [¹⁴C]ethacrynic acid and the amount of label incorporated into the protein determined as described in the Materials and methods section. Figure 2 shows that the incorporation of radioactivity into the enzyme with time corresponds to the inactivation of the enzyme. After complete inactivation, 1 mol of ethacrynic acid was covalently bound per mol of enzyme subunit.

Figure 1 Kinetics of inactivation of GST YfYf by ethacrynic acid

A representative graph is given which shows the inactivation of GST YfYf by ethacrynic acid, plotted as the natural log of the proportion of activity remaining, as a function of time, for 0.2 mM (○), 0.4 mM (●), 0.8 mM (□), 1 mM (■), 1.5 mM (△) and 2 mM (▲) ethacrynic acid. All lines drawn are from linear regression.

Figure 2 Time-dependent incorporation of [¹⁴C]ethacrynic acid into GST YfYf with the concurrent inactivation of enzyme activity

The enzyme was incubated with [¹⁴C]ethacrynic acid as described in the Materials and methods section. A sample was taken from the reaction mixture for each time point and then divided into two. One half was assayed for enzyme activity (●) and the remaining half was assayed for the incorporation of radiolabel into protein (○). The curves are drawn by hand.

Figure 3 Protective effect of DNPG on the inactivation of GST YfYf by ethacrynic acid

The apparent first-order rate constant for inactivation (k_{inact}') is plotted against ethacrynic acid concentration in the presence of 0 (○), 2.9 (●) and 8.4 (□) mM DNPG. The control data were fitted by linear regression through the origin ($y = ax$) and the other data to a second-order polynomial through the origin ($y = ax + bx^2$).

pH dependence of the inactivation of the enzyme by ethacrynic acid

The rate of reaction between ethacrynic acid and the enzyme, as measured by the apparent first-order inactivation constant, was found to be pH-dependent. The data were fitted to the equation:

$$Y = \frac{(\text{Limit}_1 + \text{Limit}_2)10^{pH-pK}}{10^{pH-pK} + 1} \quad (1)$$

where Limit_1 and Limit_2 are the start and end points of the titration and Y represents the 'effect', in this case the apparent first-order inactivation constant. From the fitting procedure, the pK_{app} was determined to be 7.3. Cysteine and ethacrynic acid were found to react readily in solution, and the rate of reaction was easily monitored at 270 nm. The pH-dependence of this reaction indicated a pK_{app} of 7.7.

Protection against ethacrynic acid inactivation of the enzyme by DNPG and S-methylglutathione

It was of interest to examine the effects, if any, of DNPG on the inactivation of the enzyme by ethacrynic acid. Figure 3 shows a plot of the first-order inactivation constant against the concentration of ethacrynic acid and it is clear that DNPG does have a protective effect. However, two important features of ethacrynic acid inactivation in the presence of DNPG were noted: (a) the concentration of DNPG required to produce any appreciable protective effect was far in excess of the previously measured inhibition constant for DNPG as a product inhibitor and (b) a plot of the first-order inactivation constant against the ethacrynic acid concentration was curved in the presence of DNPG (Figure 3) and S-methylglutathione (results not shown). Statistical analysis (F test) indicated that the data generated in the absence of DNPG fitted best to an equation of the type $y = ax$ (i.e. linear through the origin) but that the data generated in the presence of DNPG fitted best to an equation of the type $y = ax + bx^2$ (i.e. quadratic through the origin). Increasing the number of parameters from one to two (for data in the presence of DNPG) gave a statistically significant decrease in the residual sum of squares (at the 0.05 probability level). No significant decrease was found if the number of parameters was increased from two to three (i.e. fitting to an equation of the type $y = ax + bx^2 + cx^3$).

Figure 4 Analysis of [^{14}C]ethacrynic acid-labelled enzyme on SDS/PAGE after V8 digestion

A 20% (w/v) acrylamide gel is shown in (a). Lanes 1–5 represent digestion of ethacrynic acid-labelled protein (35 μg) by the glutamate-specific endopeptidase from *Staphylococcus aureus* (V8 protease; 3 μg) after 5, 10, 20, 30 and 45 min respectively. Lane 6 contains molecular-mass standards as follows: BSA (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), α -lactalbumin (14.2 kDa). A sheet of X-ray film was exposed to this gel for approx. 1 month in order to detect the location of ^{14}C label and the autoradiogram is shown in (b).

Peptide mapping of the [^{14}C]ethacrynic acid-labelled enzyme

Having identified the covalent nature of the interaction between the enzyme and ethacrynic acid, it was of interest to determine the nature of the amino acid residue involved and also, if possible, its position in the protein's sequence. After being labelled with [^{14}C]ethacrynic acid, the enzyme was subjected to proteolytic digestion with V8-protease. A time course of digestion (5–45 min) was followed and the results analysed by SDS/PAGE and subsequent autoradiography. Figure 4 shows the gel with the corresponding autoradiogram. The digestion, after 45 min, leads to two principal bands of approx. 14 and 11 kDa. These two bands persist even after 24 h of digestion, the protein proving resistant to any further breakdown. The autoradiogram in Figure 4 shows that the 11 kDa band contains radiolabel, whereas the 14 kDa band does not. (There is a transient intermediate of 12 kDa, containing radiolabel, which appears to give rise to the 11 kDa peptide.)

N-Terminal sequencing of the 11 kDa peptide from V8 digestion of the [^{14}C]ethacrynic acid-labelled enzyme

Several samples of a 45 min V8 digest of ethacrynic acid-modified enzyme were subjected to SDS/PAGE, and the protein was then transferred to Immobilon. A band with a mobility on SDS/PAGE of 11 kDa, previously identified as containing radioactivity, was then excised and introduced directly into the sequencer. Figure 5 shows the determined sequence, which clearly overlaps with the N-terminal sequence previously described (Phillips and Mantle, 1991) starting at position 16, corresponding to a cut at Glu-15. Figure 5 also shows the amino acid sequence for a full-length mouse GST Yf cDNA clone (Hatayama et al., 1990).

Thiol accessibility under native and denaturing conditions

Incubation of mouse GST YfYf with DTNB in 6 M guanidinium chloride yielded a rapid release of 2.8 molar equivalents of NTB

cDNA-derived sequence	1	14	20	30	40					
Amino acid sequence	P P Y T I V Y F P V R G R C E A M R M L L A D Q G Q S W K E E V V T I D T W M Q									
	----- N-Terminal sequence -----									
	----- V8 fragment -----									
cDNA-derived sequence continued	47	50	60	70	80					
	G L L K P T C L Y G Q L P K F E D G D L T L Y Q S N A I L R H L G R S L G L Y G									
	90	100	110	120						
	K N Q R E A A Q M D M V N D G V E D L R G K Y V T L I Y T N Y E N G K N D Y V K									
	130	140	150	160						
	A L P G H L K P F E T L L S Q N Q G G K A F I V G D Q I S F A D Y N L L D L L L									
	169	180	190	200						
	I H Q V L A P G C L D N F P L L S A Y V A R L S A R P K I K A F L S S P E H V N									
	209									
	R P I N G N G K Q									

Figure 5 N-Terminal sequence of an 11 kDa V8 fragment of GST YfYf

The amino acid sequence (20 residues) of an 11 kDa fragment derived from digestion of GST YfYf by the glutamate-specific endopeptidase from *Staphylococcus aureus* (V8 protease) is shown overlapping (residues 16–25) the previously sequenced N-terminus (Phillips and Mantle, 1991). The complete amino acid sequence for the mouse Yf subunit from Hatayama et al. (1990), derived from the cDNA sequence, is shown alongside to indicate the position of the three cysteine residues in the protein (positions 14, 47 and 169).

Figure 6 An extended time course for the release of NTB (A_{412}) from the reaction between GST YfYf and DTNB

The A_{412} (left axis) and the corresponding number of mol of NTB released per mol of enzyme subunit (ϵ_{412} $14.15 \text{ mM}^{-1} \cdot \text{cm}^{-1}$) are plotted against time for a range of pH. An initial burst of NTB release over an approximate time scale of < 2 min, which indicated 1 mol of NTB released per mol of enzyme subunit, was followed by pH > 8 by the much slower release of a second mol of NTB per mol of enzyme subunit. The reaction was carried out at pH 9.6 (○), 9.3 (●), 8.9 (□), 8.5 (■), 8.1 (△), 8 (▲), 7.5 (○), 7 (●), 6.6 (□), 6.1 (phosphate) (■), 6.1 (acetate) (△), 5.6 (▲), 5.1 (◇) and 4.6 (◆). It must be noted that the pK for NTB is 4.5 (Riddles et al., 1979) and therefore the calculated mol for mol ratio (right axis) will be underestimated at acidic pH values.

per enzyme subunit. Incubation of the native enzyme with DTNB in Tris/HCl at pH 8 yielded the rapid release of 1.2 molar equivalents of NTB per enzyme subunit. This DTNB-modified enzyme was found to be inactive. However, if the DTNB-modified enzyme was incubated with $K^{14}\text{CN}$, 0.93 molar equivalents of NTB was released per enzyme subunit with the concomitant incorporation of 0.95 molar equivalents of cyanide. The specific activity of the *S*-cyanylated enzyme was 35 % that of the native enzyme.

Stoichiometry and pH-dependence of the reaction between DTNB and enzyme under native conditions

In order to follow the kinetics of thiol modification, it was necessary to reduce the molar excess of DTNB from 50- to 2-fold. The data that described the reaction of the first protein thiol fitted well to a curve of the type:

$$A_{412} = \max. + ce^{-kt}$$

A plot of the apparent first-order rate constant k against pH indicated a pK_{app} of 7.7. If the reaction is allowed to continue after the 'first' mol of NTB has been released, a second mol is released, but only at alkaline pH, as can be seen in Figure 6. This process is a great deal slower with completion after about 3 h.

DISCUSSION

Ethacrynic acid covalently modified GST YfYf (1 mol per mol enzyme subunit) with concomitant inactivation of the enzyme. Peptide mapping indicates that the modification is to a residue between Glu-15 and Glu-112. If ethacrynic acid is modifying a cysteine residue, as seems probable, then it follows that the residue involved is Cys-47. These findings contrast with the report of Ploemen et al. (1990) for the rat enzyme in which no evidence was found for irreversible inhibition by ethacrynic acid.

It is assumed that the inactivation of the enzyme by ethacrynic acid does not occur at the active centre, since there is no evidence

for saturation in Figure 1 and the K_m for ethacrynic acid is 60 μ M (Phillips, 1992). However, the protection against inactivation provided by DNPG and *S*-methylglutathione suggests that the inactivation occurs near the active site or that multiple conformational states exist.

The lowest concentration of DNPG required to achieve effective protection (approx. 1 mM) is 300-fold higher than the K_i (3.2 μ M) determined for DNPG as a product inhibitor (Phillips and Mantle, 1991). This implies one of two possibilities. (a) Protection is not achieved by binding to the active site but through weak binding to the 'inactivation site'. If this model is correct, the plot of inactivation constant against the concentration of ethacrynic acid is predicted to be linear. This does not appear to be the case. (b) Protection is achieved through DNPG binding to the active site precluding access to Cys-47. To explain why such high concentrations of DNPG are required to achieve protection, we suggest that ethacrynic acid and DNPG compete at the active site with binding affinities akin to those seen in kinetic studies and that it is the subsequent reaction between the Cys-47 of the enzyme-ethacrynic acid complex and an additional molecule of ethacrynic acid that leads to inactivation. Since high concentrations of ethacrynic acid are utilized in the experiment to measure the inactivation process, equally high concentrations of DNPG are required to compete with ethacrynic acid at the active site. This model is presented in Scheme 1.

Scheme 1 Mechanism of inactivation of GST YfYf by ethacrynic acid

It is proposed that ethacrynic acid (I) and DNPG (P) compete for free enzyme (E) with approximately equal binding constants (K_i and K_p respectively). A further molecule of ethacrynic acid may then bind (EII) and react covalently with the enzyme leading to loss of activity. The inactivation is governed by the rate constant k_{inact} , and the binding of the second ethacrynic acid is governed by the dissociation constant (K'_i), which may be so large as to be kinetically insignificant. See the text for further discussion.

The kinetic implications of the model shown in Scheme 1 were examined by noting that the apparent first-order rate constant for inactivation (k_{inact}') could be expressed as:

$$k_{\text{inact}}' = \frac{[E]_t k_{\text{inact}} \frac{I^2}{K_i K'_i}}{1 + \frac{P}{K_p} + \frac{I}{K_i} + \frac{I^2}{K_i K'_i}} \quad (2)$$

where I and P refer to ethacrynic acid and DNPG respectively, K_i , K'_i and K_p are dissociation constants for EI, EII and EP respectively, $[E]_t$ is the total enzyme concentration and k_{inact} is the first-order rate constant of inactivation. When P is zero and the concentration of I is greater than K_i , then the apparent rate constant is predicted to vary hyperbolically with I (this process is governed by K'_i). Since we have no evidence for any saturation, we assume that K'_i is considerably larger than 2 mM. Eqn. (2) is also consistent with the statistical analysis on the data (see the Results section) which indicated the presence of squared terms in the equation.

An empirical analysis of eqn. (2), using the computer program EKPLLOT (Phillips, 1992), reveals that the distribution equation $EI/[E]_t$ functions to delay the saturation described by K'_i and that this effect is exacerbated by any finite value for P.

Various reports in the literature have identified the presence of a 'reactive thiol' on Pi-family GSTs that is important for catalytic activity. Tamai et al. (1990) identified the site of inactivation of rat YfYf as Cys-47 by using *N*-ethylmaleimide. Lo Bello et al. (1990) also identified the site of inactivation of human YfYf (GST π) as Cys-47 by using maleimide. Del Boccio et al. (1991) reported the modification of two thiols on a GST from horse erythrocytes (probably a Pi-family GST) with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; there was a 10-fold difference in the rate of modification, Cys-47 being identified as the faster reaction ($t_{1/2} = 8$ min).

Of the three cysteine residues in mouse GST YfYf, it is clear that one is accessible to solvent and readily reacts with ethacrynic acid and DTNB; this is probably Cys-47. The pH-dependence of this reaction with DTNB indicated a pK of 7.7 which is close to the pK of 7.3 determined for ethacrynic acid inactivation. It is equally clear that a second cysteine residue is inaccessible to solvent in the native enzyme and resistant to attack by DTNB. This is probably Cys-14 which is buried deep within the protein structure (I. Garci, M. Coll, M. F. Phillips and T. J. Mantle, unpublished work). We have some evidence that the third cysteine (Cys-169) is reactive towards DTNB, but not as reactive as Cys-47 and not at all with ethacrynic acid. However, a degree of caution must be applied to the significance of the second 'reactive thiol' (Cys-169) since the behaviour/stability of the NTB colour over such a long time period is uncertain (Riddles et al., 1979).

The observation that the *S*-cyanlated form of the enzyme is catalytically active suggests that Cys-47 is not directly involved in the catalytic mechanism but that modification of this residue may hinder substrate binding. It is significant that the ethacrynic acid-modified and the NTB-modified enzyme are inactive but that replacement of the NTB by the less bulky cyanide partially restores activity. This suggests that the bulk of the modifying reagent is important and that steric hindrance may account for the inhibition. A similar conclusion has recently been presented by Nishihira et al. (1992) on the basis of modification studies using methylmethanethiosulphonate. Using site-directed mutagenesis, Tamai et al. (1991) have demonstrated that replacement of Cys-47 with an alanine residue does not result in any loss in enzyme activity, confirming that the residue plays no direct part in catalysis. Tamai et al. (1991) have also shown that the Cys-47 $\rightarrow$ Ala mutant was insensitive to inactivation by *N*-ethylmaleimide, suggesting that modification of the second, less reactive, thiol results in no effect on enzyme activity.

It is at present unclear whether covalent modification of Cys-47 plays any physiological role, but it is curious that GSH and GSH-utilizing enzymes play an important role in protecting cells from oxidative stress and that Pi-family GSTs are susceptible to inactivation under circumstances in which oxidative stress is high.

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