Reactivity of Cysteine-49 and Its Influence on the Activation of Microsomal Glutathione Transferase 1: Evidence for Subunit Interaction†

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ABSTRACT: Microsomal glutathione transferase 1 is a homotrimeric detoxication enzyme protecting against electrophiles. The enzyme can also react with electrophiles, and when modification occurs at a unique Cys49 the reaction often results in activation. Here we describe the characterization of the chemical properties of this sulfhydryl (kinetic pK_a was 8.8 ± 0.3 and 9.0 ± 0.1 with two different reagents) and we conclude that the protein environment does not lower the pK_a. Upon a direct comparison of the reactivity of Cys49 and low molecular weight thiols [L-Cys and glutathione (GSH)], the protein sulfhydryl displayed a 10-fold lower reactivity. The reactivity was correlated to reagent concentration in a linear fashion with a polar reagent, whereas the reactivity toward a hydrophobic reagent displayed saturation behavior (at low concentrations). This finding indicates that Cys49 is situated in a hydrophobic binding pocket. In a series of related quinones, activation occurs with the more reactive and less sterically hindered compounds. Thus, activation can be used to detect reactive intermediates during the metabolism of foreign compounds but certain intermediates can (and will) escape undetected. The reactivities of the three cysteines in the homotrimer were shown not to differ dramatically as the reaction of the protein with 4,4′-dithiodipyridine could be fitted to a single exponential. On the basis of this result, a probabilistic expression could be used to relate the overall degree of modification to fractional activation. When *N*-ethylmaleimide activation (determined by the 1-chloro-2,4-dinitrobenzene assay) was plotted against modification (determined with 4,4′-dithiodipyridine), a nonlinear relation was obtained, clearly showing that subunits do not function independently. The contribution to activation by single-, double-, and triple-modified trimers, were 0 ± 0.06, 0.74 ± 0.09, and 0.97 ± 0.06, respectively. The double-modified enzyme appears partly activated, but this conclusion is more uncertain due to the possibility of independent modification of the purified enzyme upon storage. It is, however, clear that the single-modified enzyme is not activated whereas the triple-modified enzyme is fully activated. These observations together with the fact that MGST1 homotrimers bind only one substrate molecule (GSH) strongly support the view that subunits must interact in a functional manner.

Glutathione transferases (GST, E.C. 2.5.1.18) comprise a group of phase II detoxication enzymes that occur abundantly in eukaryotic cells and also in prokaryotic organisms (*1*). These enzymes are one of the main defense systems against carcinogenic, mutagenic, toxic, and pharmacologically active electrophilic compounds (*2*). Inherent to this function, these enzymes mediate the protection against carcinogenic substances and can be induced by anticarcinogens in our diet (*3*). On the other hand, glutathione transferases have also been suggested to protect tumors from chemotherapy (*4*). Attesting to the importance of this enzyme system is the presence of many cytosolic isoenzymes in several related families (*5*) as well as structurally and phylogenetically distinct membrane-bound glutathione transferases. Microsomal glutathione transferase 1 (MGST1)[1] (*6*) is a homotrimeric membrane protein that also forms part of the so-called MAPEG superfamily. This superfamily contains structurally and phylogenetically related enzymes (including six human forms) involved in detoxication, protection from oxidative stress, and synthesis of prostaglandin E and cysteinyl leukotrienes (*7, 8*).

Microsomal GST1 displays a unique ability to be activated (up to 15-fold) by sulfhydryl reagents such as *N*-ethylmaleimide (NEM), redox events, and proteolysis (*9*−*11*). This activation could thus be a rapid adaptive response to

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[1] Abbreviations: MGST 1, microsomal glutathione transferase 1; NEM, *N*-ethylmaleimide; CDNB, 1-chloro-2,4-dinitrobenzene; MAPEG, membrane-associated proteins in eicosanoid and glutathione metabolism; DTP, 4,4′-dithiodipyridine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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toxic insult and oxidative stress. The activated enzyme displays an increased efficiency at low glutathione concentration that can occur during toxic insult (*12*). Microsomal GST1 contains a single cysteine residue per subunit (*13, 14*). This sulfhydryl, Cys49, resides on the cytoplasmic side of the endoplasmic reticulum and was unequivocally demonstrated as the site of activation since mutation to alanine prevented activation by NEM (*15*). The present study was undertaken to answer several questions regarding the activation process. Is the reactivity and pK_a of the thiol in Cys49 particularly favorable for reaction with electrophiles? Which properties of electrophiles are important for activation, such as reactivity and steric factors? Since MGST1 is a homotrimer, what is the stoichiometry of covalent modification in relation to activation? The results give important insight into a possible functional interaction between subunits, indicated by earlier substrate binding experiments (*16*).

EXPERIMENTAL PROCEDURES

Materials

Glutathione (Sigma), 1-chloro-2,4-dinitrobenzene (Merck), *N*-ethylmaleimide (Sigma), 2,6-di-*tert*-butyl-1,4-benzoquinone (Aldrich Chemical Co.), 2,6-dimethyl-*p*-benzoquinone (Aldrich), 2,6-dimethoxy-*p*-benzoquinone (Aldrich), 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Aldrich), 2-hydroxy-1,4-naphthoquinone (Aldrich), 4,4′-dithiodipyridine (Sigma), and iodoacetamide (Sigma) were from the suppliers indicated. All other chemicals were from common suppliers.

Methods

Enzyme Purification. Microsomal glutathione transferase was purified from male Sprague-Dawley rat livers as described previously (*17*).

Removal of GSH. Glutathione was removed from the purified enzyme by two methods as described (*18*) , either by ion-exchange chromatography or by gel filtration. In the former procedure, the enzyme was diluted in 10 volumes of 10 mM potassium phosphate, pH 7.0, 0.1 mM EDTA, 1% Triton X-100, 20% glycerol (v/v), and 1 mM GSH (buffer A) and applied to a CM-Sepharose cation-exchange column equilibrated with the same buffer. The column was washed with 5 volumes of buffer A (without GSH), and the glutathione-free enzyme was eluted by the addition of 0.2 M KCl in GSH-free buffer A. Removal of GSH was verified as described (*18*).

For gel filtration, desalting chromatography columns (10 DG, 10 mL, Bio-Rad Laboratories, Hercules, CA) were utilized. The columns were washed with buffer A devoid of GSH. After the addition of 1.5 mL of purified enzyme to the column, 1.5 mL of the same buffer was added, and then 2 mL of buffer was used to elute the GSH-free enzyme, which was collected in a single tube and kept on ice until use. In some experiments buffer A devoid of GSH and with a Triton X-100 concentration of 0.2% was used; this will be referred to as buffer X.

Enzyme Assay. Glutathione transferase activity was measured with glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates essentially according to the method of Habig et al. (*19*). The activity of the enzyme was determined in a 0.1 M potassium phosphate buffer, pH 6.5, containing 0.1% Triton X-100, 5 mM GSH, and 0.5 mM CDNB. The rate of product formation was monitored by measuring the change in absorbance at 340 nm on a single-beam Philips PU8700 UV/visible spectrophotometer (Philips Scientific & Analytical Equipment, Cambridge, Great Britain). Enzyme activities were calculated after correction for the nonenzymatic reaction.

pH Dependence of Activation and Modification. The purified enzyme (in buffer A) was desalted as previously described in 5 mM buffer X (the concentration given refers to potassium phosphate). Aliquots were mixed with the same amount of 0.2 M buffer X in a pH range of 6−9.5 (at pH > 8, Tris-HCl was used instead of potassium phosphate) and incubated for a few minutes, whereafter samples for determining basal activity were withdrawn. Activation was started by the addition of 0.1 mM NEM and followed by the CDNB assay. Data were fitted to a single exponential to give the apparent first-order rate constant k_{obs} at a given pH. The pH was verified with a pH-meter.

The time course of NEM or DTP reaction with Cys49 was monitored on an Applied Photophysics stopped-flow spectrophotometer at 5−5.5 °C. The reaction was followed as a change in absorbance at 302 or 324 nm for NEM or DTP, respectively, after desalted, purified enzyme in 10 mM buffer X was mixed with 2 mM NEM or 0.5 mM DTP dissolved in 0.2 M buffer X (pH 6−9.5) (final concentration of reagent was 1 or 0.25 mM). Rate constants were obtained by fitting the data to a single or a double exponential. Fitting to a double exponential was performed when a rapid low-amplitude reaction indicative of low amounts of residual GSH in the preparation was observed. Upon double gel filtration of the enzyme, this part of the reaction was greatly diminished.

Chemical Reactivity of Cys49. To study chemical modification, GSH-free enzyme in 0.1 M buffer X (pH 7 or 7.5) was rapidly mixed with the same buffer containing various concentrations of sulfhydryl reagent (NEM, DTP, or DTNB) at 4 °C. The reaction was followed until completion at 302, 324, and 412 nm for NEM, DTP, and DTNB, respectively (extinction coefficients $\epsilon_{NEM} = 600\ M^{-1}\ cm^{-1}$, $\epsilon_{DTP} = 19\,800\ M^{-1}\ cm^{-1}$, and $\epsilon_{DTNB} = 14\,150\ M^{-1}\ cm^{-1}$) (*20*−*22*). The concentration of sulfhydryl reagent was always held in excess (>5 times the concentration of Cys49). Reaction progress could be fitted to a single exponential except in some cases, where the reaction progress was fitted to a double exponential due to reaction of trace amounts of GSH, which reacts much faster as explained above.

Stoichiometry Study. GSH-free enzyme was incubated with different (mostly substoichiometric) concentrations of NEM (0.5−50 μM) on ice for 4 h. After this time enzymatic activities were measured in the CDNB assay. In addition, a 4,4′-dithiopyridyl disulfide (DTP) assay was employed to determine remaining unreacted sulfhydryl (*21*). In short, the absorbance of the sample after addition of SDS (10% final concentration) was recorded at 324 nm; after 30 s, 4,4′-DTP (0.25 mM) was added; and the reaction was followed until completion.

Experiments with Quinones. The purified GSH-free enzyme and quinone (1 mM) were incubated on ice for a few minutes (in 0.1 M buffer X). Enzyme activities were measured by the CDNB assay. Subsequent addition of 2 mM NEM to the incubation, followed by enzyme assays was

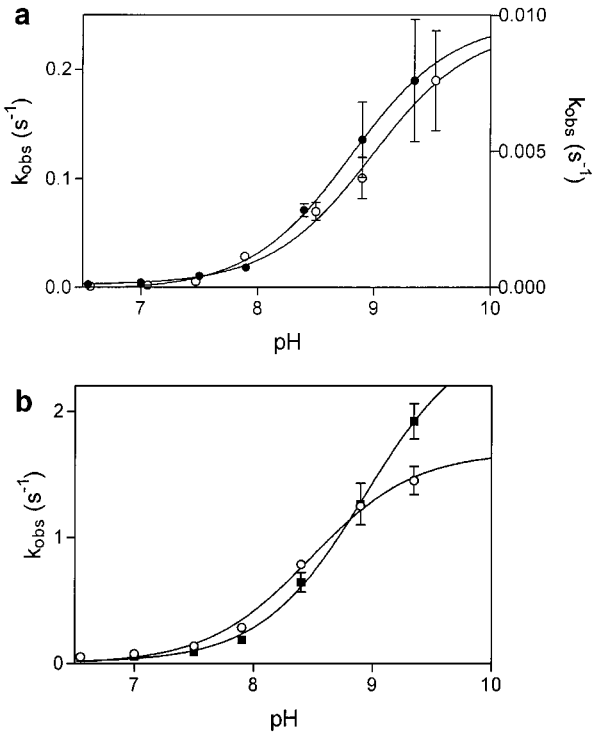

FIGURE 1: (a) Rate of activation (k_{obs}) of MGST1 by 0.1 mM NEM as a function of pH at 0 °C (○), measured by following increased activity of MGST1 toward CDNB. p*K*a = 9.0 ± 0.1, and kobs corresponds to the right *y*-axis. Kinetic p*K*a of Cys49 (●), where k_{obs} was determined by following the reaction between Cys49 (15−30 μM) and 0.25 mM DTP at 324 nm at 5 °C. p*K*a = 8.8 ± 0.3, and k_{obs} corresponds to the left *y*-axis. (b) Kinetic p*K*a of L-cysteine and GSH. k_{obs} was determined by following the reaction of L-cysteine (○) or GSH (■) with 0.25 mM DTP at 324 nm and 5 °C. $pKa_{L\text{-}Cys}$ = 8.5 ± 0.1; pKa_{GSH} = 8.9 ± 0.1.

utilized to determine the extent of chemical modification. Controls received EtOH, or dimethyl sulfoxide (DMSO) in the case of 2,6-dimethoxy-*p*-benzoquinone.

Protein was determined by the method of Peterson (*23*) with bovine serum albumin as standard.

Kinetic Data Analysis. Nonlinear regression analyses were performed with the program package Graphpad Prism 3 (Graphpad Software Inc.). Stopped-flow experiment progress curves were fitted with the software provided.

RESULTS AND DISCUSSION

pK_a of Cys49. MGST1 is activated in vitro by sulfhydryl reagents (15-fold by NEM) (*17*). Cys49 in the enzyme has been unequivocally demonstrated as the site of modification correlated to activation (*15*). This allows for two approaches to study the progress of activation/modification: either the activity increase of the enzyme is measured in enzyme assays or the reaction of the Cys49 sulfhydryl is monitored directly by a spectrophotometric assay. First, k_{obs} of activation as a function of pH displayed a pK_a of 9.0 ± 0.1 (Figure 1a) when the enzyme was incubated with 0.1 mM NEM at different pH values from 6 to 9.5. Second, determination of the direct reaction of DTP and NEM with Cys49 at varying pH yielded pK_as of 8.8 ± 0.3 (Figure 1a) and 8.8 ± 0.1 (not shown), respectively. For comparison, the pK_as of nonprotein model thiols, L-cysteine (8.5 ± 0.1) and GSH (8.9 ± 0.1) (Figure 1b), were determined under the same reaction conditions (with DTP). These pK_a values agree well with literature data (*24*). The pK_a of Cys49 is thus similar to those of low molecular weight thiols and not lower, as might have been suspected of a reactive sulfhydryl.

Table 1: Apparent Second-Order Rate Constants for the Reaction between MGST1, GSH, and Sulfhydryl Reagents at pH 7

	k_2 (M^{-1} s^{-1})	
thiol	DTNB	DTP
GSH	120 ± 4	240 ± 5
Cys49	12 ± 0.4	22 ± 1.0[a]

[a] Obtained from fit of the linear portion in Figure 2a.

Reactivity of Cys49. The reaction rate of Cys49 toward DTP and DTNB was 10-fold lower than that for model thiols (Table 1), showing that Cys49 is clearly unreactive. The concentration dependence of the reaction with DTP and DTNB at pH 7 and 7.5 is shown in Figure 2. A strictly linear dependence was observed with the hydrophilic reagent DTNB. In contrast, there appeared to be a nonlinear concentration dependence in the reaction with the more hydrophobic reagent DTP at low concentrations. This suggests that there is a hydrophobic binding site in close proximity to Cys49, displaying affinity for hydrophobic compounds. Many examples exist where MGST1 has been shown to become activated in vivo (*25*−*28*). Furthermore, substoichiometric additions of NEM to rat liver microsomes suggested that the MGST1 sulfhydryl reacted preferentially in comparison to other protein sulfhydryls (*9*). These observations are difficult to reconcile with the normal pK_a and low reactivity of the MGST1 sulfhydryl. However, the presence of a hydrophobic binding site could provide a rationale for the efficient in vitro and in vivo activation of MGST1 by hydrophobic electrophiles (*29*, *30*). There is even a possibility that the enzyme has evolved a specific function where precise interactions with this binding site are required.

Traditionally, electrophilic substrates for GST enzymes are often effective inducers (transcriptional activators) of GST genes. The exposure to such compounds induces the synthesis of enzymes that catalyze their detoxication. In many cases, novel isoenzymes that are not expressed constitutively appear after the administration of the inducer (*3*). As rat MGST1 does not respond to traditional inducers of drug metabolism, the presence of a nucleophilic group, Cys49, provides an alternate more rapid mechanism of activation.

Reactivity of Cys49 toward Quinones. As such the high concentration of the enzyme in the liver endoplasmic reticulum (1 mM; *31*) can serve as a protective sink for electrophiles. Once damage is very pronounced, the enzyme is activated and can in addition protect by increased catalytic efficiency. The presence of the Cys49 thiol in a hydrophobic binding site makes it increasingly apparent that the nature and characteristics of the electrophile will of course govern the outcome and extent of modification. It has been established that MGST1 in rat liver microsomes can be activated by reactive metabolites of phenol (*32*), which is in turn a metabolite of benzene. The mechanism of activation is probably analogous to the effect of sulfhydryl reagents. In fact, it has been found that phenol, hydroquinone, and catechol, metabolites of benzene, give rise to similar toxicity and cellular effects as sulfhydryl reagents (*33*).

To further investigate the mechanism of activation of MGST1, we have studied the possible chemical interaction

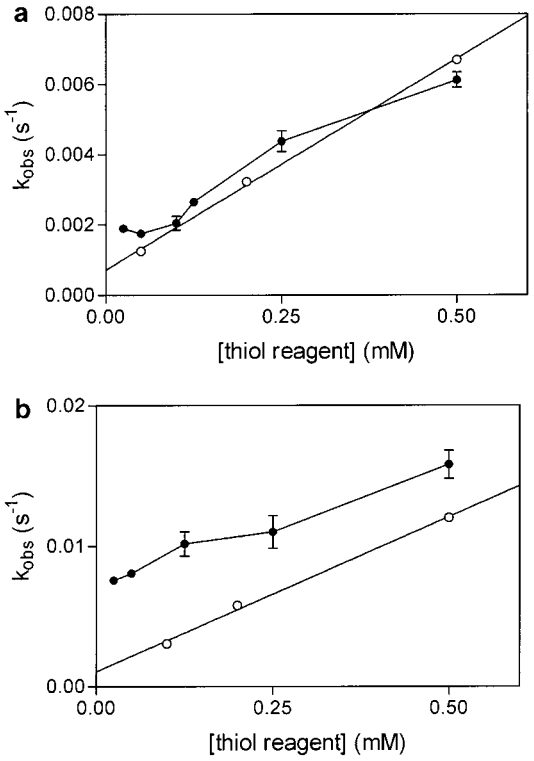

FIGURE 2: Concentration dependence of the reaction between Cys49 in MGST1 and sulfhydryl reagents of DTNB (○) and DTP (●) at pH 7 (a) and pH 7.5 (b). Experimental conditions are given under Methods.

Table 2: Activity of MGST1 Treated with Quinones[a]

treatment (1 mM quinone)	change in activity[b]	change in activity after NEM
2,6-di-*tert*-butyl-1,4-benzoquinone	0.7 ± 0.09	11 ± 2.3
2,6-dimethyl-*p*-benzoquinone	13 ± 0.86	16 ± 0.96
2,6-dimethoxy-*p*-benzoquinone	0.6 ± 0.19	7.3 ± 1.4
2,3-dimethoxy-5-methyl-1,4-benzoquinone	7.4 ± 0.95	5.9 ± 0.33
2-hydroxy-1,4-naphthoquinone	0.9 ± 0.35	9.5 ± 3.2
control (EtOH)	1 ± 0.37	17 ± 5.3
control (DMSO)	1.2 ± 0.9	11 ± 2.3

[a] The enzyme was incubated with the indicated quinone and CDNB activity was determined as described under Methods. At the end of the incubations 2 mM (final concentration) NEM was added to determine the maximum amount of activation that could be achieved. [b] Unactivated enzymatic activity was 1.5−2 μmol min^{-1} mg^{-1}. Changes in activity are given as *x*-fold ± SD, for $n = 3$.

between the purified enzyme and five different quinones displaying different degrees of steric hindrance and electrophilicity. The quinones were 2,6-di-*tert*-butyl-1,4-benzoquinone, 2,6-dimethyl-*p*-benzoquinone, 2,6-dimethoxy-*p*-benzoquinone, 2,3-dimethoxy-5-methyl-1,4-benzoquinone, and 2-hydroxy-1,4-naphthoquinone.

In our experiments quinones (1 mM) were added to purified MGST1. Activity was recorded and NEM (2 mM) was added subsequently to the incubation mixture, whereafter maximal activation was determined.

The results presented in Table 2 indicate that no reaction (and no activation) of the enzyme occurred with 2,6-di-*tert*-butyl-1,4-benzoquinone, full reaction and activation occurred with 2,6-dimethyl-*p*-benzoquinone, partial reaction and no activation occurred with 2,6-dimethoxy-*p*-benzoquinone, partial reaction and activation occurred with 2,3-dimethoxy-5-methyl-1,4-benzoquinone, and partial reaction and no activation occurred with 2-hydroxy-1,4-naphthoquinone.

It is a relevant observation that these quinones give rise to different responses (reaction/activation) of MGST1. The quinone that most strongly activated MGST1 is 2,6-dimethyl-*p*-benzoquinone, probably because it displays high reactivity and the least steric hindrance of the quinones tested. In fact, the analogue 2,6-di-*tert*-butyl-1,4-benzoquinone does not react with the enzyme at all. No inhibition by any of the quinones was observed in the assay system.

Less reactive quinones do not activate MGST1, except for 2,3-dimethoxy-5-methyl-1,4-benzoquinone, which activated MGST1 to a lower extent than NEM. It appears that this quinone can lock the enzyme in a partially activated state. Such a state is an interesting candidate for electron crystallography [a projection structure has been determined to 3 Å of the unactivated enzyme (*34*)].

Stoichiometry of Modification. The reaction with DTNB was only about 60% complete, whereas close to 100% modification was obtained with DTP under these experimental conditions (e.g., 0.5 mM, 500 s, 5 °C). Modification with NEM is also complete (vide infra). Since it has been proposed that the cysteines in the trimer could be closely situated (*35*), it is possible that steric and/or electrostatic factors (e.g., DTNB is charged and larger than DTP) prevent full modification in some cases.

Cys49 Reactivity Is Equal in the Different Subunits of the Homotrimer. No a priori assumption regarding the reactivity of cysteines in different subunits of the trimeric enzyme can be made. From symmetry one would predict equal reactivity, but cooperativity could alter reactivity during the course of modification. It is also known that each homotrimer binds only one substrate molecule of GSH under equilibrium conditions, suggesting the possibility of functional subunit interactions. A spectrophotometric method was employed in order to directly measure the reactivity of all cysteine sulfhydryls during the course of modification. The reaction between Cys49 and DTP (Figure 3) appeared to follow a double exponential. We found that the minor fast phase was due to trace amounts of GSH since double gel filtration decreased this phase. The amplitude for the slow phase was also in agreement with the actual Cys49 concentration (based on protein determination and active-site titration). The data thus support that the Cys49 sulfhydryl groups in an MGST1 homotrimer react independently and at equal rates.

Stoichiometry of Activation. Chemical modification of Cys49 in MGST1 with sulfhydryl reagents yields an activated enzyme. The relationship between extent of modification and activation has not, however, been determined previously. Covalent modification of a homotrimer containing one target site in each subunit can in principle yield several outcomes. For instance, if subunits function independently, a linear relationship between degree of activation and modification is expected. Since it is known that MGST1 binds only one GSH per trimer under equilibrium conditions (*16*), the possibility of cooperativity or shared mutually exclusive binding sites on the enzyme must be considered. In such a situation, nonlinear relationships between modification and activation can occur. In fact, when MGST1 activity (strictly speaking, fraction of maximal activation) was plotted against relative amounts of Cys49 modified by NEM (Figure 4),

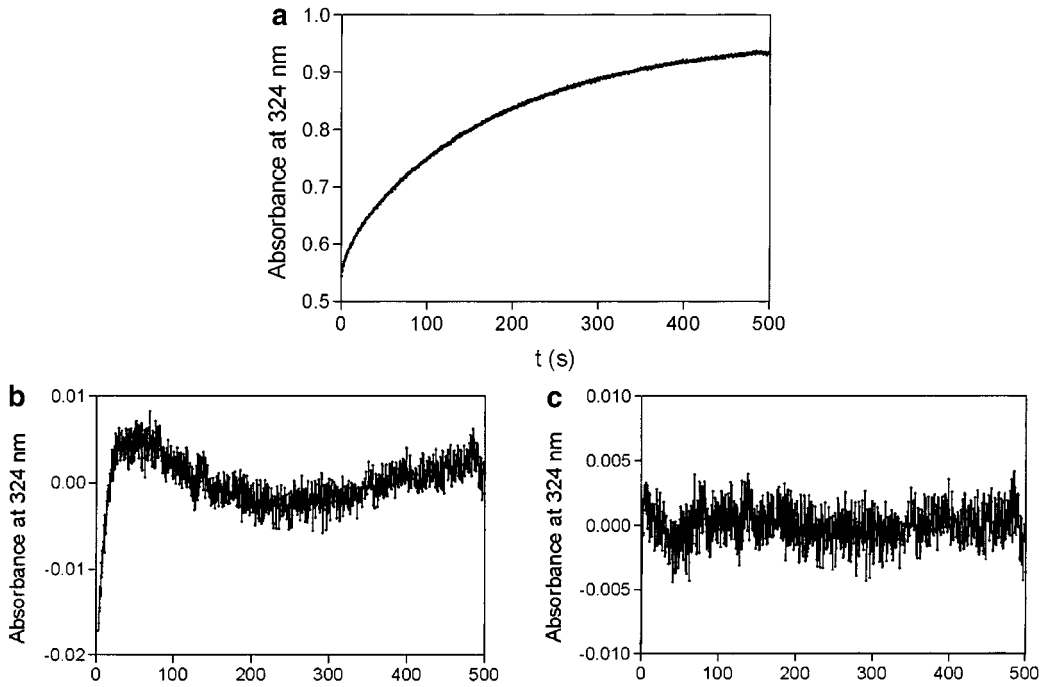

FIGURE 3: Time course of the reaction between MGST1 and DTP. (a) DTP (0.5 mM) and 17 μM MGST1 subunit at pH 7 and 5.5 °C at 324 nm. (b) Residuals from the fit of panel a to a single-exponential function. (c) Residuals from the fit of panel a to a double-exponential function.

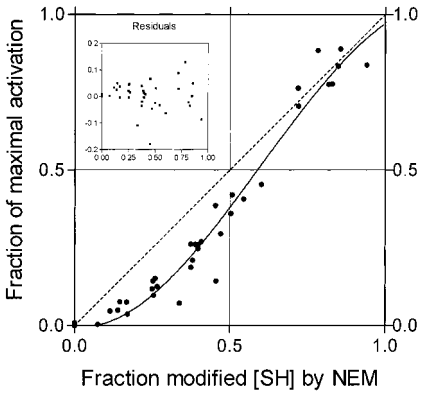

FIGURE 4: Relationship between the fractional degree of activation (Y) and the fractional extent of covalent modification of MGST1 (X). Data points were fitted to the probabilistic expression $Y = k_1[3X(1 - X)^2] + k_2[3X^2(1 - X)] + k_3X^3$, where k_1, k_2,and k_3 refer to the fractional contribution to activation by single-, double-, and triple-modified MGST1 trimers, respectively.

nonlinear behavior was evident. The same relationship was also evident when iodoacetamide was used (not shown), except that data at high modification (where higher concentrations (>10 mM) of iodoacetamide had to be used) had to be excluded since the enzyme became inactivated (iodoacetamide can react with Tyr, His, and Met in addition to Cys). Our data indicate that a single-modified trimer is not activated at all, whereas the double-modified enzyme could be partly activated and the triple-modified MGST1 is fully activated. The first and last conclusions can be arrived at simply by inspection of the graph (Figure 4). As we have shown that cysteine sulfhydryls in the different subunits are equally reactive, a probabilistic expression[2] ($Y = k_1[3X(1 - X)^2] + k_2[3X^2(1 - X)] + k_3X^3$) could be used to describe the distribution of variously modified trimers in terms of total modification (X) and their contribution to fractional activation (Y) (Figure 4). The constants that are fitted to the probabilistic expression correspond to the fraction of maximal activation carried by the differently modified enzyme forms and were 0 ± 0.06, 0.74 ± 0.09, and 0.97 ± 0.06, respectively. Fractional activation that can be ascribed to the double-modified trimer is close to 0.7. It thus appears that the double-modified enzyme actually is partly activated. This fractional activation varies somewhat between experiments with different enzyme preparations (0.3−0.7). Since we have observed that MGST1 self-activates, albeit very slowly, upon prolonged storage (a process that could be independent of the sulfhydryl group and probably undetectable when only one subunit is affected), an alternate interpretation concerning activation is possible in this case. Some double-modified enzyme (as measured in the experiment) was perhaps already fully modified by this combination of events. If this were true, only the fully modified enzyme is activated.

Regardless of the state of activation of the double-modified enzyme it appears clear that the single-modified MGST1 is not activated. This is an important observation showing that subunits do not operate independently. It is thus a fair assumption that the trimer forms an active site that has to be influenced by at least two covalent modifications to become activated. Perhaps this active site is built from three overlapping but mutually exclusive symmetric sites. The fact that GSH binds to only one site under equilibrium conditions (*16*) is consistent with this suggestion.

In conclusion, the sulfhydryl group of MGST1 displays an unperturbed pK_a and is comparatively unreactive toward sulfhydryl reagents. Instead there is a possibility that this enzyme has adopted an alternate mechanism to facilitate

[2] If the three sites react independently and at equal rates ("probability") the probability for zero modifications is $(1 - X)^3$, for one modification $3X(1 - X)^2$, for two modifications $3(X^2)(1 - X)$, and for three modifications X^3, where X is the total fractional modification (or "probability of modification"). k_1–k_3 are the conditioned probabilities that the corresponding modification leads to activation (Y).

activation, i.e., a more or less specific binding site for hydrophobic molecules directing modification of Cys49. The exciting possibility of general affinity for hydrophobic electrophiles and/or highly developed specificity for particular (perhaps endogenous) electrophiles thus forms an interesting area for future explorations. Even though the Cys49 is unreactive, the enzyme can be employed to investigate whether electrophilic (unknown) metabolites arise during the metabolism of xenobiotics and this concept has been used (*32*, *36*). Sterically hindered or less reactive electrophiles can, and will, escape undetected, whereas certain electrophiles that display affinity for the activation site can probably be detected with high sensitivity. Activation occurs only when at least two subunits in the homotrimeric MGST1 have been modified. This result demonstrates that functional interaction between subunits must occur.

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