Epitope mapping of a monoclonal antibody to human glutathione transferase P1–1 the binding of which is inhibited by glutathione

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Although the three-dimensional structure of human glutathione transferase (GST) P1–1 crystallized with a GSH analogue has been reported, its structure in the non-complexed form has not been determined. Four monoclonal antibodies to GST P1–1 were produced to facilitate structural analysis. Of these, one, clone d-1 of IgG_{2a} isotype, dose-dependently inhibited the activity of GST P1–1 but did not affect the activities of either GST A1–1 or M1–1. On immunoblotting, the antibody reacted strongly with GST P1–1 and weakly with rat GST-P and mouse GST-II, indicating cross-reactivity with Pi-class forms but preferential reactivity with GST P1–1. When GST P1–1 and the antibody were incubated in the presence of 60 μ M GSH, no inhibition of activity was found, whereas 1-chloro-2,4-dinitrobenzene had no effect at concentrations up to 10 μ M. The binding of GST P1–1

to antibody adsorbed to Protein A–Sepharose was also prevented by both 0.1 mM GSH and *N*-ethylmaleimide treatment. Trypsin digests of GST P1–1 were resolved by HPLC and a peptide that reacted with the antibody was detected by absorption experiments. N-Terminal amino acid sequencing revealed the peptide to be in the C-terminal portion of the enzyme, stretching from amino acid residues 198 to 208. A synthetic peptide of this sequence also absorbed the antibody. These results suggest that both GSH bound to the active site and *N*-ethylmaleimide bound to the cysteine residue repress antibody binding to the C-terminal region. Thus this antibody may be useful for examining the steric configuration of the C-terminal and other regions of GST P1–1 in the absence of GSH.

INTRODUCTION

The glutathione transferases (GSTs) (EC 2.5.1.18) are a family of multifunctional proteins that act as enzymes and also as binding proteins in various detoxication processes [1]. Many molecular forms of cytosolic GST have been identified from various organs in a variety of species, and these are grouped into five classes, Alpha, Mu, Pi, Theta and Sigma [2,3]. Previous studies from our laboratories showed that the Pi-class GST forms, rat GST-P (7–7) and human GST P1–1, are strongly expressed in neoplastic and pre-neoplastic tissues [4–6]. Furthermore an increased expression of Pi-class forms has been noted in many cancer cell lines resistant to several anticancer agents [7,8].

These enzymes have dimeric structures as the result of non-covalent association of identical or different subunits. Each subunit contains one binding site for GSH (G-site) and another for hydrophobic substrates (H-site) [1]. Since the involvement of GSTs in drug resistance was first suggested, the three-dimensional structures of these enzymes crystallized with GSH or its analogues have been extensively studied to aid development of specific inhibitors [9–12]. Several amino acid residues have been identified, the replacement of which results in marked decreases in activity or alterations in the K_m values for GSH [13–16]. Most residues constituting the G-site are localized in the N-terminal half of the subunit (domain I) [9,11]. The crystal structure of GSTs not complexed with GSH has not yet been determined but binding of GSH analogues is suggested to induce a conformational change in the vicinity of the G-site [17–20]. For example, the 47th Cys residue of GST P1–1 in this location is highly reactive with SH-blocking reagents, including *N*-ethylmaleimide (NEM), when the enzyme is free but becomes unreactive on complexation with GSH analogues [17,19].

In the present study, we produced monoclonal antibodies to human GST P1–1 to facilitate its structural analysis. One clone that was found to inhibit enzyme activity was blocked from binding to the enzyme by the presence of GSH. We identified the epitope of the antibody to be in the C-terminal region and examined the relationship between this and the active sites.

MATERIALS AND METHODS

Materials

Protein A–Sepharose CL-4B and Sepharose 4B were obtained from Pharmacia Biotech (Uppsala, Sweden), diphenylcarbamyl chloride (DPCC)-treated trypsin and papain attached to agarose from Sigma (St Louis, MO, U.S.A.), an ABC Vector stain kit from Vector Laboratories (Burlingame, CA, U.S.A.), nitrocellulose membranes from Bio-Rad (Richmond, CA, U.S.A.), complete and incomplete Freund's adjuvants from Iatron Laboratories (Tokyo, Japan), horseradish peroxidase-conjugated goat anti-mouse immunoglobulins from Tago (Burlingame, CA, U.S.A.) and anti-mouse subclass-specific antibodies from Southern Biotechnology (Birmingham, AL, U.S.A.). All other chemicals were of analytical grade. *S*-Hexylglutathione–Sepharose was prepared as described by Guthenberg and Mannervik [21].

Purification of GST forms

Human GST P1–1 was purified from placental tissue (120 units/mg of protein) and GST A1–1 and M1–1 from liver as reported previously [22]. Rat GST-P (7–7) and mouse GST-II,

Abbreviations used: GST, glutathione transferase; G-site, binding site for GSH; H-site, binding site for hydrophobic substrates; TPBS, PBS containing 0.02% (w/v) Tween 20; CDNB, 1-chloro-2,4-dinitrobenzene; NEM, *N*-ethylmaleimide; DPCC, diphenylcarbamyl chloride.

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both Pi-class forms, were also purified from hyperplastic nodule-bearing livers and normal liver respectively, as reported previously [4,23]. The purity of these preparations was established by SDS/PAGE.

Production of monoclonal antibodies to GST P1-1

Eight-week old male Balb/c mice were immunized with 100 μ g aliquots of GST P1-1 emulsified with complete Freund's adjuvant. They then received two booster injections at 2-week intervals with antigen solution emulsified with incomplete adjuvant. At 7–10 days after the last booster injection, the mice were injected intravenously with 50 μ g of the antigen solution. After 3 days their spleen cells were fused with mouse myeloma cells, P3x63Ag8U1, at a ratio of 4:1 by centrifugation with poly(ethylene glycol) by the method of Galfre et al. [24]. The fused cells were placed in 96-well culture plates (Nunc, Roskilde, Denmark) with 100 000 cells/well, incubated in a humidified 5% CO₂ atmosphere at 37 °C overnight, and selected with HAT medium supplemented with 100 μ M hypoxanthine, 0.4 μ M aminopterin and 16 μ M thymidine [25]. Antibody-producing hybrid cells were screened twice by ELISA [26] and subcloned twice by limiting dilution.

Screening for antibody-producing hybrid cells

Culture supernatant (50 μ l portions) was added to wells of plates, which had been coated with 100 μ l of 20 μ g/ml GST P1-1 solution overnight at 4 °C and blocked with 1% (w/v) BSA for 2 h at room temperature, before incubation for 2 h at room temperature. After a wash with PBS containing 0.02% (w/v) Tween 20 (TPBS), 50 μ l of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin diluted 1000-fold with 1% (w/v) BSA was added to each well, and the incubation was continued for 1 h at room temperature. The plates were then washed thoroughly with TPBS. After this, 50 μ l of 0.3 mM 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) in 0.1 M sodium citrate buffer, pH 4.0, containing 0.03% (v/v) H₂O₂ was added as the substrate for horseradish peroxidase with incubation for 30 min at 37 °C. *A*₄₀₅ was read with a spectrophotometer (MTP-22; Corona Electric Co., Katsuta, Japan).

Purification of monoclonal antibodies to GST P1-1

Each clone producing antibody to GST P1-1 was injected into pristane-primed Balb/c mice and 7 days later ascites fluid was tapped from the mice. γ -Globulin prepared from ascites by (NH₄)₂SO₄ fractionation (0–50% saturation) was dialysed against PBS, and then applied to a GST P1-1-Sepharose column (1 cm $\times$ 10 cm) previously equilibrated with PBS. GST P1-1-Sepharose was prepared by coupling GST P1-1 to Sepharose 4B activated with CNBr by the method of Cuatrecasas and Anfinsen [27]. After a wash with PBS, anti-(GST P1-1) antibody bound to the column was eluted with 0.1 M glycine/HCl, pH 2.8, and dialysed against PBS. For some experiments employing Fab fragments, the antibody (200 μ g of protein) was further digested with insoluble papain attached to agarose (10 units/0.1 ml of gel) in 0.1 M sodium phosphate buffer, pH 7.0, containing 10 mM cysteine and 2 mM EDTA, at 37 °C for 18 h.

Immunoblotting of GST P1-1 with monoclonal antibodies

SDS/PAGE was performed by the method of Laemmli [28] and immunoblotting as described by Towbin et al. [29] using monoclonal antibodies to GST P1-1 and the avidin-biotin-peroxidase complex method [30].

Monoclonal antibody inhibition of GST P1-1 activity

Activity inhibition testing using monoclonal antibodies was performed as follows: antibody (up to 10 μ g of protein) was added to GST P1-1 (0.02–0.04 unit) or other forms, and the final volume was made up to 400 μ l with PBS containing 0.1% (w/v) BSA. The mixture of enzyme and antibody was left at 4 °C overnight, and then assayed for GST activity with 1-chloro-2,4-dinitrobenzene (CDNB) as described by Habig et al. [31]. In some experiments GSH or CDNB was added before the addition of the antibody. For immunoprecipitation reactions, anti-mouse IgG or IgM antibodies were added to the mixture as secondary antibodies.

Inhibition of GST P1-1 binding to monoclonal antibody by GSH

The monoclonal antibody, d-1, affinity-purified as described above (2.8 mg of protein/3 ml of PBS) was applied to a Protein A-Sepharose column (1 cm $\times$ 2 cm) previously equilibrated with PBS. After a wash with PBS containing 0.1 mM GSH, 2 units of GST P1-1 with 0.1 mM GSH were applied to the column. Flow-through fractions were assayed for GST activity. A similar experiment was also performed in the absence of GSH.

NEM treatment of GST P1-1

GST P1-1 (1 mg) was incubated with 0.2 mM NEM in 2 ml of 0.1 M Tris/HCl, pH 7.8, at 25 °C for 10 min. The mixture was dialysed against 10 mM Tris/HCl, pH 7.8, containing 0.2 M NaCl, and then subjected to *S*-hexylglutathione-Sepharose chromatography (column size 1 cm $\times$ 5 cm) as described previously [17]. Unbound fractions were used as NEM-modified GST P1-1.

Epitope mapping

To map the epitope of the monoclonal antibody d-1, an absorption experiment was performed. GST P1-1 peptides were prepared by digestion of 2 mg of GST P1-1 in 0.5 ml of 10 mM Tris/HCl, pH 7.4, with 10 μ g of DPCC-treated trypsin at 30 °C for 16 h. Each peptide was resolved by HPLC under the same conditions as used previously for rat GST-P peptides [17]. After lyophilization, each peptide was dissolved in 100 μ l of PBS. Then 20 μ l of peptide solution was added to 80 μ l of PBS containing up to 5 μ g of the antibody, and incubated at 4 °C for 2 h. After addition of 0.2 μ g of GST P1-1, the final volume was made up to 400 μ l with PBS containing 0.1% BSA. As in the inhibition test, the mixtures were left overnight at 4 °C, and then assayed for GST activity.

N-Terminal amino acid sequencing

N-Terminal amino acid sequencing was performed as reported previously [32].

Peptide synthesis

The C-terminal peptide of GST P1-1 between Tyr-198 and Lys-208 was synthesized by the solid-phase method [33] with an automated peptide synthesizer (Applied Biosystems Synergie, Foster, CA, U.S.A.). After the peptide chain had been formed, the protecting groups were removed and the peptide resin anchoring bond was cleaved by trifluoroacetic acid in the presence of 1,2-ethanedithiol and thioanisole [34]. The crude peptide was purified by preparative HPLC. N-Terminal amino acid sequencing confirmed the presence of the desired peptide.

RESULTS

Production of monoclonal antibodies to GST P1-1

Three monoclonal antibodies, c-8, d-1 and f-1, were produced which were shown to react by immunoblot analysis with GST P1-1 but not with GST A1-1 or M1-1, as shown in Figure 1. Another antibody, b-2, reacted with GST P1-1 as assessed by ELISA but not by immunoblotting. Isotype determination revealed b-2 and c-8 to be IgM, d-1 to be IgG_{2a} and f-1 IgG₁. Incubation of antibody d-1 with GST P1-1 at 4 °C for 16 h resulted in a dose-dependent inhibition of enzyme activity, with 2.5 μ g of the antibody causing about 70 % inhibition (Figure 2). It did not affect the activities of either GST A1-1 or M1-1, indicating specificity for GST P1-1. The other antibodies did not inhibit GST P1-1 activity in the absence of anti-mouse IgG or IgM antibodies. However, in the presence of the second antibodies, b-2 and c-8 demonstrated about 30 % inhibition (results

Figure 1 Immunoblot analysis of reactivity of monoclonal antibodies to human GSTs

Purified preparations of GST P1-1 (5 μ g of protein, lane 1), A1-1 (10 μ g of protein, lane 2) and M1-1 (10 μ g of protein, lane 3) were subjected to SDS/PAGE using a 12.5% acrylamide gel. (a) Protein staining with Coomassie Brilliant Blue; (b), (c) and (d) immunoblots with monoclonal antibodies, c-8, d-1 and f-1 respectively.

Figure 2 Inhibition of enzymic activity of human GSTs by monoclonal antibody d-1

GST P1-1 (●), A1-1 (▲) and M1-1 (△) in PBS containing 0.1% (w/v) BSA were incubated with the indicated amounts of the monoclonal antibody d-1 at 4 °C overnight, and then assayed for GST activity with CDNB as a substrate.

Figure 3 Immunoblot analysis of reactivity of monoclonal antibody d-1 to Pi-class GSTs

GST P1-1 (lane 1), rat GST-P (lane 2) and mouse GST-II (lane 3) (5 μ g of protein in each case) were subjected to SDS/PAGE. (a) Protein staining; (b) immunoblotting with antibody d-1.

Figure 4 Effects of GSH and CDNB on the inhibition of GST P1-1 activity by the antibody

(a) GST P1-1 in PBS/0.1% BSA (0.08 unit/ml) containing 0 (●), 0.06 (○), 0.6 (▲), 6 (△) and 60 (■) μ M GSH was mixed with 0–10 μ g of antibody d-1, incubated at 4 °C overnight, and then assayed for activity. (b) GST P1-1 in PBS/0.1% BSA (0.05 unit/ml) containing 0 (●), 1 (○), 10 (▲) and 100 (△) μ M CDNB was used as the enzyme solution. Other conditions were the same as for (a).

not shown). Papain-treated Fab fragments of the d-1 antibody also inhibited the activity of GST P1-1 (results not shown), suggesting that inhibition by the antibody is not due to immunoprecipitation. On immunoblotting, the d-1 antibody only faintly reacted with rat GST-P and weakly with mouse GST-II, both of which belong to the same Pi class as GST P1-1 (Figure 3), indicating cross-reactivity with Pi-class forms but preferential reactivity with GST P1-1.

Effects of GSH on the binding of GST P1-1 to the monoclonal antibody

In the process of this study, we found no inhibition by antibody d-1 when GST P1-1 preparations contained *S*-hexylglutathione, but there was inhibition of the purified preparation free from *S*-hexylglutathione. As the assay mixture for GST contained 1 mM GSH and 1 mM CDNB, GSH itself did not appear to affect the inhibition of enzyme activity by the antibody. Therefore, to examine the effect of GSH on the binding of GST P1-1 to d-1, enzyme solutions containing various concentrations of GSH were incubated with the antibody at 4 °C for 16 h, and then assayed for activity. As shown in Figure 4(a), addition of GSH before the formation of immune complexes dose-dependently

Figure 5 Effects of GSH on the binding of GST P1-1 to the antibody

Antibody d-1 (2.8 mg of protein) was applied to a Protein A-Sepharose column (1 cm × 2 cm) previously equilibrated with PBS. After a wash with PBS containing 0.1 mM GSH, GST P1-1 in the same solution (2 units) was applied to the column. Fractions of volume 2 ml were collected and flow-through fractions were assayed for GST activity. ● and ○ denote the results with and without 0.1 mM GSH respectively.

Figure 6 Non-binding of NEM-treated GST P1-1 to the antibody

NEM-treated GST P1-1 (0.5 mg) was applied to a column of antibody-bound Protein A-Sepharose previously equilibrated with PBS. After a wash with PBS, bound fractions were eluted with 0.1 M glycine/HCl, pH 2.8. Unbound and bound fractions were subjected to SDS/PAGE. Lane 1, GST P1-1 (10 µg of protein); lane 2, unbound fraction (4 µg); lane 3, bound fraction (10 µg); lane 4, antibody d-1 (4 µg). H and L denote the heavy and light chains of IgG, respectively. (a) Protein staining; (b) immunoblotting with the d-1 antibody.

prevented inhibition by the antibody, the prevention being complete with a 60 µM concentration. On the other hand, CDNB did not affect the inhibition at concentrations up to 10 µM (Figure 4b), although the enzyme was inactivated to a large extent by 100 µM CDNB. *S*-Hexylglutathione in place of GSH also prevented inhibition by the antibody (results not shown), suggesting that reduction of the immunoglobulin by GSH is not involved in this prevention. The pH of solutions was not changed by the addition of GSH or *S*-hexylglutathione at concentrations up to 100 µM. Partial inhibition of GST P1-1 activity by other antibodies, b-2 and c-8, in the presence of the second antibodies were not significantly affected by GSH (results not shown).

The effects of GSH on the binding of GST P1-1 to d-1 were also studied by using columns of antibody-bound Protein A-Sepharose. When GST P1-1 solution containing 0.1 mM GSH was applied to a column previously equilibrated with PBS containing 0.1 mM GSH, over 90 % of activity was recovered in breakthrough fractions, whereas only 10 % of the enzyme amount

Figure 7 HPLC pattern of trypsin digests of GST P1-1

GST P1-1 (2 mg in 0.5 ml of 10 mM Tris/HCl, pH 7.4) was digested with 10 µg of DPCC-treated trypsin at 30 °C for 16 h. Trypsin digests were separated by HPLC as described in the text. The flow rate was 1 ml/min and peptides were detected at 210 nm.

applied was recovered in the same fractions when GSH was not included (Figure 5). In the latter case, SDS/PAGE revealed that most of the GST P1-1 was bound to the column and eluted with 0.1 M glycine/HCl, pH 2.8 (results not shown).

Reactivity of NEM-treated GST P1-1 with the antibody

NEM-treated GST P1-1 was applied to a column of antibody-bound Protein A-Sepharose. The modified enzyme was recovered only in unbound fractions whereas the antibody alone was eluted from the column with 0.1 M glycine/HCl, pH 2.8 (Figure 6a, lane 3), indicating that NEM-treated GST P1-1 did not bind to the antibody. However, immunoblot analysis revealed that the antibody could bind NEM-treated enzyme subjected to SDS/PAGE (Figure 6b, lane 2).

Epitope mapping

To map the epitope of antibody d-1, trypsin digests of GST P1-1 were resolved by HPLC (Figure 7), and the reactivity of each peptide with the antibody was examined by absorption testing. The antibody was only absorbed when the peak-12 peptide was present (Figure 8a). N-Terminal amino acid sequencing indicated the peak to be an 11-amino acid peptide (Table 1). Comparison with the sequence deduced from the GST P1-1 cDNA [35] revealed that it is in the C-terminal portion of the enzyme, stretching from residues 198 to 208. This C-terminal peptide was then synthesized and used for absorption instead of peak 12 (Figure 8b). The antibody was also dose-dependently absorbed with the peptide, and inhibition by the antibody was completely blocked at a concentration of 15 nmol/ml, about 1700-fold higher than the GST P1-1 concentration (0.05 unit/ml = 8.7×10^{-3} nmol/ml).

DISCUSSION

In the present study, one monoclonal antibody to human GST P1-1, d-1, belonging to the IgG_{2a} class, almost completely inhibited enzyme activity (Figure 2). This inhibition occurred when GST P1-1 was previously incubated with the antibody before assay for activity. It did not seem to be due to immunoprecipitation because it did not require the presence of the second antibody and also occurred with papain-treated Fab fragments.

Figure 8 Absorption of antibody d-1 with the peak-12 peptide

(a) Antibody d-1 (0–5 μg) in PBS/0.1% BSA was incubated with the peak-12 peptide of Figure 7 (6 nmol) at 4 °C for 2 h. After addition of 0.2 μg of GST P1-1, the mixtures were further incubated for 16 h at 4 °C, and then assayed for GST activity. ● and ○ denote the results with and without the peptide respectively. (b) Antibody d-1 (5 μg) was incubated with the indicated amounts of the synthetic peptide. Other conditions were the same as for (a). Synthesis of the peptide is described in the text. ● and ○ denote enzyme activities when the antibody was incubated together with the peptide and those when only the peptide was added to the enzyme respectively.

Table 1 N-Terminal amino acid sequence of the peak-12 peptide

N-Terminal amino acid sequencing of the peptide derived from 200 μg of GST P1-1 was performed twice with a gas-phase protein sequencer as described in the text.

Position	Residue	Content (pmol)
1	Tyr	907
2	Val	645
3	Asn	713
4	Leu	1170
5	Pro	852
6	Ile	1270
7	Asn	1340
8	Gly	636
9	Asn	682
10	Gly	1020
11	Lys	802
12	—	—

However, when the enzyme was incubated with the antibody in the presence of 60 μM GSH, no inhibition of its activity was found (Figure 4). This did not significantly alter the GSH concentration of the actual assay mixture (1.006 compared with 1.000 mM). Since the K_m value of GST P1-1 for GSH was 0.1 mM (results not shown), such a small difference in GSH concentration (around 1 mM) was unlikely to be responsible for the difference in activity. The binding of GST P1-1 to the antibody was prevented by 0.1 mM GSH (Figure 5), in line with the K_m value for GSH. These results indicate that GSH, even at a low concentration, represses the binding of the enzyme to the antibody but does not affect antigen-antibody complexes after they have formed.

The epitope of the antibody was demonstrated to be localized in the C-terminal portion of the enzyme between Tyr-198 and Lys-208 (Figures 8a and 8b). Compared with the whole enzyme, about a 1700-fold higher concentration of the peptide was required to absorb the antibody. Such a large difference suggests that the conformation of the C-terminal portion that makes it particularly suitable for binding to the antibody may be partly

lost in the peptide. This result also raises a possibility that not only the C-terminal portion but also other portions of the enzyme may constitute the epitope of the antibody. However, the addition of other peptides resolved by HPLC did not facilitate absorption of the antibody by the C-terminal peptide (results not shown). The antibody weakly reacted with mouse GST-II and only faintly with rat GST-P, as compared with GST P1-1, indicating cross-reactivity with Pi-class forms (Figure 3). A comparison of the amino acid sequences of the C-terminal portions of GST P1-1 and GST-P [36] revealed three different residues (His-198, Leu-199 and Arg-201 for GST-P). Two residues are different between GST P1-1 and GST-II [37] (His-198 and Arg-201 for GST-II). The other nine residues are identical. Thus the N-terminal side (Tyr-198 to Leu-201) of the 11-amino acid peptide may play an important role in the preferential reactivity with GST P1-1.

Previous studies have revealed that the C-terminal sequences of rat Alpha-class subunits incorporate *S*-(*p*-azidophenacyl)-glutathione [38], and a mutant of the human GST A1-1, with 12 residues deleted from its C-terminus, exhibits diminished specific activity [39]. The C-terminal segment is suggested to form an H-site for electrophilic substrates rather than a G-site [38,39]. Recent X-ray-crystallographic studies have indicated that active sites are located in the vicinity of the C-terminal segments in the individual GST forms of the three major classes [9–12]. The C-terminal region of GST P1-1 is reported to exhibit high conformational flexibility which is partly controlled by a hydrogen bond formed between Gly-205 and Tyr-108 [40]. Although our present results revealed that binding of the antibody to the C-terminal region resulted in inhibition of activity with CDNB and GSH, only GSH blocked binding of the antibody to the enzyme and prevented its inhibition of GST activity. CDNB up to a concentration of 10 μM did not influence the inhibition (Figure 4b), and as GST P1-1 was inactivated by 100 μM CDNB, effects at high concentrations could not be evaluated.

Several amino acid residues of the Pi-class forms including GST P1-1 have been identified in the G-site [9,11,13–16,41], but none of them is contained in the C-terminal region between Tyr-198 and Lys-208 recognized by the antibody. Thus it is unlikely that GSH directly binds to the region, thereby inhibiting the interaction with the antibody. In fact, the peptide was not retained by *S*-hexylglutathione-Sepharose or glutathione-

Sepharose (results not shown). Although the Cys-47 residue is not essential for catalytic activity, it is suggested to be located in the vicinity of the G-site [17–19]. The present finding that the NEM-treated enzyme did not bind to the antibody (Figure 6a) suggests the spatial proximity of the C-terminal region and the Cys residue, consistent with crystallographic results [9,11]. After SDS/PAGE, NEM-treated enzyme was bound to the antibody (Figure 6b). In this case, the C-terminal region may be located at a distance from the Cys residue as the result of denaturation by the SDS treatment. Thus the finding that GSH inhibited the enzyme–antibody binding (Figures 4 and 5) raises the possibility of steric hindrance caused by GSH occupation of the G-site. However, the other possibility, that conformation of the C-terminal peptide located in the vicinity of the G-site may be altered by the binding of GSH, cannot be ruled out. The latter is consistent with the high conformational flexibility of the C-terminal region [40] and binding of GSH has indeed been suggested to induce conformational change at or near the G-site [17–19].

In conclusion, these results indicate that the monoclonal antibody described here recognizes the C-terminal region of GST P1–1. This antibody should prove useful for examining the steric configuration of the C-terminal and other regions in the absence of GSH.

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