Glutathione Peroxidase Activity of Glutathione S-Transferase in Rabbit Liver\textsuperscript{1,2)}

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Glutathione peroxidase (GP, EC 1.11.1.9) activity of glutathione S-transferase (GST, EC 2.5.1.18) in rabbit liver was investigated by using hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and cumene hydroperoxide (C-OOH) as substrates for GP and 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate for GST.

The crude extract from rabbit liver was applied to a diethylaminoethyl (DEAE)-cellulose column equilibrated with 10\,mM Tris–HCl (pH 8.0) and the adsorbed proteins were eluted with the same buffer containing 0.5\,mM KCl. The DEAE-non-adsorbed fraction showed 63–87\% of the GST activity of the crude extract and 27–36\% of the GP activity toward C-OOH, but no GP activity toward H\textsubscript{2}O\textsubscript{2}. The DEAE-adsorbed fraction showed 2–11\% of the GST activity, 28–48\% of the GP activity toward C-OOH and 54–87\% of the GP activity toward H\textsubscript{2}O\textsubscript{2}. GST and GP in the DEAE-adsorbed fraction could be completely separated as two different activity peaks by gel filtration. This GP had a molecular weight of about 84000, a value similar to that of the well-known selenium-dependent GPs. However, activities of GST and GP in the DEAE-non-adsorbed fraction were eluted as a single peak on gel filtration. The proteins in the peak had a molecular weight of about 52000, a value corresponding to those of the rabbit hepatic GST forms in the previous study.

The DEAE-non-adsorbed fraction was resolved into at least seven GST activity peaks (R0, R1, R2, Rx, R3, Rz, R4) by carboxymethyl (CM)-cellulose chromatography or at least eight GST activity peaks (I, II, III, IV, VI, VII, VIII, IX) by isoelectric focusing. These peaks, excepting R2 and VII–IX, showed a high GP activity toward C-OOH.

The present study and the previous studies indicate that GST forms composed of Y1 and/or Y3 subunit have a high GP activity toward C-OOH and that these forms account for most of the non-selenium-dependent GP activity in rabbit liver.

Keywords—glutathione; glutathione S-transferase; glutathione peroxidase; substrate specificity; subunit; rabbit liver

Introduction

Glutathione peroxidase (GP, EC 1.11.1.9) was first discovered in erythrocytes\textsuperscript{3)} and later in the liver of various species.\textsuperscript{4)} This enzyme is capable of reducing H\textsubscript{2}O\textsubscript{2} or organic hydroperoxides to the corresponding alcohols, simultaneously oxidizing reduced glutathione (GSH). GP generally requires selenium for the expression of its activity.\textsuperscript{5,6)} Sies et al.\textsuperscript{7)} discovered that perfusion of the liver with H\textsubscript{2}O\textsubscript{2} results in the release of oxidized glutathione. O’Brein and Little\textsuperscript{8,9)} showed that GP effectively reduces lipid peroxides, but catalase does not. These findings suggest that GP should protect tissues from oxidation by hydroperoxides.

Lawrence and Burk\textsuperscript{11)} reported that rat liver contains another GP without activity toward H\textsubscript{2}O\textsubscript{2} and that its activity is not dependent upon selenium. This GP is co-induced with GST in rats fed on a selenium-free diet.\textsuperscript{12)} Prohsaka and Ganther\textsuperscript{13)} discovered that some glutathione S-transferase (GST, EC 2.5.1.18) forms in guinea pig liver have GP activity toward organic hydroperoxides and other properties different from those of selenium-
dependent GP. The rat liver has relatively low non-selenium-dependent GP activities. In contrast, the sheep liver has relatively high non-selenium-dependent GP activities. Deficiencies of selenium and vitamin E lead to hepatic necrosis in rat but not in sheep. Therefore, the non-selenium-dependent GP activity may be important for the protection of tissues from oxidation by hydroperoxides.

GST plays an important role in the detoxication and excretion of xenobiotics. This enzyme can catalyze the conjugation of GSH not only with many exogenous electrophiles but also with some endogenous ones. The most extensive studies on GST have been done on rat liver GST and many forms have been purified to homogeneity. All of them are apparently dimers involving at least five different subunits, having molecular weights of 22000 (Ya), 23000 (Yn), 23500 (Yb1 and Yb2) and 25000 (Yc). Mannervik and Jansson suggested that the non-selenium-dependent GP activity in rat liver is primarily due to three GST forms, 1–1, 1–2 and 2–2 (nomenclature by Jakoby et al.), which are composed of YaYa, YaYc and YcYc subunits, respectively.

Although the rabbit as well as the rat is a very useful laboratory animal for in vivo experiments on the metabolism of xenobiotics, GST in rabbit liver has hardly been investigated except for a few reports. We have recently indicated that seven GST forms (R1a, R1b, R1c, R1d, R2, R3a and R3b) with basic isoelectric points could be purified to homogeneity from rabbit liver, and all of them were apparently dimers involving four different subunits with molecular weights of 24500 (Y1), 25000 (Y2), 26500 (Y3) and 28000 (Y4). Namely, R1a, R1b, R1c, R1d, R2, R3a and R3d are composed of Y1Y1, Y2Y4, Y2Y4, Y2Y4, Y2Y2, Y1Y3 and Y3Y3 subunits, respectively.

The present study was designed to elucidate the following points: 1, the activity ratio of the two kinds of GP in rabbit liver; 2, the involvement of GST in non-selenium-dependent GP activity; 3, the correlation between the GP activity of GST and the subunit composition of GST.

**Experimental**

**Materials**—Diethylaminoethyl (DEAE)- (DE-52) and carboxymethyl (CM)-cellulose (CM-52) were obtained from Whatman Chemical Separation and Sephadex G-75 (superfine) and Pharmalyte (pH 10.5–8 and pH 9–6.5) were from Pharmacia Fine Chemicals. Rabbit phosphorylase b, human transferrin, bovine serum albumin, ovalbumin, α-chymotrypsinogen-A, glutathione reductase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. 1-Chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH) and hydrogen peroxide (H₂O₂) were obtained from Wako Pure Chemical Industries and cumene hydroperoxide (C-OOH) from Nakarai Chemicals. All other chemicals were of the highest purity available.

**Animals**—Female Japanese white rabbits (2.5–3.8 kg) from Sankyo Labo. were fed on a standard diet (100 g/head/d, Clea Japan Inc.) and tap water ad libitum. Rabbits were killed by injecting 10 ml of air into an ear vein. The liver was removed and perfused with ice-cold 0.25 M sucrose in order to eliminate blood.

**Enzyme Assays**—GST activity was spectrophotometrically determined by measuring the rate of conjugation of GSH with CDNB according to the method of Habig et al. One unit of enzyme activity was defined as the amount of enzyme conjugating 1 μmol of substrate per min at 25°C. GP activity was assayed by the procedure described by Tappel with some modifications. The reaction mixture contained 0.25 mM GSH, 0.2 mM C-OOH or 0.2 mM H₂O₂, 0.12 mM NADPH, 0.1 mM ethylenediaminetetraacetic acid (EDTA) and one unit (1 μmol of NADPH oxidized per min) of glutathione reductase per ml in 50 mM Tris–HCl (pH 7.6). The rate of disappearance of NADPH was spectrophotometrically measured at 340 nm and 37°C. One enzyme unit was defined as the amount of enzyme that transformed 1 μmol of NADPH to NADP per min at 37°C.

**Protein Concentration**—Protein concentration was measured at 280 nm for crude extracts and column fractions, while for the purified GST the method of Lowry et al. was used with bovine serum albumin as a standard.

**Activities of GP and GST in Rabbit Liver**—The procedures were carried out at 4°C. Five-gram proteins of five different rabbit livers were separately homogenized with 25 ml of distilled water in a Teflon-glass homogenizer. The homogenates were centrifuged at 20000 × g for 1 h and the supernatants were adjusted to pH 8.0 with 1 M Tris base. These crude extracts (25 ml) were applied to DEAE-cellulose columns (2 × 16 cm) equilibrated with 10 mM Tris–HCl (pH 8.0). After washing of the column with 200 ml of the same buffer, the proteins were eluted with 200 ml of the
above buffer containing 0.5 m KCl. The activities of GST and GP in the crude extracts, and DEAE-non-adsorbed and DEAE-adsorbed fractions were examined.

**CM-Cellulose Chromatography of DEAE-Non-adsorbed Fraction** — The crude extract was prepared from 50 g of rabbit liver as described above and applied to a DEAE-cellulose column (4 x 44 cm) equilibrated with 10 mm Tris-HCl (pH 8.0). The DEAE-non-adsorbed fraction was packed in cellophane tubing and concentrated by dialysis against polyethylene glycol 20000. The concentrated sample was dialyzed against four changes, each of 4 l, of 10 mm potassium phosphate buffer (pH 6.7) for 8 h. Insoluble materials precipitated during the dialysis were removed by centrifugation at 20000 x g for 15 min. The dialyzed sample was applied to a CM-cellulose column (2.8 x 25 cm) equilibrated with 10 mm potassium phosphate buffer (pH 6.7). After washing of the column with about 250 ml of the above buffer, the adsorbed proteins were eluted with a 0—60 mm KCl linear gradient in the above buffer in a total volume of 1.2 l.

**Isoelectric Focusing of DEAE-Non-adsorbed Fraction** — The DEAE-non-adsorbed fraction equivalent to 7 g of liver was applied to an S-hexylglutathione-linked Sepharose 6B column (2 x 5 cm) equilibrated with 20 mm Tris-HCl (pH 8.0) containing 0.2 m KCl. After washing of the column with 100 ml of the same buffer, the adsorbed proteins were eluted with 5 mm S-hexylglutathione and 2.5 mm GSH in the same buffer. The active fractions were combined in cellophane tubing and dialyzed against two changes, each of 5 l, of 1 mm potassium phosphate buffer (pH 6.7) for 12 h. The dialyzed sample was subjected to isoelectric focusing in a 110 ml column. A mixture of Pharmalytes with pH ranges of 10.5—8 and 9—6.5 (3:1, v/v) was used at 1% final concentration as the carrier ampholyte. Isoelectric focusing was performed with a 0—50% sorbitol density gradient at 3 W for 46 h at 2°C. Fractions of 1.5 ml were collected, and their pH values were measured immediately at 2°C.

**Results**

**Activities of GST and GP in Rabbit Liver**

The activities of GST and GP were examined in the crude extracts from five rabbit livers (Table I). Five grams of rabbit liver contained GST activity of 720—1610 units toward CDNB, GP activity of 192—235 units toward C-OOH and GP activity of 60—138 units toward H₂O₂. Most of the GST activity (63—87%) in the crude extract could be recovered in the DEAE-non-adsorbed fraction. The amount of GP activity toward C-OOH in this fraction was roughly equal to that in the DEAE-adsorbed fraction. However, GP activity toward H₂O₂ could not be detected in the DEAE-non-adsorbed fraction and 54—87% of this activity in the crude extract was found in the DEAE-adsorbed fraction.

By Sephadex G-75 gel filtration, GST and GP in the DEAE-adsorbed fraction were completely separated as two different activity peaks, whereas the activities of GST and GP in the DEAE-non-adsorbed fraction were eluted as a single peak (Fig. 1). The molecular weight of GP in the DEAE-adsorbed fraction was estimated to be about 8400 from the elution

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Crude extract</th>
<th>DEAE-non-adsorbed</th>
<th>DEAE-adsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDNB</td>
<td>C-OOH</td>
<td>H₂O₂</td>
<td>CDNB</td>
</tr>
<tr>
<td>A</td>
<td>720</td>
<td>198</td>
<td>127</td>
</tr>
<tr>
<td>B</td>
<td>920</td>
<td>235</td>
<td>138</td>
</tr>
<tr>
<td>C</td>
<td>1610</td>
<td>203</td>
<td>60</td>
</tr>
<tr>
<td>D</td>
<td>1300</td>
<td>192</td>
<td>97</td>
</tr>
<tr>
<td>E</td>
<td>1330</td>
<td>212</td>
<td>96</td>
</tr>
</tbody>
</table>

These values were obtained with 5 g of wet liver. Activities are in units/ml. Recoveries (%) of activity from crude extracts are given in parentheses.
volume. This value is similar to those of the well-known selenium-dependent GPs. The GP in the DEAE-non-adsorbed fraction showed a molecular weight of about 52000, a value corresponding to those of the rabbit hepatic GST forms in the previous study.1)

**CM-Cellulose Chromatography of DEAE-Non-adsorbed Fraction**

The DEAE-non-adsorbed fraction of the rabbit crude extract could be resolved into at least seven GST activity peaks (Fig. 2) as reported previously.1,2) These peaks were designated as R0, R1, R2, Rx, R3, Rz and R4 in order of elution. R2, one of the main GST forms, hardly showed GP activity toward C-OOH, while all of the other GST forms showed high GP activity. Furthermore, GST peaks corresponded to the GP peaks as regards elution volume.

**Isoelectric Focusing of DEAE-Non-adsorbed Fraction**

The DEAE-non-adsorbed fraction could be resolved into at least eight GST activity

**Fig. 1. Sephadex G-75 Gel Filtration of DEAE-Adsorbed and DEAE-Non-adsorbed Fractions**

Panel A, DEAE-adsorbed fraction. Panel B, DEAE-non-adsorbed fraction. Each sample was separately chromatographed on a Sephadex G-75 column (2.7 x 82 cm) equilibrated with 50 mM potassium phosphate buffer (pH 6.7) containing 0.5 M KCl. The eluate was collected in 3.65 ml fractions, which were monitored at $A_{280}$ (-----) for protein concentration and assayed with CDNB (O) for GST activity and with C-OOH (●) and $H_2O_2$ (△) for GP activity. Their molecular weights were estimated with the same column, using rabbit phosphorylase b (94000), human transferrin (80000), bovine serum albumin (66000), ovalbumin (45000) and α-chymotrypsinogen-A (25000) as marker proteins.

**Fig. 2. CM-Cellulose Chromatography of DEAE-Non-adsorbed Fraction**

The CM-adsorbed proteins were eluted with a 0-60 mM KCl gradient (-----) in 10 mM potassium phosphate buffer (pH 6.7). The eluate was collected in 7 ml fractions, which were monitored at $A_{280}$ (-----) for protein concentration and assayed with CDNB (O) for GST activity and with C-OOH (●) for GP activity. No GP activity was found in any fraction when $H_2O_2$ was used as a substrate (not shown on graph).
Fig. 3. Isoelectric Focusing of DEAE-Non-adsorbed Fraction

The sample partially purified by S-hexylglutathione affinity chromatography was subjected to isoelectric focusing. The contents were collected in 1.5 ml fractions, which were monitored at A_{340} (-----) for protein concentration and assayed with CDNB (○) for GST activity and with C-OOH (●) for GP activity. No GP activity was found in any fraction when H$_2$O$_2$ was used as a substrate (not shown). The pH values (▲) in the fractions were measured at 2°C.

<table>
<thead>
<tr>
<th>GST</th>
<th>Molecular weight</th>
<th>Isoelectric point</th>
<th>Subunit composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1a</td>
<td>51000</td>
<td>9.72</td>
<td>Y1Y1</td>
</tr>
<tr>
<td>R1b</td>
<td>51000</td>
<td>8.56</td>
<td>Y2Y4</td>
</tr>
<tr>
<td>R1c</td>
<td>51000</td>
<td>7.64</td>
<td>Y2Y4</td>
</tr>
<tr>
<td>R1d</td>
<td>51000</td>
<td>7.07</td>
<td>Y2Y4</td>
</tr>
<tr>
<td>R2</td>
<td>51000</td>
<td>8.34 (8.1)</td>
<td>Y2Y2</td>
</tr>
<tr>
<td>R3a</td>
<td>51000</td>
<td>10.35 (9.7)</td>
<td>Y1Y3</td>
</tr>
<tr>
<td>R3b</td>
<td>51000</td>
<td>9.95 (9.3)</td>
<td>Y3Y3</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>(1-1)$^a$</td>
<td></td>
<td>YaYa</td>
</tr>
<tr>
<td>III</td>
<td>(3-4)</td>
<td>47000</td>
<td>(8.2)</td>
</tr>
<tr>
<td>IV</td>
<td>(1-2)</td>
<td>47000</td>
<td>(9.6)</td>
</tr>
<tr>
<td>V</td>
<td>(3-3)</td>
<td>46000</td>
<td>(8.9)</td>
</tr>
<tr>
<td>VI</td>
<td>(2-2)</td>
<td>45000</td>
<td>(9.2)</td>
</tr>
</tbody>
</table>

R1a—R1d were purified by isoelectric focusing of R1 fraction separated on CM-cellulose chromatography. R3a and R3b were purified by isoelectric focusing or hydroxylapatite chromatography of R3 fraction separated on CM-cellulose chromatography. $^a$ These values were estimated by gel filtration and the molecular weights of rat GST were taken from the report of Jakoby and Habig. $^b$ Isoelectric point was determined at 2°C with a pH meter for GST fractions obtained by isoelectric focusing in a column. The values in parentheses were determined by isoelectric focusing in an ultrathin-layer polyacrylamide gel plate using a high pH calibration kit (Pharmacia Fine Chemicals). $^c$ The molecular weights of subunits were obtained as 24500 (Y1), 25000 (Y2), 26500 (Y3), 28000 (Y4), 24000 (Ya), 25500 (Yb1, Yb2) and 27500 (Yc) by sodium dodecyl sulfate (SDS)/polyacrylamide-gel electrophoresis. $^d$ The nomenclature is that of Jakoby et al.$^{29}$

peaks and into at least six GP activity peaks by isoelectric focusing, and these were focused at pHs of 10.43—10.55, 10.14, 9.84, 9.47, 8.79, 8.60, 8.33, 7.76, 7.36 and 7.00 (Fig. 3). These activity peaks were designated as I—X in decreasing order of isoelectric point. GP peaks V and X could not be followed as GST peaks. GST peaks I—IV showed high GP activity toward C-OOH, while GST peaks VI—IX showed little GP activity. The GST peaks corresponded to the GP peaks with regard to the fraction focused as well as in CM-cellulose chromatography (Fig. 2). This result suggests that some GST forms may have intrinsic GP activity.
TABLE III. Substrate Specificities of Purified Glutathione S-Transferase Forms

<table>
<thead>
<tr>
<th>GST</th>
<th>GST activity</th>
<th>GP activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDNB</td>
<td>DCNB</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1a</td>
<td>26</td>
<td>N.M.</td>
</tr>
<tr>
<td>R1b</td>
<td>51</td>
<td>N.M.</td>
</tr>
<tr>
<td>R1c</td>
<td>64</td>
<td>N.M.</td>
</tr>
<tr>
<td>R1d</td>
<td>41</td>
<td>N.M.</td>
</tr>
<tr>
<td>R2</td>
<td>91</td>
<td>0.186</td>
</tr>
<tr>
<td>R3a</td>
<td>18</td>
<td>0.044</td>
</tr>
<tr>
<td>R3b</td>
<td>18</td>
<td>0.039</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>22</td>
<td>0.010</td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>1.8</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>0.005</td>
</tr>
<tr>
<td>V</td>
<td>54</td>
<td>3.4</td>
</tr>
<tr>
<td>VI</td>
<td>9</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Activities are in units/mg. DCNB, 1,2-dichloro-4-nitrobenzene; p-NBC, p-nitrobenzyl chloride; BA, benzalacetone; EA, ethacrynin acid; ENPP, 1,2-epoxy-3-(p-nitrophenoxy)propane; N.M., activity was not measured; N.D., no activity was detected with about 0.1—0.4 mg of protein.

Substrate Specificities of Purified Different GST Forms

We have succeeded in purifying seven GST forms from rabbit¹ ² and also from rat liver.³ ⁴ Table II shows the properties of the rabbit GST forms and five authentic rat GST forms. In order to clarify the correlation between GP activity and subunit, we examined the substrate specificities of the purified GST forms. As shown in Table III, all of the rabbit GST forms showed low specific activities toward the conjugating substrates except CDNB, and they could not be distinguished from each other in terms of substrate specificity. The levels of GST activity toward CDNB were in the order of R2 > R1b, R1c, R1d > R1a > R3a, R3b. From this result and the subunit compositions, the subunits dominating GST activity toward CDNB seemed to be Y2 > Y4 > Y1, Y3. In the case of GP activity toward C-OOH, the activities were in the order of R1a > R3a > R3b > R1c > R2 and the dominant subunits were Y1 > Y3 > Y4 > Y2 subunit. In fact, R2 (composed of Y2Y2 subunits) showed the highest specific activity toward CDNB and R1a (composed of Y1Y1 subunits) showed the highest specific activity toward C-OOH.

Rat hepatic GST was different from rabbit hepatic GST in terms of the specificities for the conjugating substrates. In the rat, 1–2 and 2–2 (containing Yc subunit) showed higher specific activity toward EA and 3–4 (containing Yb2 subunit) was most active toward BA, while 3–3 and 3–4 (containing Yb1 subunit) were active toward every substrate, CDNB, 1,2-dichloro-4-nitrobenzene (DCNB), p-nitrobenzyl chloride (p-NBC) and 1,2-epoxy-3-(p-nitrophenoxyl)propane (ENPP). The activity toward CDNB was dominated by Yb1 > Yb2 > Ya > Yc subunit. The GP activity toward C-OOH was dominated by Yc > Ya > Yb2 > Yb1 subunit.

When the subunits of rabbit hepatic GST were compared with those of rat hepatic GST in regard to activities toward CDNB and C-OOH, Y1, Y2, Y3 and Y4 subunits of rabbit GST resembled Yc, Yb1, Ya and Yb2 subunits of rat GST, respectively.

Discussion

As little is known about the correlation between GST and two kinds of GP activities in
rabbit liver, we studied the GP activity of GST in rabbit liver. Assay of GP with C-OOH measures both selenium-dependent GP and non-selenium-dependent GP activities, while the assay with H₂O₂ measures selenium-dependent GP activity alone. CDNB was the most suitable substrate for the purpose of detecting all GST forms.

Gel filtration of the DEAE-adsorbed fraction could separate GP and GST. This GP showed activity toward both C-OOH and H₂O₂, but not toward CDNB. Its molecular weight was similar to those of selenium-dependent GP in rat liver²⁵ and bovine erythrocyte. On the other hand, activities of GST and GP in the DEAE-non-adsorbed fraction were co-eluted in the same fraction on gel filtration. The molecular weight was estimated to be about 52000 and was similar to those of GST forms obtained from other species.¹⁹,³⁶⁻⁴³ These findings suggest that the GP activity in the DEAE-adsorbed fraction is attributable to selenium-dependent GP and that the GP activity in the DEAE-non-adsorbed fraction is dependent upon some GST forms, namely, non-selenium-dependent GP.

We reported that the DEAE-non-adsorbed fraction give four major GST peaks [R₁(R₁a, R₁b, R₁c, R₁d), R₂, R₃(R₃a, R₃b) and R₄] by CM-cellulose chromatography, or eight GST peaks, having pls of 10.95—10.77 (I), 10.35 (II), 9.95 (III), 9.40 (IV), 8.88 (V), 8.48 (VI), 8.36 (VII) and 7.66 (VIII), by isoelectric focusing.² The relationship between GST forms separated by both procedures was indicated to be as follows: I (R₄), II (R₃a), III (R₃b), IV and V (not identified), VI (R₁b), VII (R₂), VIII (R₁c). Furthermore, we indicated that GST peaks I—V contain Y₁ and/or Y₃ subunit and GST peaks VI—VIII contain Y₂ and/or Y₄ subunit.

The present result of CM-cellulose chromatography was in complete agreement with the previous one with regard to the number of GST peaks and the KCl concentrations at which they were eluted. The isoelectric focusing profile was also very similar to the previous one except for the absence of GST peak V and the additional GST peak IX in the present study. Accordingly, GP activity toward C-OOH may be dependent upon Y₁ and/or Y₃ subunit in R₁, R₃ and I—VI. This correlation between GP activity and subunit was confirmed by investigating the substrate specificities of the purified GST forms (Table III). All of the GST forms were inactive toward H₂O₂. Relatively high activity toward C-OOH was observed in GST forms which were composed of Y₁Y₁ (R₁a), Y₁Y₃ (R₃a) and Y₃Y₃ subunits (R₃b). GST forms composed of Y₂Y₂ (R₂) and Y₂Y₄ subunits (R₁b, R₁c, R₁d) had a high specific activity toward CDNB, but a low specific activity toward C-OOH. Therefore, the GST forms containing Y₂ and/or Y₄ subunits may play an important role in GSH-conjugation with xenobiotics. The GST forms containing Y₁ and/or Y₃ subunit, as well as selenium-dependent GP, may be important for the protection of tissues from oxidation by organic hydroperoxides.

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References