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Glutathione S-Transferases in Rat Extrahepatic Tissues: Immunologic Relation to Hepatic Neutral Glutathione S-Transferase in the Diethylaminoethyl-Cellulose-Bound Fraction¹⁾

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Each extract of liver, kidney, heart, lung, small intestine and brain of rat was applied to a diethylaminoethyl (DEAE)-cellulose column (10 mm Tris-HCl, pH 8.0), and glutathione S-transferase (GST) activity in the DEAE-cellulose-unbound and -bound fractions was assayed by using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The ratio of the bound to unbound GST activity differed considerably from tissue to tissue. In lung, kidney, liver and small intestine, the DEAE-cellulose-unbound fraction contained more GST activity than the DEAE-cellulose-bound fraction, but the opposite was the case in heart and brain.

We examined GSTs in the DEAE-cellulose-bound fraction of rat liver in detail. Most GSTs in this fraction had YbYb subunits, and in addition, this fraction contained GSTs having YbYn subunits or YaYa subunits. The antiserum was raised against GST having YbYb subunits (peak II). The double immunodiffusion studies showed that the DEAE-cellulose-bound fraction of each tissue contained GST(s) antigenically similar to peak II. This was confirmed by the result that each GST activity was inhibited by anti-peak II serum on immunotitration.

Keywords—glutathione S-transferase; rat tissue; enzyme purification; rat liver; diethylaminoethyl-cellulose; carboxymethyl-cellulose; substrate specificity; subunit; double immunodiffusion; immunotitration

Introduction

Glutathione S-transferases (GSTs) exist mainly in cytosol. They are a group of enzymes which play an important role in the detoxication and excretion of xenobiotics.²⁾

When the extract of rat liver is applied to a diethylaminoethyl (DEAE)-cellulose column (10 mm Tris-HCl, pH 8.0), the activity of GST is mostly recovered in the DEAE-cellulose-unbound fractions.³⁾ Many studies have been done on GSTs in the DEAE-cellulose-unbound fraction.⁴⁾ Recently some works have been done on GSTs in the DEAE-cellulose-bound fraction.⁵⁾ The DEAE-cellulose-bound fraction was reported to contain mainly GST(s) having YbYb subunits.⁵⁾

In this study, to examine whether or not most activity in each extrahepatic tissue of rat could be similarly recovered in the DEAE-cellulose-unbound fraction, as in the case of liver, we divided GSTs into the DEAE-cellulose-unbound and -bound fractions and assayed GST activity in each fraction by using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The result showed that the DEAE-cellulose-unbound fraction contained more activity than the DEAE-cellulose-bound fraction in lung, kidney and small intestine, as well as liver, but the opposite was the case in brain and heart. Then we produced rabbit antiserum against the purified GST (peak II) having YbYb subunits, which originated in the DEAE-cellulose-bound fraction of rat liver. By using this antiserum, we examined whether the DEAE-cellulose-unbound and -bound fractions of extrahepatic tissues contained GSTs which were antigenically similar to peak II of liver.

Experimental

Materials—Glutathione (GSH), CDNB, *trans*-4-phenyl-3-buten-2-one (*t*-PBO), *p*-nitrobenzyl chloride (*p*-NBC) and *p*-nitrophenethyl bromide (*p*-NPB) were purchased from Wako Pure Chemical Ind., Ltd. 1,2-Epoxy-3-(*p*-nitrophenoxy) propane (ENPP) was from Eastman Kodak Co. 1,2-Dichloro-4-nitrobenzene (DCNB) was from Tokyo Kasei Ind., Ltd. Ethacrynic acid (EA) was from Sigma Chemical Co. Poly(ethyleneglycol) 20000 was from Nakarai Chemical Ltd. DEAE-cellulose and carboxymethyl (CM)-cellulose were from Whatman Chemical Separation Ltd.

Assay Method—GSTs were assayed according to the procedures of Habig *et al.*,³⁾ except that the wavelength and $\Delta\epsilon$ ($\text{mm}^{-1} \text{cm}^{-1}$) used for *p*-NBC were 320 nm and 1.75, respectively. Enzyme activity was monitored through the purification steps by measuring the conjugation of CDNB with GSH. The assay mixture (3 ml) consists of 1 mM CDNB, 1 mM GSH and 0.1 M potassium phosphate buffer, pH 6.5. The rate of increase in absorbance at 340 nm was monitored at 25 °C. One unit of activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product/min at 25 °C. Protein content was determined by measuring the absorbance at 260 and 280 nm,⁶⁾ or by the method of Bradford⁷⁾ with bovine serum albumin as the standard.

A) Division of GSTs in Several Tissues of Rat into the DEAE-Cellulose-Unbound and -Bound Fractions

Male Wistar strain rats were killed by exsanguination. The liver, kidney, lung, heart, small intestine and brain were removed, washed with 0.25 M sucrose and stored at -80 °C. The following procedures were carried out at 4 °C. Twenty percent (w/v) homogenates were prepared in 10 mM Tris-HCl, pH 8.0 (20 °C) (buffer A), with a Teflon-glass homogenizer. The homogenates were centrifuged at $20000 \times g$ for 60 min. The supernatant fractions were adjusted to a concentration of 10 mM Tris and a pH of 8.0. The absorbance at 280 nm of each supernatant fraction was measured to estimate the concentration of protein. Then an amount of each supernatant solution such that the numerical value of A_{280} multiplied by the volume amounted to 50 was taken and applied to a DEAE-cellulose column (DE-23, 1.4×6.5 cm). The column was washed with 100 ml of buffer A and then the bound activity was eluted with 100 ml of buffer A containing 0.5 M KCl. GST activity was assayed with CDNB.

B) Purification of GSTs from Rat Liver

This was done according to the procedures of Habig *et al.*³⁾ with minor modifications. Male Wistar strain rats (body weight 295–355 g) were killed by exsanguination. The following procedures were carried out at 4 °C. Livers (307 g) were washed with 0.25 M sucrose and homogenized in 5 vol of distilled water with a Teflon-glass homogenizer. The homogenate was centrifuged at $20000 \times g$ for 60 min. The supernatant fraction was filtered through a plug of glass wool to remove floating materials. The filtrate was adjusted to a concentration of 10 mM Tris and a pH of 8.0.

Step 1 DEAE-Cellulose (DE-23) Column Chromatography: The liver extract (1.5 l) was applied to a DEAE-cellulose column (DE-23, 9.7×43 cm) equilibrated with buffer A. The column was washed with 6 l of buffer A to remove the unbound GSTs and then the bound GSTs were eluted with a linear gradient composed of 4 l each of 50 and 400 mM KCl in buffer A. Fractions having transferase activity were pooled, concentrated by dialysis against poly(ethyleneglycol) 20000 and dialyzed against four changes, each of 3 l, of buffer A for 22 h. The dialyzed solution was centrifuged at $20000 \times g$ for 20 min to remove the precipitate formed during dialysis.

Step 2 DEAE-Cellulose (DE-52) Column Chromatography: The enzyme solution obtained from DE-23 column chromatography was applied to a DEAE-cellulose column (DE-52, 5.5×39.5 cm) equilibrated with buffer A. The column was washed with 1.23 l of buffer A and then the bound activity was eluted with a linear gradient formed from 3 l each of buffer A and buffer A containing 110 mM KCl. Fractions of 20.5 ml were collected every 10 min. Fractions having transferase activity were pooled, concentrated by dialysis against poly(ethyleneglycol) 20000 and dialyzed against three changes, each of 3 l, of 10 mM potassium phosphate buffer, pH 6.7 (buffer B) for 22 h.

The following steps were the purification procedures for peak II. Other peaks were also purified by means of the following steps, using procedures which were almost the same as those for peak II.

Step 3 CM-Cellulose (CM-52) Column Chromatography: The enzyme solution from DE-52 column chromatography was centrifuged at $20000 \times g$ for 20 min to remove the precipitate formed during dialysis. The supernatant fraction was applied to a CM-cellulose column (CM-52, 2×15 cm) equilibrated with buffer B. The column was washed with buffer B until no further activity was detected and then a linear gradient formed from 250 ml each of buffer B and buffer B containing 80 mM KCl was applied. Fractions having activity were pooled and concentrated by ultrafiltration through a PM-10 membrane.

Step 4 Hydroxylapatite Column Chromatography: The enzyme solution from the unbound fraction on CM-52 column chromatography was supplemented to give final concentrations of 2 mM GSH, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 10% (v/v) glycerin and 50 mM potassium phosphate buffer, pH 6.7. It was then applied to a hydroxylapatite column (2.1×10 cm) equilibrated with 50 mM potassium phosphate buffer, pH 6.7, containing 2 mM GSH, 0.1 mM EDTA and 10% (v/v) glycerin (buffer C). The column was washed with buffer C and then the bound activity was eluted with a linear gradient of 50 to 400 mM potassium phosphate buffer, pH 6.7, containing 2 mM GSH, 0.1 mM EDTA and 10% (v/v) glycerin. Fractions were pooled and concentrated by ultrafiltration through a PM-10 membrane.

Step 5 Sephadex G-75 Column Chromatography: The enzyme solution from hydroxylapatite column chromatography was applied to a Sephadex G-75 column (superfine grade, 2.85 × 89 cm) equilibrated with buffer C and eluted with buffer C.

Gel Electrophoresis—Sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis was done by the method of Laemmli.⁸⁾ Protein samples were subjected to electrophoresis in slab gel containing 12.5% polyacrylamide with SDS/Tris/glycine electrode buffer. Proteins were stained with 0.025% Coomassie brilliant blue R 250–50% (v/v) methanol–10% (v/v) acetic acid in water.

Determination of Molecular Weight—The molecular weight of glutathione S-transferase was estimated by Sephadex G-75 (superfine grade, 2.8 × 92 cm) column chromatography in 0.1 M potassium phosphate buffer, pH 6.7. Bovine serum albumin (M_r 66000 Sigma), ovalbumin (M_r 45000 Sigma) and myoglobin (M_r 17200 Sigma) were used as standards.

Isoelectric Focusing—Purified peak II (3 mg) was applied to a 110 ml isoelectric focusing column. Ampholine carrier ampholytes of pH 3.5 to 10 (final conc. 1% w/v) were used in a 0–50% sucrose density gradient. After electrofocusing at 600 V for the first 20 h and then at 700 V for 24 h at 3 °C, 0.9 ml fractions were collected and the pH was determined at 3 °C. Enzyme activity was assayed with CDNB.

C) Immunological Procedures

Preparation of Antiserum—Antiserum against purified peak II was raised in two rabbits; 0.6 mg each of antigen (peak II total dose 1.8 or 2.4 mg for antiserum obtained on days 40 and 51, respectively; see below) in 1 ml of 10 mM potassium phosphate, pH 6.7, was emulsified with an equal volume of complete Freund's adjuvant and injected intracutaneously into all foot pads and subcutaneously into the backs of two rabbits on days 1, 15, 30 and 43. They were bled on days 40 and 51. The prepared antiserum was heat-inactivated at 56 °C for 30 min and then stored at –80 °C. Immunoglobulin (Ig) G fraction was obtained as follows: anti-peak II serum (9 ml) was applied to a DE-23 column (1.45 × 6.7 cm) equilibrated with 15 mM Tris-phosphate buffer, pH 8.3 and Ig G was eluted with the same buffer.

Double Immunodiffusion—Double immunodiffusion studies were done on 1.1% agar plates by the method of Ouchterlony.⁹⁾

Immunotitration—Immunotitration studies were done by incubating a fixed amount of enzyme with various amounts of antiserum. The reaction mixtures, total volume 0.6 ml, were incubated overnight at 4 °C and then for 2 h at room temperature. Subsequently, goat anti-(rabbit Ig G) serum (20 μ l) was added. The reaction mixtures were again incubated overnight at 4 °C and then they were centrifuged at 20000 × *g* for 20 min to precipitate the antigen-antibody complex. The supernatant solutions were used for enzyme activity determination. The incubation mixtures contained various volumes of a bovine serum albumin solution (57 mg/ml in 0.1 M potassium phosphate, pH 6.7) in order to obtain a constant protein concentration.¹⁰⁾

SDS/Polyacrylamide Gel Electrophoresis of Immunoprecipitates—Each reaction mixture, containing purified peak IIIb (30 μ g in 75 μ l) and Ig G solution (0, 300 or 600 μ l), was incubated overnight at 4 °C and then for 2 h at room temperature, then centrifuged at 20000 × *g* for 15 min. The supernatant solution were used for enzyme activity determination. The precipitates were washed five times with distilled water and then supplemented to give final concentrations of 1% SDS, 1% β -mercaptoethanol, 10 mM Tris-HCl (pH 6.8) and 20% glycerine. The solution was mixed and boiled for 4 min and then subjected to SDS/polyacrylamide gel electrophoresis.

Results

A) Division of GSTs in Several Tissues of Rat into the DEAE-Cellulose-Unbound and -Bound Fractions

As shown in Table I, the ratio of bound to unbound GST activity differed considerably from tissue to tissue. The ratio decreased in the order, heart > brain > lung > kidney > liver > small intestine.

B) Purification of GSTs from Rat Liver

Three different elution patterns of GST activity were obtained with CDNB, DCNB and *t*-PBO as substrates (Fig. 1). The group of GSTs that could be bound by DEAE-cellulose (DE-52, 10 mM Tris-HCl, pH 8.0) was found to be resolved into at least four peaks under the conditions of a 0–110 mM KCl linear gradient and a gradient solution of 6.4 times the volume of the column bed. The peaks were named peaks I, II, III and IV. These peaks had little activity toward ENPP or *p*-NPB. When each peak obtained from the DE-52 column was subjected to CM-52 column chromatography, the activity of peak II mostly passed through the CM-52 column, but peak I, III and IV were each subdivided into unbound and bound

TABLE I. DEAE-Cellulose (DE-23) Column Chromatography (10 mM Tris-HCl, pH 8.0) of GST in Six Tissues

	Activity (units: $\mu\text{mol}/\text{min}$)			Starting material	Recovery (%)	Ratio (B/UB)	Activity (units/g tissue)
	UB ^{a)}	B ^{b)}	Total (UB+B)				
Liver	10.4	1.1	11.5	18.2	63	0.11	54
Kidney	1.61	0.21	1.82	2.02	90	0.13	4.9
Heart	0.15	0.52	0.67	0.80	84	3.47	1.0
Lung	0.36	0.21	0.57	0.97	59	0.58	2.0
Small intestine	1.06	0.06	1.12	0.80	140	0.06	3.0
Brain	0.50	1.00	1.50	1.46	103	2.00	1.4

Enzyme activity was measured by using 1 mM CDNB and 1 mM GSH as substrates. a) DE-23-unbound fraction. b) DE-23-bound fraction.

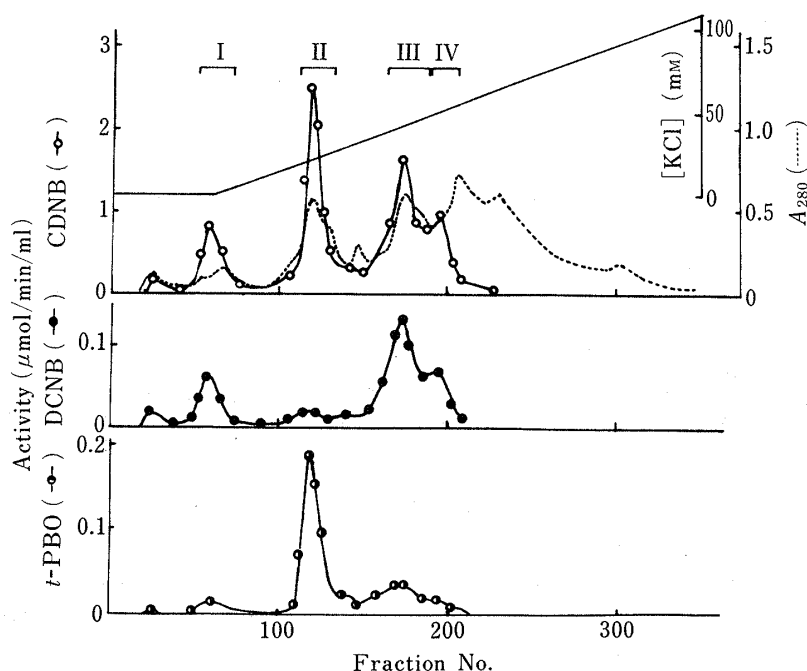


Fig. 1. Elution Pattern of Rat Liver GST from a DEAE-Cellulose Column

—○—, activity toward CDNB; —●—, activity toward DCNB; —●—, activity toward *t*-PBO; ·····, absorbance at 280 nm.

fractions. The CM-52-unbound and -bound fractions were named "a" and "b," respectively. On Sephadex G-75 gel filtration, the activities of peaks Ia—IVb showed a single peak in each case. On hydroxylapatite column chromatography, the activities of peak Ib, II, IIIb and IVb were each eluted as a single sharp peak at approximately 150 mM potassium phosphate concentration in the gradient, while the activity of peak IVa was eluted as a single broad peak at 170 mM and the activity of peak IIIa appeared as three overlapping peaks (150, 160, 190 mM). Each purified sample was stored frozen at -80°C in potassium phosphate buffer, pH 6.7, containing 2 mM GSH, 0.1 mM EDTA and 10% glycerin. The concentration of potassium phosphate buffer was not constant, because if the final purification step was hydroxylapatite column chromatography, the sample was stored frozen at the potassium phosphate concentration at which it was eluted. Each stored sample was dialyzed against 10 mM potassium phosphate buffer, pH 6.7, and applied again to a CM-52 column. GST activity of the CM-52-unbound fraction (a fraction) was again recovered in the CM-52-

TABLE II. Substrate Specificities of Rat Liver GST
(Peaks Ia, Ib, II, IIIa, IIIb, IVa and IVb)^{a)}

Substrate	Specific activity ($\mu\text{mol}/\text{min}/\text{mg protein}^{\text{b)}$)						
	Ia	Ib	II	IIIa	IIIb	IVa	IVb
CDNB	6.37	6.91	7.48	2.04	21.8	6.49	2.36
DCNB	0.494	0.614	0.005	0.189	1.29	0.369	0.039
<i>t</i> -PBO	0.290	0.142	1.12	0.238	0.332	0.252	0.012
<i>p</i> -NBC	5.16	4.76	10.52	3.15	3.87	3.03	0.28
EA	0.07	0	0.158	0.062	0.261	0.139	4.17

a) The samples used were the enzyme fractions obtained from the final step. b) Protein content was determined by the method of Bradford. c) Zero activity indicates no significant enzymatic reaction under the conditions used.

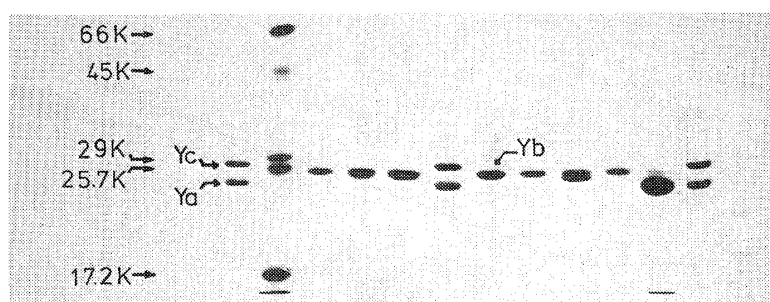


Fig. 2. SDS/Polyacrylamide Gel Electrophoresis of Peaks Ia, Ib, II, IIIa, IIIb, IVa and IVb from Rat Liver

The slots, from left to right are GST B, standards, peak Ia, Ib, II, GST B, GST A, peak IIIa, IIIb, IVa, IVb and GST B.

Standards are bovine serum albumin (M_r 66000), ovalbumin (M_r 45000), carbonic anhydrase (M_r 29000), α -chymotrypsinogen-A (M_r 25700) and myoglobin (M_r 17200).

unbound fraction, but on this rechromatography, the CM-52-bound fraction (b fraction) did not bind to the CM-52 column.

Substrate Specificities—Table II shows the substrate specificities of peaks Ia to IVb at the final step of purification. All the GSTs obtained showed different substrate specificities. Thus, peak II had low activity toward DCNB and high activities toward *t*-PBO and *p*-NBC. Peak IIIb had high activities toward CDNB and DCNB. Peak IVb had high activity toward EA. However, peak Ia and Ib had similar substrate specificities.

SDS/Polyacrylamide Gel Electrophoresis—As shown in Fig. 2, peaks Ia, Ib, II, IIIa and IVa contained one band corresponding to YbYb subunits, but peak IIIb contained two bands with equal concentrations. The one with larger molecular weight migrated to the position of Yb subunit and the other migrated between Ya and Yb subunits. The main band of peak IVb corresponded to YaYa subunits. The calculated molecular weights of Ya, Yb and Yc subunits were 23000, 25000 and 27000, respectively. The molecular weight of the smaller band of peak IIIb was 24500.

Determination of the Molecular Weight—On Sephadex G-75 gel filtration of peak II, a single activity peak was eluted a little behind the ovalbumin peak and its molecular weight was estimated to be about 44000.

Isoelectric Focusing—Purified peak II had a single sharp activity peak, which was located at pH 7.4.

C) Immunological Studies

Antiserum raised against peak II reacted with GSTs which had Yb subunit, but did not react with GSTs which had YaYa, YaYc or YcYc subunits. The precipitin line of peak II with

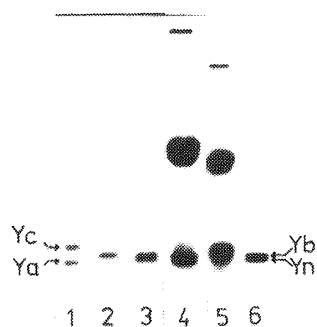


Fig. 3. SDS/Polyacrylamide Gel Electrophoresis of the Immunoprecipitate of Peak IIIb

1, GST B; 2, peak II; 3, 6, peak IIIb; 4, immunoprecipitate (peak IIIb + anti-peak II Ig G); 5, immunoprecipitate (anti-peak II Ig G + anti-rabbit Ig G serum).

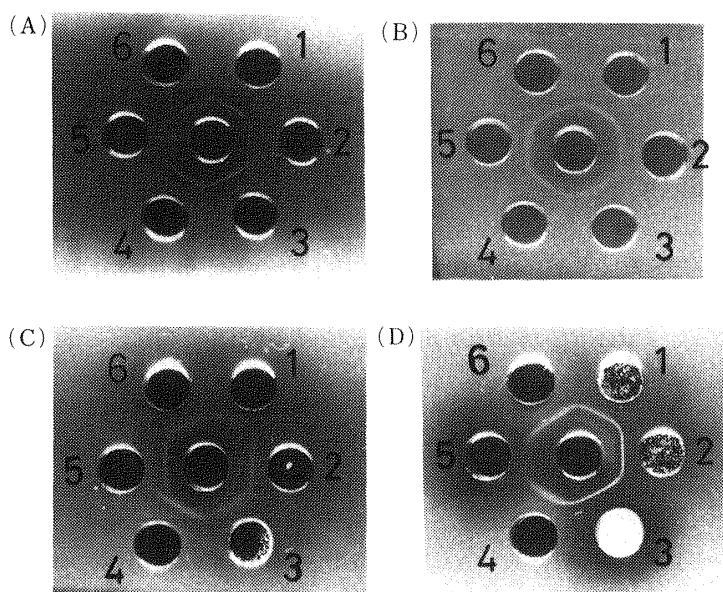


Fig. 4. Double Immunodiffusion of GST

Each center well contained anti-peak II serum. (A) 1, 3, GST C; 2, 5, GST A; 4, peak II; 6, GST A + GST C. (B) 1, 5, peak II; 2, GST C; 3, GST A + GST C; 4, 6, peak IIIb. (C) (D) DEAE-cellulose unbound (C) and bound (D) fractions of GSTs from six tissues. 1, heart; 2, kidney; 3, lung; 4, liver; 5, brain; 6, small intestine.

anti-peak II serum fused smoothly with the line of GST C and the two formed a spur with the line of GST A (Fig. 4A). The activity of peak IIIb was completely inhibited by anti-peak II Ig G and two bands of equal concentration corresponding to the original bands of peak IIIb were obtained on SDS/polyacrylamide gel electrophoresis of immunoprecipitates between peak IIIb and anti-peak II Ig G (Fig. 3). In a double immunodiffusion study, peak IIIb gave a single precipitin line, which fused smoothly with the line of peak II or GST C (Fig. 4B). The activity of peak IVb (YaYa) was not inhibited by anti-peak II serum.

On double immunodiffusion, each DE-23-bound fraction from the six tissues gave a precipitin line which fused smoothly with the others (Fig. 4D) and with the line of peak II or GST C (data not shown). The DE-23-unbound fraction gave one or two precipitin lines (Fig. 4C). The inside line fused with the line of GST A and the outside line fused with the line of peak II or GST C (data not shown). The DE-23-unbound fractions of liver, lung and small intestine each gave two precipitin lines, that of brain gave the inside line, and those of kidney and heart each gave the outside line.

Immunotitration studies showed that each tissue contained GST(s) which were antigenically similar to peak II.

Discussion

Mannervik and Jensson,^{5c)} Reddy *et al.*^{5d)} and Friedberg *et al.*^{5g)} reported that the DEAE-cellulose-bound fraction of rat liver contained only a GST which had YbYb subunits, but Hayes and Chalmers^{5f)} reported that it contained five GSTs (GST Q, R, S, T, U), which could be resolved by using PBE 94 chromatofocusing, and that GSTs Q, R, T, U had YbYb subunits, while GST S had YbYn subunits. Our result, like Hayes and Chalmers' result, suggested that there are many GSTs in the DEAE-cellulose-bound fraction. Though the possibility that this was an artifact could not be excluded, on the basis of the differences of substrate specificities and subunits, we considered that the DEAE-cellulose-bound fraction probably contained many GSTs. In our study, in addition to peak II, peaks Ia, Ib, IIIa and IVa had YbYb subunits. The GST having YbYb subunits which was reported by Mannervik and Jensson, Reddy *et al.*, and Friedberg *et al.* may be peak II or a mixture of GSTs from among peaks Ia, Ib, II, IIIa and IVa, because all of them have YbYb subunits and we could not clearly resolve them in our early experiments. On the other hand, peak IIIb had two bands of equal concentration. These two bands were probably identical with the bands of GST S reported by Hayes and Chalmers. In addition, peak IVb had YaYa subunits, though its substrate specificity differed from that of ligandin, which also had YaYa subunits. Recently, Boyer *et al.*¹¹⁾ reported that "Ya" subunit could be resolved into Ya₁ and Ya₂. Therefore, rat liver may contain some GSTs having "Ya" subunits and peak IVb may be a new GST different from ligandin. Further studies are needed on this GST (peak IVb) having YaYa subunits.

Peaks Ib, IIIb and IVb which had been purified and stored frozen at -80°C in potassium phosphate buffer, pH 6.7, containing 2 mM GSH, 0.1 mM EDTA and 10% glycerin, did not bind to a CM-52 column on rechromatography. The cause of this phenomenon remains unexplained. Purification might cause a change of binding ability. On the other hand, Fjellstedt *et al.*¹²⁾ reported that when GST E was stored frozen in the presence of 30% glycerin and 5 mM GSH (in the absence of EDTA), its pI and activity decreased. Our samples might have changed similarly during storage. The possibility that a large quantity of peak IIIb (YbYn) or peak IVb (YaYa) was present in peak IIIa (YbYb) or peak IVa (YbYb), respectively, on the first CM-52 column chromatography can be ruled out, because peak IIIa and peak IVa did not have Yn and Ya subunit, respectively, on SDS/polyacrylamide gel electrophoresis. On the other hand, peak Ia and peak Ib had YbYb subunits and similar substrate specificities. Therefore, peak Ia might be identical with peak Ib. Further study is necessary on this phenomenon.

As shown in Table I, the ratio of the DEAE-cellulose-bound to -unbound activity differed from tissue to tissue in the rat. Human GSTs as well as those of the rat have tissue specificity. Koskelo¹³⁾ reported that the transferase activities of human liver and kidney consisted mainly of basic enzymes, whereas those of lung, spleen and placenta each consisted virtually of a single acidic enzyme which seemed to be closely related or even identical in the three tissues. The transferase activity of liver seems to consist mainly of basic enzymes in rat,³⁾ mouse,¹⁴⁾ guinea pig,¹⁵⁾ hamster,¹⁶⁾ chicken,¹⁷⁾ monkey¹⁸⁾ and human.¹⁹⁾ In order to explain the cause and the significance of such tissue-specific distributions of acidic, neutral and basic GST, further studies of GSTs in various tissues and species are needed.

In immunodiffusion studies, GST A and peak II (GST C) formed different precipitin lines with anti-peak II serum (Fig. 4A). We designated precipitin lines which fused smoothly with that of GST A and that of peak II (GST C) as GST A type and peak II (GST C) type, respectively. GSTs in the DEAE-cellulose-bound fraction of each tissue which react with anti-peak II serum formed a peak II type precipitin line. The precipitin line of the DEAE-cellulose-bound fraction of brain was fainter than those of other tissues, though a similar amount of

enzyme activity was contained in each well. This suggests that GST in the DEAE-cellulose-bound fraction of brain may be somewhat different from that of the liver. Among GSTs in the DEAE-cellulose-unbound fraction, those from the liver, lung and small intestine formed precipitin lines of both GST A type and peak II (GST C) type, while that of the brain gave a GST A type line, and those of kidney and heart gave peak II (GST C) type lines. The results of immunotitration confirmed that every tissues examined contained GSTs which were antigenically similar to peak II. Further studies on GSTs in the DEAE-cellulose-unbound and -bound fractions of extrahepatic tissues are planned.

It should be noted that we used bovine serum albumin in order to obtain a constant protein concentration in immunotitration studies. We found that bovine serum albumin increased GST activity to different extents from isomer to isomer of GST. The increase of GST activity of ligandin was greater than that of any other isomer, being up to about 300%. A study on this activation by bovine serum albumin is also planned.

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