Soluble Forms of γ-Glutamyltransferase in Human Adult Liver, Fetal Liver, and Primary Hepatoma Compared

P. Selvaraj and K. A. Balasubramanian

We isolated the soluble forms of γ-glutamyltransferase (EC 2.3.2.2; γ-GT) from adult and fetal human liver and primary hepatoma and compared their properties. The $K_m$ value for L-γ-glutamyl-p-nitroanilide and glycglycine, the $K_i$ for antithrombin, and the pH optimum were identical for the enzyme from all three sources. Nor were significant differences observed among the three in their heat stability, inhibition by serine and borate, or ability to transfer the γ-glutamyl moiety to various amino acids and dipeptides. Unlike membrane-bound γ-GT, the soluble form from all three sources entered polyacrylamide gel and showed identical electrophoretic mobilities. Treatment with neuraminidase decreased the electrophoretic mobilities to a similar extent. The relative molecular mass of the enzyme from each of the three sources is about 84 000. Immuno- and immunoprecipitation of γ-GT from the three sources by antibody to fetal liver γ-GT followed an identical pattern. γ-GT from fetal liver and hepatoma differed significantly from that of adult liver in affinity for wheat-germ agglutinin and Ricinus communis agglutinin (RCA-120). In many of the properties studied, soluble γ-GT resembles the papain-digested form of membrane-bound γ-GT.

Additional Keyphrases: enzyme activity • cancer • membrane-bound vs soluble form of enzyme, and possible relation

The activity of γ-glutamyltransferase [γ-GT; (5-glutamyl)-peptide:amino-acid 5-glutamyltransferase, EC 2.3.2.2] is increased in most hepatobiliary diseases, including malignancy of the liver (1). In rat hepatoma and fetal liver the activity of γ-GT reportedly is 100-fold that of adult liver (2). Reports from our laboratory (3) and others (4, 5) show that in human fetal liver and primary hepatoma, the activity of γ-GT is three- to 13-fold that of adult liver. Sobiech and Szewczuk (6) described a variant form of γ-GT found during development of human intestine. Köttgen et al. (7) reported the presence of a fetal form of γ-GT in rat fetal liver and intestine but not in adult rat liver. Therefore, identification of such a variant form of γ-GT associated with the development of human liver and primary hepatoma might serve as a useful diagnostic tool in the differential diagnosis of human primary hepatomas.

γ-GT exists in tissues as membrane-bound and “soluble” forms (8). The membrane-bound form can be solubilized by use of detergents such as Triton X-100 and deoxycholate or by treatment with papain (9). The membrane-bound γ-GT has been purified and extensively characterized in various tissues (9-11), but relatively little information is available on soluble γ-GT. Earlier, we reported (3) that the membrane-bound γ-GT in human adult liver, fetal liver, and hepatoma exhibits identical catalytic properties. Fujisawa et al. (12) reported a soluble form of this enzyme in human primary hepatoma and in sera of patients with hepatoma, which is immunologically identical to the soluble form of γ-GT in fetal liver but differs from the enzyme in normal serum. However, they failed to compare the soluble γ-GT from adult liver with that of hepatoma. This prompted us to study and compare the properties of soluble γ-GT in human adult liver, fetal liver, and hepatoma, to see whether there is any alteration in γ-GT during oncologic development of liver and, if so, whether this would be useful in the diagnosis of hepatoma.

Materials and Methods

Reagents

Phenyl-Sepharose CL-4B was from Pharmacia Fine Chemicals, Uppsala, Sweden, and DEAE-cellulose (DE-52) from Whatman Ltd., Maidstone, U.K. L-γ-Glutamyl-p-nitroanilide, glycglycine, reduced glutathione, dipeptides, bovine serum albumin, wheat germ agglutinin (WGA), Ricinus communis lectin (castor bean, RCA-120), and tris(hydroxymethyl)aminomethane (Tris) were from Sigma Chemical Co., St. Louis, MO 63178. Neuraminidase (EC 3.2.1.18) of Vibrio cholerae was from Boehringer, Mannheim, F.R.G. All the chemicals used in polyacrylamide gel and Triton X-100 were from Eastman Kodak Co., Rochester, NY 14650. We prepared concanavalin A (Con A) from Canavalia ensiformis (jack beans) as described (13). Soybean agglutinin (SBA) was from Hy-gro Chemical (P) Ltd., Calcutta, India. Anththrin was a generous gift from Dr. S. Minato, Fermentation Research Laboratories, Sanky Co. Ltd., Tokyo, Japan. All other chemicals used were AR grade.

Enzyme Assay and Unit

The enzyme was assayed by a modification of the method of Szasz (14). We added 50 μL of partly purified γ-GT in Tris HCl buffer (50 mmol/L, pH 7.5) to 0.75 mL of substrate mixture and incubated for 2 h at 25 °C. The final concentration of each substance in the incubation mixture was, per liter, 200 mmol of Tris HCl buffer (pH 8.3), 4 mmol of L-γ-glutamyl-p-nitroanilide, and 40 mmol of glycglycine. We stopped the reaction by adding 0.5 mL of a 105 g/L solution of trichloroacetic acid, and measured the absorbance at 405 nm in a Vitatron Colorimeter. During the purification procedure, the enzyme was assayed as described (3).

One unit (U) is the amount of enzyme that converts 1 μmol of the substrate per minute at 25 °C (i.e., not corrected to 30 °C). Specific activity is expressed as units per milligram of protein. Protein was estimated by the method of Lowry et al. (15), with bovine serum albumin as standard.

Procedures

Electrophoresis on polyacrylamide gel. Electrophoresis was carried out according to Davis (16) on polyacrylamide (70 g/L) slab gels. γ-GT was located on the gels as described (17), by using L-γ-glutamyl-p-nitroanilide as substrate.

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Nonstandard abbreviations: γ-GT, γ-glutamyltransferase; WGA, wheat-germ agglutinin; SBA, soybean agglutinin; Con A, concanavalin A; RCA-120, castor-bean lectin.

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Neuraminidase treatment. We incubated 1 mg of partly purified enzyme with 0.2 U of neuraminidase (EC 3.2.1.18) at 30 °C for 6 h in Tris HCl buffer (50 mmol/L, pH 7.5; buffer A). Controls were subjected to the same treatment, but without neuraminidase.

Immunological studies. For immunoinhibition and immunoprecipitation studies we used rabbit antibody to membrane-bound γ-GT that had been purified from human fetal liver. The γ-GT was purified from human fetal liver by a procedure involving deoxycholate extraction of membrane-bound γ-GT, precipitation with acetone and ammonium sulfate, chromatography on Phenyl-Sepharose CL-4B, digestion with papain (EC 3.4.22.2) and preparative electrophoresis on polyacrylamide gel. We mixed 300 μg of this purified γ-GT with Freund’s complete adjuvant and injected it subcutaneously into a six-month-old rabbit. On the 22nd day after the first injection we injected 150 μg of the enzyme subcutaneously, without adjuvant, as booster. On the seventh, eighth, and ninth day after this booster injection, the rabbit was bled, and IgG from the antiserum was purified (18) and used for inhibition and precipitation of γ-GT. As a control, IgG was also purified from non-immune rabbit serum.

We mixed 4 mU of partly purified soluble γ-GT with 0.1 mL of various concentrations of antiserum to fetal liver γ-GT (IgG), incubated this at 4 °C for 16 h, re-mixed, and took a 50-μL aliquot for inhibition studies. To the rest of the incubation mixture we added 20 μL of a 100 g/L formaldehyde-fixed Staphylococcus aureus (Cowan I) bacterial suspension (19) and centrifuged at 15 300 × g in a Beckman/Spinco Microfuge (Model 152A) for 5 min to remove the enzyme–antibody complex (20). The supernate was assayed for γ-GT activity. The enzyme–antibody complex was completely precipitated in all the experiments with the amount of bacterial suspension used.

We performed three types of control experiments. In the first and second, the antibody was replaced by non-immune IgG and buffer A, respectively. In the third, the IgG and bacterial suspension were replaced by buffer A.

Lectin-binding studies. To a constant amount of enzyme, various concentrations of soluble lectins were added and incubated for 24 h at 4 °C. After incubation, the mixture was centrifuged at 15 300 × g in the Microfuge for 10 min. The supernate obtained was assayed for γ-GT activity. We varied the ratio of protein to lectin from 1:0.25 to 1:5. The controls were subjected to the same treatment, but without lectins. For Con A, the incubation mixture was buffer A containing 0.1 mol of NaCl and 0.1 mol each of CaCl2 and MgCl2 per liter. For WGA and SBA the incubation mixture was buffer A containing 50 mmol of NaCl per liter, and for RCA-120 buffer A containing 0.1 mol of NaCl per liter.

Dissociation of lectin–enzyme complex by specific sugars. The enzyme was precipitated by incubating it with various lectins as described above. The concentration of lectin used was twice that of the enzyme protein for the Con A and RCA-120 experiments and fivefold greater for WGA. The precipitate obtained after centrifugation was suspended in 50 μL of various concentrations of sugars in buffer A and incubated for 2 h at 4 °C (for WGA and RCA-120) or 25 °C (for Con A). After incubation, we centrifuged the mixture as described before (15 300 × g) and assayed the supernate for γ-GT activity.

The sugars used were α-methyl-D-mannoside (10 to 100 mmol/L), N-acetylglucosamine (1 to 100 mmol/L), and lactose (1 to 100 mmol/L) in buffer A for dissociating the lectin–enzyme complexes formed by Con A, WGA, and RCA-120, respectively.

Determination of molecular mass. Relative molecular mass was estimated by gel filtration (21) on Sephadex G-150 equilibrated with Tris HCl buffer (50 mmol/L, pH 8.0) containing 0.15 mol of NaCl per liter.

Isolation of soluble γ-GT from human adult liver, fetal liver, and primary hepatoma. Normal adult liver, pooled fetal livers, and primary hepatoma of human—all of which had been stored at −20 °C—were used for enzyme purification. The fetal livers were collected from human fetuses at 16 to 30 weeks of gestation. Unless otherwise stated, γ-GT was isolated at 4 °C.

The livers were homogenized for 3 min at high speed in a Waring Blender with 10 volumes of a solution containing, per liter, 80 mmol of MgCl2 and 0.75 mmol of NaOH. This homogenate was then stirred for 2 h and centrifuged (40 000 × g, 30 min). The supernate was taken as the "soluble" fraction (12). To the supernate we added solid (NH4)2SO4 (to a final concentration of 250 g/L), stirred for 30 min, centrifuged, and discarded the pellet. The (NH4)2SO4 concentration in the supernate was further increased to 800 g/L, and it was stirred for 30 min, then centrifuged again (12 000 × g, 30 min). The resulting pellet was dissolved in Tris HCl buffer (50 mmol/L, pH 7.0) and dialyzed against the same buffer. The dialyzate was loaded on a DE-52 column equilibrated with the buffer used for dialysis and the unbound proteins were washed out with more of the same buffer. The bound enzyme was eluted as a single peak with dialysis buffer containing 0.2 mol of NaCl per liter. The active fractions were pooled, dialyzed against buffer A, and loaded on a Phenyl-Sepharose CL-4B column equilibrated with buffer A. The unbound γ-GT that eluted was collected and concentrated by use of Aquacide II (Calbiochem, San Diego, CA 92112). The concentrated enzyme was dialyzed against buffer A and used for all studies described unless otherwise stated.

Results

Enzyme Characterization

Purification. Storage of tissue at −20 °C did not result in any significant loss of γ-GT activity. The specific activities of the γ-GT in homogenates of adult liver, fetal liver, and primary hepatoma were 0.015, 0.096, and 0.183 kU/g, respectively. The proportions of γ-GT recovered in the 40 000 × g supernates of homogenates of adult or fetal liver and hepatoma were 7, 5, and 7%, respectively. Because of the small amount of enzyme in the supernate we were only able to partly purify it. The enzyme obtained from adult liver, fetal liver, and hepatoma in the final step of purification showed a specific activity of 0.055, 0.07, and 0.146 kU/g with 26, 92, and 39% recoveries, respectively, from the 40 000 × g supernate.

Kinetic constants and catalytic properties. As shown in Table 1, the pH optimum for γ-GT from adult liver, fetal liver, or hepatoma was similar for transpeptidation and autotransfer reactions. The enzymes from all the three sources exhibited a similar Km value for L-γ-glutamyl-p-nitroanilide and glycyglycine and Kl for antithion. Amino acids and dipeptides as γ-Glutamyl acceptors. Table 2 shows the activity of γ-GT when glycyglycine was replaced by various amino acids and dipeptides. Of the amino acids we tested, cystine and glutamine were good acceptors. Other amino acids (not shown in the Table) had no significant influence on enzyme activity. Of the dipeptides tested, glycyglycine and histidylglycine acted as good acceptors. There was no significant difference observed among the γ-GTs from the three sources.

Influence of metal ions and EDTA. Monovalent cations—Na+, K+, Li+, Cs+, and NH2H (up to 150 mmol/L)—did not influence the enzyme activity. Of the divalent cations we tested, Mn2+, Zn2+, and Fe2+ (each at 5 mmol/L) showed 80, 47, and 54% inhibition, respectively, whereas Ca2+ and Mg2+
had no influence on enzyme activities. We observed no difference in the behavior of the enzyme from each of the three sources towards metal ions. EDTA at 5 mmol/L did not influence enzyme activity.

Effect of serine and borate. Figure 1 illustrates the inhibition pattern of γ-GT activity by various concentrations of serine plus borate. Serine and borate, each at 15 mmol/L, in the reaction mixture, completely inhibited the enzyme activity from all three sources. Borate (15 mmol/L) alone had no influence on the enzyme activity, whereas serine alone (15 mmol/L) inhibited γ-GT activity by about 20%.

Effect of urea, sodium dodecyl sulfate, iodoacetamide, and p-hydroxymercuribenzoate. Inclusion of urea (1 mol/L), iodoacetamide (1 mmol/L), or p-hydroxymercuribenzoate (0.1 mmol/L) in the assay mixture did not affect the enzyme activity from any of the three sources. Activity of the enzyme from any of the three sources was inhibited by 30% by iodoacetamide (10 mmol/L) and 100% by sodium dodecyl sulfate (5 g/L) in the assay mixture.

Thermal stability. Heat-inactivation studies showed that the enzyme from all three sources was inactivated at 58 °C within 10 min and that the addition of reduced glutathione (20 mmol/L) protected γ-GT against heat inactivation for up to 50 min.

Molecular mass. Figure 2 shows results of the molecular mass determination of γ-GT by gel filtration. The estimated relative molecular mass of γ-GT from all the three sources was identical, about 84,000. The relative molecular mass of the papain-digested form of membrane-bound γ-GT from adult liver was 82,000.

Electrophoretic mobility. The partly purified γ-GT from all the three sources entered the polyacrylamide gel and electrophoresed as a single band with mobility identical to that of the papain-digested form of membrane-bound γ-GT from human adult liver (Figure 3). Treatment with neuraminidase decreased the mobility of γ-GT from all the three sources to

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### Table 1. Kinetic Constants for Soluble γ-GT from Adult Liver, Fetal Liver, and Hepatoma

<table>
<thead>
<tr>
<th>Properties</th>
<th>Adult liver</th>
<th>Fetal liver</th>
<th>Hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH optimum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ-Glutamyl transfer reaction</td>
<td>8.4</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Autotransfer reaction</td>
<td>8.8</td>
<td>8.8</td>
<td>8.8</td>
</tr>
<tr>
<td>$K_m$ value, mol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-γ-Glutamyl-p-nitroanilide</td>
<td>1.17 $\times 10^{-3}$</td>
<td>1.0 $\times 10^{-3}$</td>
<td>1.05 $\times 10^{-3}$</td>
</tr>
<tr>
<td>Glycylglycine</td>
<td>12.65 $\times 10^{-3}$</td>
<td>12.5 $\times 10^{-3}$</td>
<td>12.5 $\times 10^{-3}$</td>
</tr>
<tr>
<td>$K_i$ for anthglin</td>
<td>12.0 $\times 10^{-6}$</td>
<td>15.0 $\times 10^{-6}$</td>
<td>14.0 $\times 10^{-6}$</td>
</tr>
</tbody>
</table>

Tris HCl buffers in the pH range 7.0–9.0 were used to determine pH optima. For autotransfer reactions glycylglycine was omitted from the usual reaction mixture. The $K_m$ values were determined by the double-reciprocal method. The $K_i$ for anthglin was determined by Dixon plots with 1, 2, and 4 mmol/L concentrations of L-γ-glutamyl-p-nitroanilide.

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### Table 2. Relative Effectiveness of Some Amino Acids and Dipeptides as γ-Glutamyl Acceptors

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Relative enzyme activity, %</th>
<th>Adult</th>
<th>Fetal</th>
<th>Hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Cystine</td>
<td>338</td>
<td>400</td>
<td>371</td>
<td></td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>169</td>
<td>191</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>62</td>
<td>64</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>L-Methionine</td>
<td>113</td>
<td>127</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Dipeptides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly-Gly</td>
<td>458</td>
<td>568</td>
<td>580</td>
<td></td>
</tr>
<tr>
<td>Gly-Pro</td>
<td>142</td>
<td>148</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>His-Ala</td>
<td>220</td>
<td>247</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>His-Gly</td>
<td>350</td>
<td>420</td>
<td>430</td>
<td></td>
</tr>
</tbody>
</table>

To the usual reaction mixture, we added various amino acids and dipeptides (20 mmol/L) instead of glycylglycine.
Fig. 3. Results of polyacrylamide slab-gel electrophoresis of \( \gamma \)-GT

A, F, and H represent soluble form of adult liver, fetal liver, and hepatoma \( \gamma \)-GT, respectively. 2 and \( \bar{I} \) represent the \( \gamma \)-GT before and after neuraminidase treatment. 5, 4, and 3 show the mobility of adult liver membrane-bound \( \gamma \)-GT before papain digestion, after papain digestion, and treated with neuraminidase after papain digestion, respectively. Other experimental details are given in the text.

a similar extent, without affecting enzyme activity. In contrast to the soluble form, the undigested membrane-bound form remained in the origin of the gel.

Immunoinhibition and immunoprecipitation studies. Figure 4 shows the inhibition and precipitation of \( \gamma \)-GT by antibody to membrane-bound \( \gamma \)-GT purified from human fetal liver. The soluble form of \( \gamma \)-GT from all the three sources was completely precipitable by anti-fetal liver \( \gamma \)-GT. The inhibition and precipitation curves for adult liver, fetal liver, and hepatoma \( \gamma \)-GT followed an identical pattern and were superimposable. Figure 4 also shows that \( \gamma \)-GT activity was not completely inhibited by antiserum to fetal liver \( \gamma \)-GT. In other words, the enzyme–antibody complex of adult liver, fetal liver, and hepatoma each retained 50% of the free enzyme activity.

Lectin-Binding Studies

Interaction of \( \gamma \)-GT with soluble lectins. As Table 3 shows, 80, 73, and 68% of the adult liver, fetal liver, and hepatoma enzymes, respectively, were precipitated by Con A when the concentration of lectin was twice that of enzyme protein. Increasing the ratio of lectin to protein to 10:1 did not produce any further precipitation.

WGA precipitated 100, 80, and 71% of the adult liver, fetal liver, and hepatoma \( \gamma \)-GT, respectively, when the ratio of lectin to protein was 5:1. This difference in precipitation between fetal and hepatoma \( \gamma \)-GT and that of adult \( \gamma \)-GT was more pronounced (Table 3) when the ratio of lectin to enzyme protein was reduced to 1:1.

A significant difference between adult and fetal liver \( \gamma \)-GT was observed by RCA-120 precipitation when the ratio of lectin to enzyme protein concentration was 1:2, but this difference diminished on increasing the lectin concentration (Table 3).

Fig. 4. Inhibition (——) and precipitation (----) of the soluble form of \( \gamma \)-GT from adult liver (O), fetal liver (△), and hepatoma (□) by antibody to fetal liver \( \gamma \)-GT

Control activity taken as 100%.

Table 3. Precipitation of \( \gamma \)-GT by Soluble Lectins

<table>
<thead>
<tr>
<th>Source of ( \gamma )-GT</th>
<th>Precipitation of ( \gamma )-GT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (A)</td>
</tr>
<tr>
<td>Adult liver</td>
<td>80 (72)</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>73 (31)</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>68</td>
</tr>
</tbody>
</table>

Each value is the average of data from five separate experiments. The ratio of lectin to protein was 2:1 for Con A and RCA-120, 5:1 for WGA. Values in parentheses indicate: for WGA, the precipitation obtained when the lectin/protein ratio was 1:1; for RCA-120, the precipitation obtained when the lectin/protein ratio was 1:2. Other experimental conditions are given in the text.

Fig. 5. Dissociation of lectin–enzyme complex by various sugars

The WGA-enzyme complex (A) was dissociated by the indicated (abscissa) concentrations of N-acetylglucosamine and the RCA-120-enzyme complex (B) was dissociated by lactose. O, adult liver; △, fetal liver; and □, hepatoma \( \gamma \)-GT.

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SBA did not precipitate γ-GT from any of the three sources. Precipitation of the enzyme by Con A, WGA, and RCA-120 was inhibited by α-methyl-D-mannoside (0.2 mol/L), N-acetylglucosamine (0.2 mol/L), and lactose (0.15 mol/L), respectively.

**Dissociation of lectin–enzyme complexes by specific sugars.** The pattern of dissociation of Con A–enzyme complex by various concentrations of α-methyl-D-mannoside (10 to 200 mmol/L) did not reveal any differences among the three enzyme preparations.

Figure 5A shows the dissociation of WGA–enzyme complex by N-acetylglucosamine. The pattern for fetal liver and hepatoma enzyme differed from that of adult liver enzyme. At an N-acetylglucosamine concentration of 1 mmol/L, 17% of the adult enzyme, 98% of the fetal enzyme, and 81% of the hepatoma enzyme were released from the complex; at 100 mmol/L, the dissociation of adult liver enzyme from the complex was complete.

The pattern of dissociation of RCA-120–enzyme complex by lactose is shown in Figure 5B. The pattern for the adult-liver enzyme differed from that for fetal liver and hepatoma for lactose concentrations as high as 5 mmol/L, but not when the lactose was increased to 10 mmol/L.

**Discussion**

The partly purified soluble γ-GT has a molecular mass similar to that of hepatoma-specific soluble form of γ-GT described by Fujisawa et al. (12). Szewczuk (8), who first demonstrated the soluble form of γ-GT in various tissues, also found its relative molecular mass to be 80 000 to 90 000. We have observed that the papain-digested membrane-bound form of γ-GT does not bind to Phenyl-Sepharose CL-4B and had an electrophoretic mobility and molecular mass similar to that of soluble γ-GT. Moreover, the electrophoretic mobility of the soluble and papain-digested membrane-bound γ-GT is decreased to the same extent by neuraminidase treatment. Thus the enzyme may originate from the membrane-bound γ-GT by a mechanism similar to that of papain digestion.

We find the soluble form of γ-GT from adult liver, fetal liver, and hepatoma to be identical in its kinetic constants and catalytic properties, and similar to those reported for membrane-bound γ-GT (3). The soluble enzyme from all three sources also shows similarities in heat stability, molecular mass, and electrophoretic mobility. Reportedly (22), γ-GT derived from various organs from the same species differs in electrophoretic mobility because of differences in sialic acid content. Köttgen et al. (7) demonstrated in rats that the γ-GT in fetal liver is more sialylated than that in adult liver. However, our results suggest that γ-GT from human adult liver, fetal liver, and hepatoma are sialylated to an equal extent.

Our immunoinhibition and immunoprecipitation studies demonstrated that the enzyme from any of the three sources possesses identical antigenic determinants, and that the soluble and membrane-bound γ-GT have common antigenic determinants, the soluble γ-GT being completely precipitated by the antibody to membrane-bound γ-GT. This observation does not agree with that of Fujisawa et al. (12), who found that the membrane-bound and soluble γ-GT of hepatoma differed immunologically.

Partly purified membrane-bound γ-GT from human adult liver, fetal liver, and hepatoma binds to Con A–Sepharose 4B (3). Shaw et al. (23) and Huebey (24) reported that membrane-bound γ-GT in adult human liver can be precipitated by soluble Con A. In accordance with these observations, we also found that the soluble form of γ-GT from adult human liver can be precipitated by soluble Con A. As observed for membrane-bound enzyme (3), the soluble form of human adult and fetal liver γ-GT were not differentiated by Con A. Köttgen et al. (7), using Con A–Sepharose, found a sialic acid-rich (fetal form) and a sialic acid-poor (adult form) of γ-GT in fetal and adult rat liver, respectively. They also showed that the fetal form of γ-GT binds to Con A–Sepharose after treatment with neuraminidase. These contradictory observations may result from differences in the degree of sialylation of γ GT in rat and human, or from differences in the experimental conditions used. However, Ellison et al. (25) failed to differentiate fetal and adult forms of γ-GT from rat liver when they used Con A–Sepharose under the experimental conditions described by Köttgen et al. (7).

Precipitation of γ-GT with WGA and RCA-120 and the dissociation of the enzyme–lectin complex with specific sugars showed significant differences in γ-GT from the three sources. The γ-GT from fetal liver and hepatoma showed a weaker affinity for WGA than did γ-GT from adult liver, a higher concentration of N-acetylglucosamine being needed to dissociate the WGA–enzyme complex formed by the adult enzyme. RCA-120 precipitation studies showed that the fetal enzyme can be precipitated with a lower concentration of lectin than the adult liver enzyme. Differences were also observed between fetal and adult enzyme in the dissociation of RCA-120–enzyme complex at lower concentrations of lactose.

These results suggest that the carbohydrate composition of the fetal liver enzyme may differ from that of adult liver enzyme, and that the carbohydrate moiety of hepatoma γ-GT may be very similar to (if not the same as) that of fetal enzyme. Reportedly (23, 24), γ-GT in human kidney, liver, and pancreas differ in their lectin-binding properties, indicating a difference in their glycosylation. Similarly, our results with lectin-binding studies indicate that the glycosylation of γ-GT during development of fetal liver may be different from that of adult liver and that, during development of hepatoma, the glycosylation pattern of γ-GT may resemble that of fetal liver.

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**References**