Complexes of Serum Gamma-Glutamyltransferase with Apolipoproteins and Immunoglobulin A

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We have detected complexes between γ-glutamyltransferase and apolipoproteins or immunoglobulin A in sera from patients with hepatobiliary diseases but not in sera from healthy individuals. An average of 52.4% of the enzymatic activity was precipitated by antiserum against apolipoprotein A, 29.9% by antiserum against apolipoprotein B, and 9.7% by antiserum against immunoglobulin A. Fifty to 60% of the enzyme activity was inhibited in the immunoprecipitates from the transferase fraction bound to apolipoprotein A or immunoglobulin A, and 21% in the fraction bound to apolipoprotein B. We identified the complexes transferase fractions by electrophoresis.

Additional Keyphrases: hepatobiliary disease · electrophoresis, cellulose acetate · immunoprecipitation · enzymic catalytic activity

Heterogeneity of serum γ-glutamyltransferase (GGT, EC 2.3.2.2), first described about 20 years ago (1), apparently results from posttranslational modifications of a single form of the enzyme molecule (2–7). To explain this heterogeneity, several authors demonstrated the association of serum GGT with lipoproteins. De Broe et al. (8) found plasma membrane fragments with GGT activity in the serum of cholestatic patients. Freise et al. (9) demonstrated the presence in some sera of complexes between GGT and chylomicrons and postulated that the distribution of the multiple forms of the enzyme could be explained by modifications of the distribution of the serum lipoproteins (10). Others (11, 12) also observed the binding of GGT to lipids or lipoproteins. Recently, Huseby (13), studying sera from cholestatic patients, reported that an important fraction of the enzyme was bound to lipoprotein X or to high-density lipoprotein.

We studied the association of GGT with apolipoproteins A and B (apo A and B), and with immunoglobulin A (IgA) in 10 patients with hepatobiliary disease. Association of IgA with serum enzymes has been previously demonstrated for alkaline phosphatase (14, 15), aspartate aminotransferase (16), creatine kinase (17), and amylase (18).

Materials and Methods

Human samples. Sera with high GGT activities (>350 U/L) were obtained from 10 patients (four women and six men, ages 49 to 70 years) with various hepatobiliary diseases: four with alcoholic cirrhosis, and one each with acute hepatitis, extensive fibrosis with intrahepatic cholestasis, post-hepatitic cirrhosis, pancreatitis with extra-hepatic cholestasis, cancer, and congestive heart failure.

We also studied sera from five healthy subjects matched for age and weight with the patients and having GGT activities <50 U/L.

Antisera. Rabbit antisera against human apo A or apo B and against human IgA (alpha chain) were obtained from Behring, Marburg, F.R.G. The purified GGT (human hepatic and renal enzymes) used for immunizations and antisera against these enzymes were prepared as described previously (6).

Immunoprecipitations by the various antisera. Sera were incubated with antisera in NaCl (150 mmol/L) for 16 h at 37 °C. Immunoprecipitates were separated by centrifugation (7.5 min, 10 000 × g), washed three times, and suspended in NaCl (150 mmol/L) for determination of GGT activity. After we measured the GGT activity in the supernates, we subjected them to electrophoresis (see below).

Appropriate controls for nonspecific binding were treated in the same way by using serum from nonimmunized rabbits in place of specific antisera. To account for the respective GGT activities of rabbit serum and antisera, we used the following calculations: Percentage of GGT activity precipitated = 100 (Aa – Aa)/A0, where A0 is the GGT activity in the supernate after centrifugation of the mixture of human serum with serum from nonimmunized rabbit, and Aa is the GGT activity in the supernate of the mixture of human serum with the specific antisera.

The percentages inhibition of the enzyme in the immunoprecipitates were equal to 100 (1 – IAM/IAC), where IAM is the GGT activity measured in the immunoprecipitate, and IAC is the expected GGT activity calculated as IAC = A0 – Aa.

Determination of enzyme activity. We measured GGT activities at 30 °C, using a Cary 219 spectrophotometer (Varian Associates, Palo Alto, CA) as previously described (6), Tris and glycyglycine (Merck, Darmstadt, F.R.G.), and gamma-L-glutamyl-3-carboxy-4-nitroanilide (Bohringer, Mannheim, F.R.G.).

Electrophoresis. We applied 5 µL of each supernate, prepared as described above, to Titan III Zip Zone cellulose acetate plates (Helena Labs., Beaumont, TX). The migration buffer was a 25 mmol/L barbital buffer (per liter, 0.69 g of barbital and 4.38 g of sodium barbital), pH 8.6. We applied a constant voltage of 180 V for 30 min. To locate the enzyme activity, we used the substrate gamma-L-glutamyl-alpha-naphthylamide (Sigma Chemical Co., St. Louis, MO), incubating the plates at 37 °C for 2 h with the substrate solution (per liter, 5 mmol of gamma-L-glutamyl-alpha-naphthylamide and 100 mmol each of Tris and glycyglycine, pH 8.25). After quickly washing the plates, we detected the released alpha-naphthylamine by applying a freshly prepared 1 g/L aqueous solution of Fast Garnet GBC salt (Serva, Heidelberg, F.R.G.) and fixed the resulting red bands with dilute (100 m/L) acetic acid.
Results

Table 1 shows the results of the immunoprecipitation experiments. Antiserum against hepatic or renal GGT precipitates about 80% of the GGT in the serum of patients with hepatobiliary disease and of healthy subjects, thus corroborating our previous observations (6). There is little variation in the results observed for the various sera. In the 10 patients' sera, a large fraction of GGT—52.4%—on the average—was bound to apo A. In nine of these sera, some (usually less) of the enzyme was also associated with apo B, but the size of this fraction varied from serum to serum.

Six of the 10 sera contained GGT bound to IgA. This fraction never exceeded 22% of the total GGT activity. Conversely, we did not observe any association of GGT with apo A, apo B, or IgA in the sera from the five healthy subjects.

We also calculated inhibition rates of the enzyme in the various immunoprecipitates. In the GGT–anti-GGT complexes, 90% of GGT's catalytic activity was inhibited, compared with about 50% in the GGT–anti-apo A complexes, and an average of 21% in the GGT–anti-apo B complexes. For three patients' sera, all of the GGT activity precipitated from the supernatants was found in the precipitate (i.e., zero inhibition). In precipitates with anti-IgA antiserum, 57.7% (mean of six sera) of GGT activity was inhibited. We could not estimate the inhibition rates of GGT in the sera from healthy subjects because of its very low activity in the immunoprecipitates.

Multiple molecular forms of the GGT remaining in the supernatants of the immunoprecipitation experiments were separated by electrophoresis, stained for enzyme activity, and located according to their relative mobilities (RM) with regard to serum albumin.

We did not obtain interpretable results with the sera from the healthy subjects.

Before precipitation by antisera, we observed three GGT bands in the 10 patients' sera: one band at the point of application (RM = 0), one "slow" band (RM between 0.46 and 0.50), and one "fast" band (RM between 0.80 and 0.89). In two sera, we found an additional band of prealbumin mobility (RM = 1.05).

Figure 1 represents a typical pattern of GGT bands from the patients' sera after immunoprecipitation and electrophoresis on cellulose acetate. Absorption of the sera with anti-apo A antiserum greatly or totally decreased the fast band for all sera, and sometimes reduced the mobility of the slow band. Conversely, absorption with anti-apo B antiserum greatly or totally decreased the slow band for all sera, without visible modification of the fast band.

With antisera against hepatic or renal GGT, both the fast and the slow bands totally disappeared in eight patients' sera; the two other sera had very weak residual activity of the slow band.

We did not observe any significant modification in distribution and intensity of the bands after treating the sera with anti-IgA antiserum.

We were unable to estimate modifications in any of the bands of zero and prealbumin mobilities because of their weak intensities.

Discussion

Our results show that sera from patients with hepatobiliary diseases contain an important fraction of GGT associated with apo A and apo B. The fraction bound to apo A seems to be greater than that bound to apo B.

Association of GGT with apo A has been previously described by Huseby (13), but he was not able to show the binding of the enzyme to apo B. Crofton and Smith (19) had previously observed the association of GGT and alkaline phosphatase with low-density lipoproteins.

We did not find any binding of the enzyme to apolipoproteins in sera from healthy subjects. In some of the serum samples from the patients we also found GGT–IgA complexes. This corroborates the findings of Sudo and Kano (20), who reported the presence in some sera of high-molecular-mass complexes containing alkaline phosphatase, arylamidase, GGT, and IgA.

By measuring GGT activity in immunoprecipitates obtained with antibodies against hepatic or renal GGT, we observed that about 80–90% of the enzyme activity was inhibited. Conversely, GGT in immunoprecipitates with anti-apo A or B and anti-IgA antisera conserved 50–80% of its activity. Perhaps this phenomenon is attributable to changes in conformation induced by the binding of specific and aspecific antibodies to the complexed GGT. Anti-apolipoprotein antibodies bind to the lipoprotein part of the complex, whereas anti-GGT antibodies bind to the antigenic sites of the enzyme. Accessibility of the substrate to the active site of the enzyme might be less modified when enzymic complexes are bound to antibodies to apo A, apo B, or IgA than when bound to antibodies to GGT.

Apo A-bound and apo B-bound GGT showed quite differ-
...electrophoretic mobilities in cellulose acetate, the apo A-bound GGT migrating faster than the apo B-bound GGT.

In addition, when determining apo A and B in the patients’ sera by electroimmunodiffusion (Laurell technique), we observed that the precipitation arcs showed GGT activity (data not shown). We noted that specific GGT staining was more intense for the "rockets" corresponding to apo B than for those corresponding to apo A. This corroborates our present results, because less GGT activity is inhibited by anti-apo B antiserum than by anti-apo A antiserum.

The combined use of electrophoresis and selective immunoprecipitation techniques can lead to a more rigorous identification of the GGT fractions bound to various serum components.

We are studying possible correlations between the different hepatic pathologies and the relative amounts of the GGT fractions complexed with various serum components.

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References


