TWO DIFFERENT GAMMA-GLUTAMYLTRANSFERASES DURING DEVELOPMENT OF LIVER AND SMALL INTESTINE: A FETAL (SIALO-) AND AN ADULT (ASIALO-) GLYCOPROTEIN [+]

E. Köttgen, W. Reutter and W. Gerok

Medizinische Klinik and Biochemisches Institut der Universität, D-7800 Freiburg / Germany

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SUMMARY

Depending on the developmental stage, the gamma-glutamyltransferase (E.C. 2.3.2.2) exists in two different types in the liver and in the small intestine: a sialic acid-rich fetal type and a sialic acid-poor adult type. The fetal type could be detected in the undifferentiated cryptal cells, in the fetal small intestine and in the fetal liver, and the adult type in the differentiated villous cells and in the adult liver. The separation of both types was performed using ConA-sepharose, which does not bind the fetal type but the adult type. Binding was reached by neuraminidase treatment.

Gamma-glutamyltransferase (E.C. 2.3.2.2) is involved in the transport of amino acids (1). Increasing interest on this enzyme is derived from its change in activity during development (2). Moreover, the subcellular distribution of the gamma-glutamyltransferase differs between fetal and adult hepatocytes (3). The enzyme is diagnostically important, because its activity is also altered in chemically induced hepatomas as shown by histochemical means (3). Isoenzyme may refine the diagnostic significance of an enzyme. However, their existence has not been described. Accounting to the glycoprotein nature of this enzyme (4), ConA-sepharose is used in the present paper in order to characterize the gamma-glutamyltransferase during development.

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Abbreviations used: ConA, Concanavalin A,
PBS, phosphate buffered solution

MATERIALS AND METHODS

Male Wistar rats (Ivanovas, Kisslegg, Germany), weighing 180 - 200 g each, were fed on a commercial diet (Altromin; Altromin G.m.b.H., Lage-Lippe, Germany) and water given ad libitum. The diet contained 18 - 20 % (w/w) of protein. Fetal rats weighing 2,3 g each were about 18 to 20 days of embryonic age. ConA-sepharose were obtained from Pharmacia, Uppsala (Sweden), neuraminidase from Vibrio cholerae from Behring-Werke, Marburg (Germany) and neuraminidase from Clostridium perfringens from Boehringer Mannheim G.m.b.H., Mannheim (Germany), methyl-α-D-mannopyranoside from Calbiochem, Luzern (Switzerland). All other chemicals of analytical grade were purchased from E. Merck AG, Darmstadt (Germany).

Liver was homogenized in 0.1 M Tris · HCl buffer, pH 8.0, containing 0.01 M $MgCl_2$. After centrifugation at 600 x g for 10 min the supernatant was incubated with Triton X 100 (1:100 v/v) for 12 h at 4°C. After centrifugation at 13,000 x g for 20 min the enzyme activity of the supernatant was tested before and after chromatographic separation on ConA-sepharose.

Small intestine of adult rats. Cell preparation of the villous and cryptal zone was performed according to Weiser (5). The method isolates only epithelial cells. After cell-dissociation by citrate, the intestinal lumen is repeatedly washed with PBS, containing EDTA and dithiothreitol. By a series of incubations and washings, sequential fractions of isolated epithelial cells were obtained that appeared to define a gradient of cells from the mature villous zone to the poorly differentiated cryptal cell areas. The washed cells were solubilized with Triton X 100 (1:100 v/v) at 4°C for 12 h. After centrifugation at 600 x g for 10 min the gamma-glutamyltransferase-activity was tested before and after separation on ConA-sepharose. The small intestine of fetal rats was homogenized in PBS containing Triton X 100 (1:100 v/v) and further treated as described above. The estimation of gamma-glutamyltransferase activity was performed according to Szasz (6). Protein was determined by Lowry's method (7). Bovine serum albumin served as standard. Affinity chromatography was performed on ConA-sepharose columns (1.5 x 20.0 cm) at 4°C.

RESULTS

Gamma-glutamyltransferase of the adult rat liver binds to ConA-sepharose and could be eluted with methyl-α-D-mannopyranoside (Fig. 1A). Under the same conditions the enzyme of the fetal liver does not bind to the lectin (Fig. 1B). The rechromatography of the adsorbed (adult) or not adsorbed (fetal) gamma-glutamyltransferase shows the same chromatographic characteristic as in the first run. The recovery of the enzyme activity ranged between 95 and 100 %. The K_M values of the fetal and the adult enzyme are identical. After treatment of the fetal liver supernatant with neuraminidase, the gamma-glutamyltransferase is retained by ConA-sepharose as is most of the adult enzyme. Also the small not

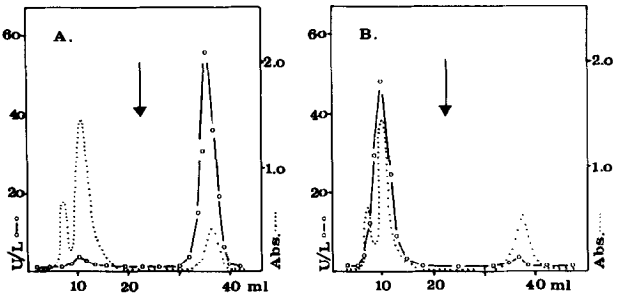

Figure 1 :

ConA-sepharose chromatography of gamma-glutamyltransferase from (A) adult and (B) fetal rat liver.

Starting buffer: 0.05 M Tris · HCl, 0.5 M NaCl, 1 mM $MgCl_2$ - $MnCl_2$ - and $CaCl_2$, 4 mM sodium-azide, Triton X 100 (0.5 : 100 v/v), pH 7.5. Elution buffer (↓) additionally 0.2 M methyl-α-D-manno-pyranoside. Fraction volume 1 ml, flow rate 30 ml/h.
o——o : Gamma-glutamyltransferase activity measured at 25°C in U/L.
----- : Absorbance measured spectrophotometrically at 280 nm.

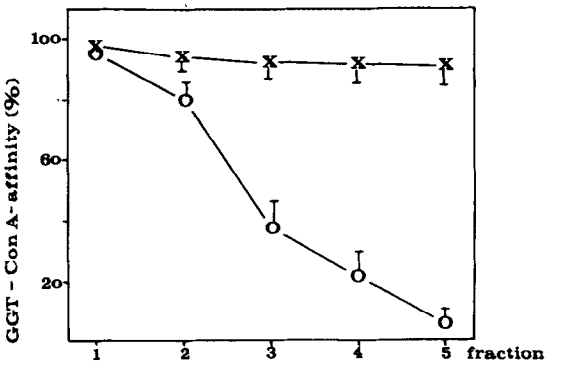

Figure 2 :

Affinity of untreated (o——o) and neuraminidase-treated (x——x) gamma-glutamyltransferase (GGT) of small intestine to ConA-sepharose.

Fract. 1 represents the villous zone and Fract. 2-5 the gradient to the cryptal area. Neuraminidase treatment: The solubilized material is dialyzed against 0.05 M acetate buffer containing 0.01 M $CaCl_2$, pH 5.0 for 12 h at 4°C and incubated for 48 h at 20°C with neuraminidase (final concentration 1 mg equivalent to 0.6 U per ml incubation solution). For control dialyzed supernatant were incubated without neuraminidase. Each point represents the mean ± S.D. of six rats.

adsorbed fraction of the adult liver (accounting to about 5 % of the total enzyme activity) does bind to ConA after treatment with neuraminidase. Incubation of the dialysed fetal supernatants at 20°C without neuraminidase binding of the gamma-glutamyltransferase does not result in detectable amount. Measurements of the activity in the intestinal cells (Fig. 2) revealed that all of the enzyme activity of the mature villous cells was retained by ConA-sepharose whereas the enzyme of undifferentiated cryptal cells was not bound to the lectin. Enzyme from cells situated in between was more or less adsorbed in relation to the distance from the villous zone. Treatment with neuraminidase of the supernatants of the cryptal cells leads also to a nearly total adsorption of the enzyme activity on ConA. Similarly, the gamma-glutamyltransferase of the fetal small intestine was bound to the lectin only after neuraminidase treatment. The total activities of the gamma-glutamyltransferase in particulate fractions of the adult small intestine are in the same range as reported by Curthoys and Shapiro (8).

DISCUSSION

By the use of ConA-sepharose columns it could be shown that the gamma-glutamyltransferase of liver and small intestine exists as two structurally different enzymes: a fetal type which is not retained and an adult enzyme which is totally bound to the lectin. The gamma-glutamyltransferase of the adult intestinal epithelial cells behaved in an analogous manner: the enzyme of the undifferentiated cryptal cells was not bound in contrast to the enzyme of the well differentiated villous cells. After treatment with neuraminidase the enzyme of the fetal or undifferentiated cells revealed the same ConA binding characteristics as the enzyme of the adult or differentiated cells. We might conclude that the fetal enzyme is a sialoprotein in contrast to the adult enzyme. This difference of the N-acetylneuraminic acid content of the fetal and adult gamma-glutamyltransferase may be due to an enhanced activity of a specific sialyltransferase or to a decreased activity of a neuraminidase. On the other hand the fetal enzyme might be a better substrate for sialyltransferase. Preliminary results indicate that the fetal type of the gamma-glutamyltransferase appears also after partial hepatectomy and in several MORRIS hepa-

toma (unpublished results). Increasing evidence for a close correlationship between metabolism of N-acetylneuraminic acid and development is shown by an enhanced content of N-acetylneuraminic acid in fetal cells (9,10) or an increased activity of sialyltransferase in the fetal liver (11). The concentration of CMP-NANA, however, did not change during development of the liver (12). Alterations of the lectin-binding capacity may be used for the characterization of cells of different developmental stage as revealed for fetal (13) and tumor cells (14-16). The gamma-glutamyltransferase in this study may serve as a model where an enzyme with a defined lectin acceptor is used in order to establish different developmental stages. Moreover, our findings could be diagnostically important since the ConA binding characteristics of the gamma-glutamyltransferase during alcoholic hepatitis, primary biliary cirrhosis and in some cases of primary hepatoma is of the fetal type (17).

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