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Biochemical Characteristics of Hepatoma-specific γ-glutamyl Transpeptidase Isozyme

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Abstract The γ-glutamyl transpeptidase (γ-GTP) isozyme specifically appears both in sera from the patients with hepatocellular carcinoma and in hepatoma tissues. The isozyme was purified from homogenates of human hepatoma tissues by chromatography on columns of Sephadex G-200, Concanavalin A-sepharose and DEAE-cellulose. Although the enzyme did not bind to the Concanavalin A-column, after treatment with neuraminidase it became to bind to the column. The results suggest that the sugar portion of the isozyme, especially the content of sialic acid, differes from that of other γ-GTP isozymes.

Key Words: Liver; hepatoma, γ-glutamyl transpeptidase isozyme, diagnostic marker, Electrophoresis; hepatoma

Introduction

It has been reported that the γ-glutamyl transpeptidase (γ-GTP) [EC 2.3.2.2] activity increases in the sera of patients with primary hepatocellular carcinoma and hepatoma tissue[1-4]. Recently, a hepatoma specific serum γ-GTP band has been separated on polyacrylamide gel[2] and this isozyme has been considered as a clinincal marker for diagnosis of hepatocellular carcinoma as well as α-fetoprotein[2-5]. Using disc electrophoresis in the double layered polyacrylamide gel (10-20%), we previously demonstrated the presence of hepatoma-specific γ-GTP isozyme called Band Ⅱ in 54% in patients with primary hepatocellular carcinoma[4]. However, different patterns of γ-GTP isozyme were observed in the sera of patients with hepatocellular carcinoma by different investigators[2,3,5]. It was, in animal experiments, reported that the γ-GTP isozyme which appeared in hepatoma tissue might be the consequence of modification of the other isozyme, since the hepatoma-specific isozyme was unable to distinguish immunologically from the other isozyme[6]. Based on the data obtained from the previous experiment[4] a possibility arose that the protein portion would be common in all γ-GTP isozymes, but the sugar portion including sialic acids would be different. In this report, this possibility was tested and biological characteristics of the isozyme will be discussed.

Materials and Methods

Fresh tumor tissues weighing 85.7 g were removed from the patients of 61-year-old and 63-year-old males with hepatocellular carcinoma at autopsy and subjected to homogenization. Both membrane binding and microsomal soluble γ-GTP were obtained together by centrifugation at 25,000×g for 30 min at 4°. The γ-GTP was purified by the slightly modified method of Huseby[7], as previously described[4]. The isozyme was purified through Sephadex G-200 (Pharmacia Fine Chemicals, Sweden) column (60×2.5 cm), Concanavalin A (Con A) sepharose (Phamacia Fine Chemicals, Sweden) column (30×1 cm) and DEAE cellulose (Nakarai Chemicals Ltd., Tokyo) column (30×1 cm) in order. Finally hepatoma-specific γ-GTP isozyme was isolated electrophoretically (Fig. 1). The Band II on disc electrophoresis of the 10-20% double layered polyacrylamide gel was proved to be hepatoma-specific γ-GTP isozyme (Fig. 2).

The purified γ-GTP isozyme was digested with neuraminidase according to the procedure reported by Köttogen at al[8], and its product was further examined by electrophoresis. Two peaks of the activity appeared on Sepahdex G-200 chromatography (Fig. 3). These lower molecular weight fractions were applied to the Con A sepharose column after treatment with or without neuraminidase.

The γ-GTP activity in gels was demonstrated by the method reported by Sawabu and his co-workers[2], using N-γ-L-glutamyl-α-naphthylamide (Sigma Chemical Co., U.S.A.), glycylglycine (Wako Chemical Ind., Osaka) and Fast Garnet GBC (Sigma Chemical Co., U.S.A.). The activity of γ-GTP was measured by Sankyo γ-GTP test pack (Sankyo Pharmaco. Co., Tokyo).

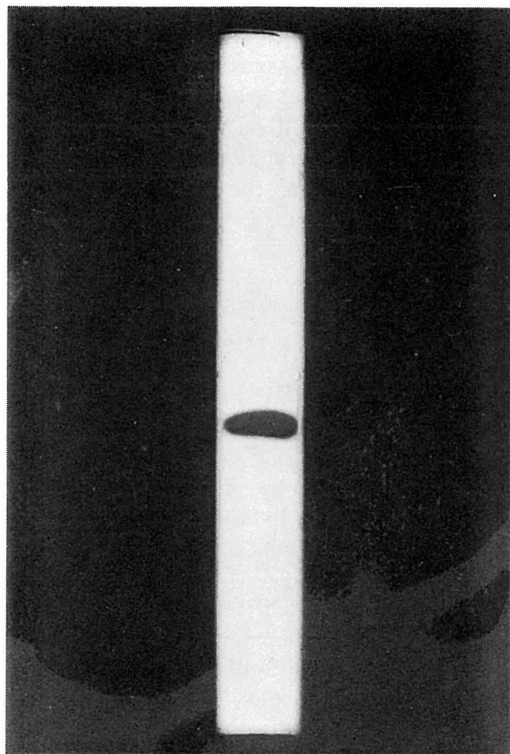

Fig. 1 Purified hepatoma-specific γ-GTP isozyme appeared as a single band on acrylamide gel disc electrophoresis. Using 7.5% polyacrylamide gel, electrophoresis was performed in 50 mmol/l Tris/glycine buffer, pH 8.3 at constant current of 2 mA/tube.

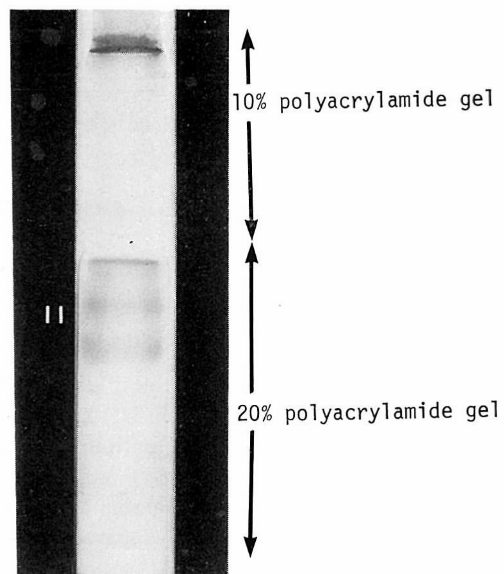

Fig. 2 Disc electrophoresis, using double layered polyacrylamide gel (10-20%), revealed hepatoma-specific γ-GTP isozyme or Band II in the case of hepatocellular carcinoma. Electrophoresis was carried out in 50 mmol/l Tris/glycine buffer, pH 8.3 at a constant current of 2 mA per tube for 6 hours. Bromphenol blue was used as a marker. The isozymes were stained with N-γ-L-glutamyl-β-naphthylamide and Fast Garnet GBC.

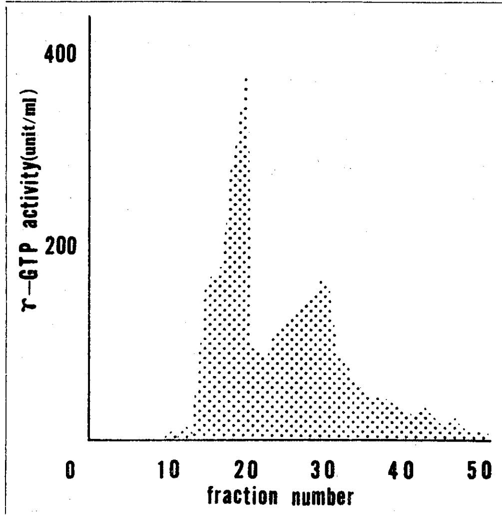

Fig. 3 Sephadex G-200 column (2.3×60 cm) chromatography for γ-GTP from human hepatoma tissue was performed in 20 mmol/l Tris/HCl buffer, pH 7.4 at 4° with flow rate of 10 ml per hour. Fractions from 15th to 22nd and from 23rd to 33rd demonstrate high activity of γ-GTP. Electrophoretically, the latter fraction is hepatoma specific γ-GTP isozyme.

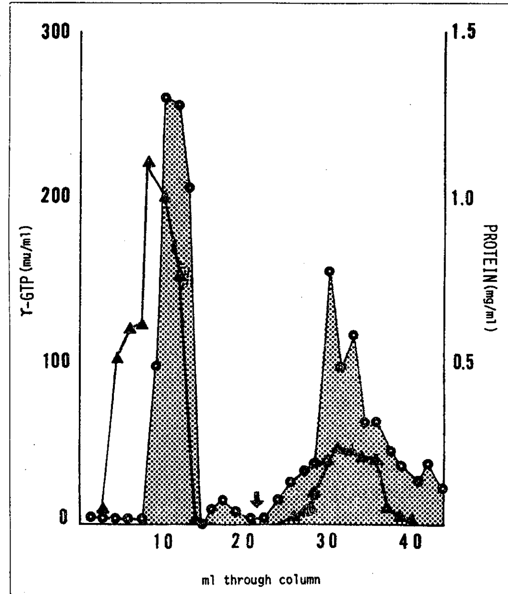

Fig. 4 The pattern of Con A-sepharose column (30×1 cm) chromatography for the fraction containing hepatomaspecific γ-GTP isozyme. Starting buffer: 50 mmol/l Tris/HCl buffer, pH 7.5, containing 0.5 mol/l NaCl, 1 mmol/l $MgCl_2$-$MnCl_2$-$CaCl_2$, 4 mmol/l sodium-azide, Triton X 100(0.5: 100 v/v). Elution buffer: the starting buffer additionally containing 0.2 mmol/l α-methyl-D-mannoside (⬇). Flow rate was 30 ml/h. 1-ml fractions were collected. ○-○: γ-GTP activity, ▲-▲: protein measured by Folin-Lowry method.

Results

Sephadex G-200 gel filtration of the hepatoma extract demonstrated two peaks of the γ-GTP activity (Fig. 3). When the lower molecular weight fractions (23rd to 33rd fractions) of the Sephadex effluent were applied the Con A column, 58% of the γ-GTP activity was not bound to the column (Fig. 4). On electrophresis, the γ-GTP activity that was not bound to the Con A column migrated as Band Ⅱ which corresponds to the hepatoma-specific γ-GTP. On the other hand, when the lower molecular weight fractions of the Sephadex effluent were treated with neuraminidase and then applied to the Con A column, 76% of the γ-GTP activity was bound to the column which was further eluted by methyl-α-D-mannoside (Fig. 5).

When the fractions unbound to the con A column was applied on the DEAE cellulose column, and the protein was eluted with a linear concentration gradient of NaCl, a single peak of γ-GTP activity appeared at 0.38M of NaCl. Electrophoresis on polyacrylamide disc gel of the purified enzyme revealed a single band corresponding to Band Ⅱ (Fig. 1). However, the purified hepatoma specific γ-GTP isozyme treated with neuraminidase migrated toward the cathods on electrophoresis.

Discussion

It has been reported that a specific γ-GTP isozyme appears electrophoretically in the

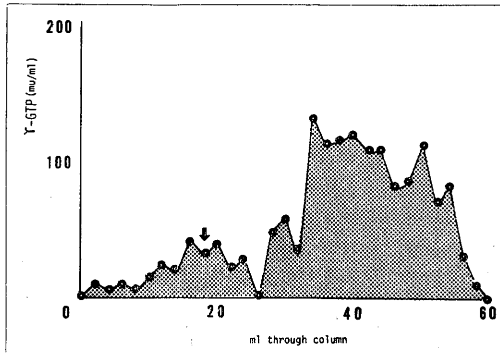

Fig. 5 The pattern of Con A-sepharose chromatography for hepatoma-sepcific γ-GTP isozyme treated with neuraminidase. The condition of chromatography was same as that in Fig. 4. ○–○: γ-GTP activity.

sera of patients with hepatocellular carcinoma[1-4], and even in the α-fetoprotein negative cases[4]. Therefore, in spite of the scanty information about its biochemical properties, it has been considered to be a useful tumor marker for the diagnosis of heptocellular carcinoma[2-4]. The studies of enzymatic deviation during malignant transformation have been accumulated from the view-points of phenotypic changes during carcinogenesis in the animal and human[9]. In this sence, it is very important to reveal the biochemical properties of this tumor related γ-GTP. According to Shaw's data[10], about 20% of the total γ-GTP activity of normal liver did not bind to the Con A affinity column, whereas for the hepatocellular carcinoma γ-GTP a much larger percentage of the total γ-GTP activity (79%) did not bind to Con A. Thus, he concluded that in this pathological liver tissue the form of γ-GTP which did not bind to Con A predominated. Therefore, firstly it was examined whether this tumor related γ-GTP isozyme was presented in the form of γ-GTP unbound to Con A column. In fact, by polyacrylamidegel electrophoresis in discontinuous gradient, γ-GTP containing fractions eluted through DEAE cellulose was completely identical with tumor related γ-GTP isozyme (Band Ⅱ). This observation indicated that this specific γ-GTP had somehow different biological character from the usual form of γ-GTP about the binding capacity to Con-A column. However, 76% in total γ-GTP activity from tumor extracts became to bind to Con-A column after neuraminidase digestion to removed terminal sialic acid residues and on the gel electrophoresis the digested enzyme migrated toward the cathode, not corresponding to Band Ⅱ. Furthermore, purified tumor related γ-GTP was separated into two subunits (molecular weight: 40,000 and 20,000) by 0.1% SDS gel electrophoresis. This data was compatible with it reported by Tate and Meister who used a purified γ-GTP from the kidney[11]. Recently, several authors reported that the antigen site of tumor related γ-GTP was identical immunologically with γ-GTP purified from the normal kidney and liver[6,12]. Thus, the tumor related γ-GTP appeared to contain terminal sialic acid residues in the carbohydrate portion of the enzyme molecule which somehow, either by direct blockage or by causing a conformational changes in the molecules, prevent the binding of Con-A to α-D-manopyranosyl moieties, and its protein portion might be common with the usual form of γ-GTP.

Based upon the results mentioned above, it may be accepted that tumor related γ-GTP is possibly due to altered glycosylation of this glycoprotein in tumor cells. However, it remains to be explored whether this altered glycosylation is depend on the enzymatic deviation of γ-GTP evoked during hepatocarcinogenesis.

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References

1) Rutengerg, A.M., Goldberg, J.A. and Pineda, E.P.: Serum-gamma-glutamyl-transpeptidase activity in Hepatobiliary pancreatic disease. *Gastroenterology*, 45 : 43–48, 1963.
2) Sawabu, N., Nakagen, M. Yoneda, M., Makino ,H., Kameda, S., Kobayashi, K., Hattori, N. and Ishii, M.: Novel γ-glutamyl transpeptidase isozyme specifically found in sera of patients with hepatocellular carcinoma. *Gann*, 69 : 601–605, 1978.
3) Fujisawa, K., Nishikawa, H., Ogura, K., Kitahara, T., Kawase, H. and Yamauchi, M.: Clinical significance of γ-glutamyl transpeptidase isozyme. *Metabolism Dis.*, 16 : 199–206, 1979.
4) Sasaki, M., Okita, K., Kodama, K., Matsuda, S., Morimoto, T. and Takemoto, T.: γ-glutamyltranspeptidase isozyme in hepatic carcinoma. *Acta Hepatol. Jpn.*, 21 : 594–601, 1980.
5) Tanaka, K.: Studies on γ-glutamyl transpeptidase. (II) The clinical evaluation of γ-glutamyl transpeptidase in hepatocellular disease. *Jpn. J. Gastroenterol.*, 70 : 1170–1181, 1973.
6) Tsuchida, S., Hoshino, K., Sato, T., Ito, N. and Sato, K.: Purification of γ-glutamyl transpeptidase from rat hepatomas and hyperplastic nodules, and comparison with the enzyme from rat kidney. *Cancer Res.*, 39 : 4200–4205, 1979.
7) Huseby, N.: Purification and some properties of γ-glutamyltranspeptidase from human liver. *Biochim. Biophys. Acta*, 483 : 46–56, 1977.
8) Kottogen, E., Reutter, W. and Gerok, T.: Two different gamma-glutamyl transpeptidase during development of liver and small intestine. *Biochem. Biophys. Res. Comm.*, 72 : 61–66, 1976.
9) Ono, T., Wakabayashi, K., Uenoyama, K. and Koyama, H.: Regulation of enzyme synthesis relating to differentiation of malignant cells. *In* Weinhouse, S. and Ono, T. (eds.), *Isozyme and Enzyme Regulation in Cancer., GANN Mongr. Cancer Res.*, Tokyo Univ. Press, Tokyo 1972, p. 19–29.
10) Shaw, L.M.: Molecular properties of γ-glutamyl transpeptidase. *In* Dermers, L.M. and Shaw, L.M. (eds.), *Evaluation of Liver Function.*, Urban & Schwarzenberg, Baltimore-Munich, 1978, p. 103–121.
11) Tate, S.S. and Meister, A.: Subunit structure and isozymic forms of γ-glutamyl transpeptidase. *Proc. Natl. Acad. Sci. U.S.A.*, 73 : 2599–2603, 1976.
12) Yamamoto, H., Sumikawa, K., Hada, T., Higashino, K. and Yamamura, Y.: γ-glutamyltransferase from human hepatoma tissue in comparison with normal liver enzyme. *Clin. Chim. Acta*, 111 : 229–237, 1981.