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Note

Chromatography of γ-glutamyl transferases from ascites hepatoma AH-66 cells and human primary hepatoma on *Phaseolus vulgaris* erythroagglutinating lectin agarose

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γ-Glutamyl transferase (EC 2.3.2.2) is one of the oncofetal proteins [1] and plays an essential role in the turnover of glutathione in mammalian tissues [2, 3]. The enzyme is highly activated in the livers of rats fed various hepatocarcinogens [4, 5], and a high activity has also been found in tumour tissues [4—6]. Previous studies in our laboratory indicated that the enzymes purified from various tissues such as fetal liver [7], normal adult liver [8], azo-dye-induced hepatoma [6], ascites hepatoma AH-66, as well yolk sac tumour cells [8, 9] are all similar with respect to kinetic, immunochemical and physicochemical properties except that the enzymes from tumour tissues contain more carbohydrate residues. Quite recently we analysed the carbohydrate structure of the enzymes from AH-66 cells and normal liver of rats and found that the enzyme from AH-66 cells contains a complex type of asparagine-linked sugar chains with bisected N-acetylglucosamine residues, while the enzyme from normal rat liver lacks these residues [10].

Phaseolus vulgaris erythroagglutinating lectin has been reported by Irimura et al. [11] to bind specifically to the bisected residues of oligosaccharides. Cummings and Kornfeld [12, 13] also reported that the following bisected

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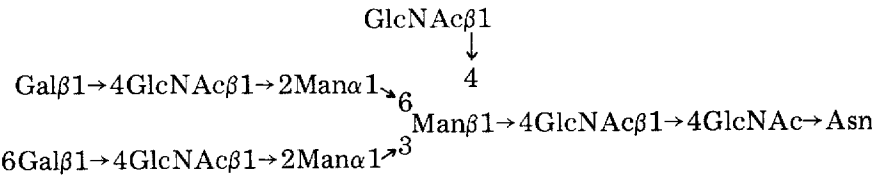
biantennary complex type asparagine-linked oligosaccharides bind to the lectin.



This was further confirmed by Yamashita et al. [14], who reported a detailed structural requirement for binding to the lectin.

In the present study we found that γ-glutamyl transferase from AH-66 cells bound to the lectin column, while the enzyme from normal rat liver did not. We also found that the neuraminidase-treated human hepatoma enzyme bound to the column, while the normal human liver enzyme did not. This is the first report to show that highly purified γ-glutamyl transferase has an affinity for E-PHA agarose. Affinity chromatography on E-PHA agarose is of value for the isolation and identification of glycoproteins with bisected N-acetylglucosamine residues.

MATERIALS AND METHODS

Affinity chromatography on E-PHA agarose

E-PHA agarose (*Phaseolus vulgaris* erythroagglutinating lectin agarose) was purchased from E-Y Labs. San Mateo. A column (10 × 1 cm) was equilibrated with 50 m*M* Tris—HCl buffer, pH 7.5 containing 0.01% sodium azide and 0.15 *M* sodium chloride. The untreated or neuraminidase-treated sample (100 μl) was loaded on the column, and 0.2-ml fractions were collected at room temperature. The flow-rate was 5 ml/h.

Purification of γ-glutamyl transferase

γ-Glutamyl transferase was purified from AH-66, spontaneous hepatoma and normal rat liver according to the method described previously [1, 9, 10]. The purified enzymes from AH-66, spontaneous hepatoma and normal rat liver had specific activities of 428, 450 and 440 U/mg of protein, respectively. The purification procedure for the enzyme from normal human liver and hepatoma was essentially the same as that for normal rat liver enzyme except that the antibody used for the immunoaffinity column was goat antibody against human kidney γ-glutamyl transferase which was purified according to the method of Tate and Meister [15]. The purified enzymes from normal human liver and hepatoma had specific activities of 456 and 480 U/mg of protein, respectively. All these enzymes were found to be homogeneous and composed of two non-identical subunits by polyacrylamide gel electrophoresis in the presence of 1% sodium dodecyl sulphate.

Assay of γ-glutamyl transferase

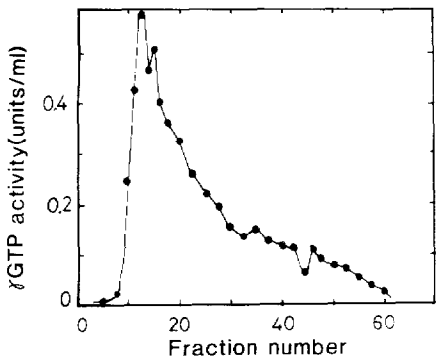
γ-Glutamyl transferase activity was assayed according to the method described previously [7] using a Gilford Model 240 recording spectrophotometer at 37°C.

Neuraminidase treatment of the purified enzymes

Neuraminidase treatment was done in the presence of 0.1 U of neuraminidase (*Arthrobacter ureafaciens*, Marukin Shoyu Japan) at pH 6.8 for 36 h as previously described [8].

RESULTS AND DISCUSSION

γ-Glutamyl transferase from normal rat liver, which lacks the bisected N-acetylglucosamine residue, was loaded on the column. However, almost all of the enzyme was washed through the column (Fig. 1). On the other hand, the enzyme from AH-66 cells, which has bisected N-acetylglucosamine residues, was retarded on the column as shown in Fig. 2. The recovery of the enzyme activity after this procedure was over 85%. These results indicate that the E-PHA column binds bisected N-acetylglucosamine residues found in the γ-glutamyl transferase from AH-66 cells [10]. We also found that the spontaneous hepatoma enzyme of the rat bound to the E-PHA column (Fig. 3). Therefore it seems that the enzyme from the spontaneous hepatoma has a bisected N-acetylglucosamine structure similar to that of the enzyme from AH-66 cells.



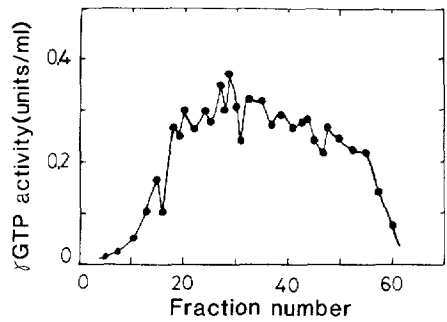


Fig. 1. Chromatography of AH-66 γ-glutamyl transferase on E-PHA agarose. Purified γ-glutamyl transferase (2 μg) from AH-66 cells was loaded on the column of E-PHA agarose, which had been equilibrated with 0.05 *M* Tris—HCl buffer, pH 7.5 containing 0.01% sodium azide and 0.15 *M* sodium chloride. γ-Glutamyl transferase activity of each fraction was assayed as described in Materials and methods. γGTP indicates γ-glutamyl transferase.

Fig. 2. Chromatography of normal rat liver γ-glutamyl transferase on E-PHA agarose. Purified γ-glutamyl transferase (2 μg) from normal rat liver was loaded on the column according to the method described in Materials and methods. γGTP indicates γ-glutamyl transferase.

The enzymes purified from normal human liver and hepatoma were also loaded on the column of E-PHA agarose. As shown in Fig. 4 (upper panel), the enzyme from human hepatoma partially bound to the column. However, the affinity for the column was not as strong as that of the AH-66 enzyme or the spontaneous hepatoma enzyme as described above. We also found that the enzyme from azo-dye-induced hepatoma of rats did not bind to the column so well as those from AH-66 and spontaneous hepatoma, while its desialylated form bound to the column very well (data not shown).

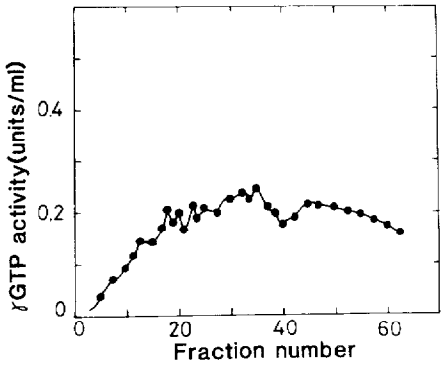


Fig. 3. Chromatography of spontaneous hepatoma of rats on E-PHA agarose. Purified γ-glutamyl transferase from spontaneous hepatoma (2 μg) was loaded on the column according to the method described in Materials and methods. γGTP indicates γ-glutamyl transferase.

Previous studies [16] in our laboratory on the affinity of γ-glutamyl transferase from hepatoma patients for E-PHA agarose indicated that the enzyme from hepatoma patients' sera was retarded on the column, while that from sera of patients with other hepatic diseases was not. Quite recently Hitoi et al. [17]

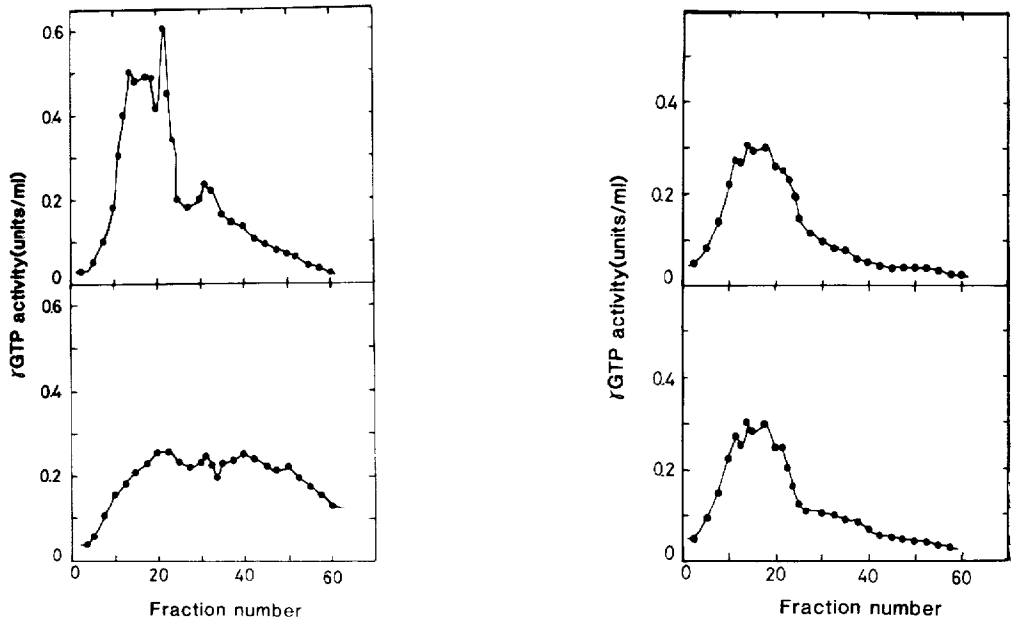


Fig. 4. Chromatography of human hepatoma γ-glutamyl transferase on E-PHA agarose. Purified γ-glutamyl transferase (2 μg) was loaded on the column according to the method described in Materials and methods. Upper panel: untreated enzyme; lower panel: enzyme treated with 0.1 U of neuraminidase at pH 6.8 for 36 h according to the method described in Materials and methods. γ-GTP indicates γ-glutamyl transferase.

Fig. 5. Chromatography of human normal liver γ-glutamyl transferase on E-PHA agarose. Purified enzyme (2 μg) from normal human liver was loaded on the column of E-PHA according to the method described in Fig. 1. Upper panel: untreated enzyme; lower panel: enzyme treated with 0.1 U of neuraminidase at pH 6.8 for 36 h according to the method described in Materials and methods. γGTP indicates γ-glutamyl transferase.

reported that the sialylated enzyme from human sera did not bind to the column, while the desialylated form bound to the column. These reports suggest that this affinity column may be of clinical value for the diagnosis of primary hepatoma.

The enzyme from human hepatoma was treated with neuraminidase and then loaded on the column. As shown in Fig. 4 (lower panel) the neuraminidase-treated enzyme was retarded on the column. On the other hand, neither neuraminidase-treated nor untreated enzyme from normal human liver was retarded on the column (Fig. 5, upper and lower panels). From the above results we could conclude that the bisected N-acetylglucosamine residues of γ-glutamyl transferase from ascites hepatoma AH-66 cells have an affinity for E-PHA agarose, and human hepatoma enzyme may have a sialylated complex type of carbohydrate chain with bisected residues. The treatment of the enzyme from normal rat liver with neuraminidase did not change the pattern or E-PHA agarose chromatography. This indicates that the normal rat liver enzyme does not contain a sialylated carbohydrate chain with bisected N-acetylglucosamine residues. E-PHA chromatography will be useful for obtaining structural information about bisected N-acetylglucosamine residues.

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