

THE SUGAR CHAINS OF γ -GLUTAMYL TRANSPEPTIDASE

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Abstract - Structures of the sugar moieties of γ -glutamyl transpeptidases purified from the kidney and the liver of rat, mouse, and cattle were studied after being chemically released as oligosaccharides. The results indicated that both organ-specific and species-specific differences exist in the sugar chains of the enzyme. Comparative studies of sugar chains of the heavy and light subunits of the rat kidney enzyme revealed that high mannose-type sugar chains are found only in the heavy subunit. By the same analysis of the oligosaccharide fractions obtained from four isozymic forms of the rat kidney enzyme, it was found that all these enzymes contain 2 mole of neutral sugar chains but different numbers of acidic sugar chains in one molecule. Comparative studies of oligosaccharides obtained from the enzymes purified from rat AH-66 hepatoma and from normal rat liver revealed that more than 40% of the sugar chains of the hepatoma enzyme contain bisecting *N*-acetylglucosamine residues which are not found in those of the liver enzyme. By making use of the structural changes associated with malignant transformation, a new diagnostic method of hepatoma was developed. In principle, the method consists of affinity chromatography of the desialylated serum enzyme by an erythroagglutinating lectin agarose column.

INTRODUCTION

There is much evidence indicating that cancer is associated with abnormalities in gene regulation expressed in multiple molecules in the plasma membrane. These molecules appear to be involved in cellular interactions such as control of growth, movement and adhesiveness and in specific binding of extracellular molecules such as hormones and growth factors. Sugar chains bound to polypeptides or to lipids are important components of the plasma membrane. Because the surface sugar chains have been shown to play a role as signals of a number of forms of cellular recognition, their structural changes associated with oncogenesis and ontogenesis of cells are a focus of everincreasing interest. Since sugar chains are synthesized by the concerted action of glycosyl transferases expressed in a cell, there are considerable possibilities that abnormal gene regulation in tumor cells produces sugar chains which are not found in normal cells. Therefore, the sugar chains of various glycoconjugates in tumors can become useful markers for diagnosis and prognosis. γ -Glutamyl transpeptidase (which will be abbreviated in this paper to γ -GTP) is one such example with which we wish to introduce the usefulness of this line of study.

PROBLEMS ASSOCIATED WITH γ -GTP

γ -GTP is a membrane-bound glycoenzyme distributed widely in the plasma membrane of epithelial cells of a variety of organs including kidney, liver, pancreas and brain of many mammals. It catalyzes the first step of glutathione catabolism and is considered to play a role in amino acid transport (1). γ -GTP is composed of two subunits (heavy and light) both of

which contain carbohydrates. The heavy subunit is associated with the plasma membrane of cells by its hydrophobic amino acid cluster located near its N-terminal region. The catalytic site of the enzyme is included in the light subunit which is associated with the heavy subunit. γ -GTP can be solubilized from plasma membrane by treatment with detergent or proteinase. The enzyme solubilized with detergent is a hydrophobic glycoprotein containing the domain that anchors the enzyme to the plasma membrane. On the other hand, γ -GTP solubilized with proteinase is a hydrophilic protein that holds the complete catalytic activity but which has lost the membrane binding segment. The difference in molecular weights of γ -GTP solubilized with detergent and γ -GTP solubilized with proteinase is approximately 5,000 (2), which is about 7% of the molecular weight of γ -GTP solubilized with detergent calculated from the amino acid composition. γ -GTP solubilized by proteinase treatment contained 92% of total hexose of γ -GTP solubilized with detergent.

The presence of isozymic forms of γ -GTP was first reported by Tate and Meister (3). They found that rat kidney γ -GTP can be separated into at least 12 isozymic forms by the isoelectric focusing method. Matsuda et al (4) isolated five of the isozymic forms by DEAE-cellulose column chromatography and compared their structures. All these enzymes gave identical amino acid compositions, identical groups at the amino and carboxy termini and very similar molecular weights. Furthermore, they were all dissociated into two glycoprotein subunits with molecular weights of 40,000 and 23,000. In contrast, all these isozymic forms contain different amounts of sialic acid indicating that the difference in the carbohydrate moiety might account for the isozymic forms.

Although γ -GTPs purified from various organs are immunologically identical, their heterogeneity varied according to tissue (5). The physicochemical characteristics of γ -GTP may also change according to the physiological state of cells, especially in malignant transformation (6-10). Fiala et al (11) found that γ -GTP activity is tremendously elevated in hepatoma induced in rats by feeding 3'-methyl-4-dimethylaminoazobenzene. Since the elevation had been observed in the preneoplastic nodule of liver (12), the enzyme was expected to be a good marker for the diagnosis of hepatoma. γ -GTPs produced in azo dye-induced rat hepatoma (6), rat malignant mammary tissue (8), and human renal carcinoma (13) had more acidic isoelectric points than those of γ -GTPs in the respective normal tissues. Since the differences mostly disappear after sialidase treatment (13), the transformational changes of γ -GTPs could mainly be induced in the carbohydrate moieties of the enzyme molecules.

A large number of recent experiments indicated the occurrence of tissue-specific and species-specific structural differences in the carbohydrate moieties of glycoproteins (14-18). γ -GTP is considered to be a useful glycoprotein for studying this problem more profoundly, because it is widely distributed in various tissues of all mammals.

Despite all this evidence which indicates the importance of studying the structure of the sugar moiety of γ -GTP, no information about the structure of this enzyme was available until 1982.

STRUCTURES OF THE ALL SUGAR CHAINS FOUND IN RAT AND BOVINE KIDNEY γ -GTP

Because of the ease in obtaining large amount of sample, γ -GTPs of rat kidney and bovine kidney were selected to obtain complete structural information of the sugar moiety of γ -GTP. As already described, γ -GTP is composed of a series of isozymic forms. Because of this, care was taken to include all isozymic forms during enzyme purification. This was successfully performed by excluding the ion-exchange column chromatographic procedures. Glucosamine but no galactosamine was found in the acid hydrolysates of both enzymes, indicating that only asparagine-linked sugar chains are present in these enzymes. Therefore, the enzyme preparations were subjected to hydrazinolysis (19) and the released oligosaccharides were converted to tritiated oligosaccharides by reduction with NaB^3H_4 . Since the analytical results obtained from both enzymes (20, 21) were almost the same, only the data of rat kidney γ -GTP will be presented below. By paper electrophoresis, the radioactive oligosaccharides were separated into a neutral and five acidic radioactive peaks (Fig. 1A). Oligosaccharides in the five acidic peaks were completely converted to neutral oligosaccharides by sialidase digestion, indicating that the acidic nature of all these acidic oligosaccharides can be ascribed to their sialic acid residues. Analysis of the six neutral oligosaccharide fractions by gel permeation chromatography revealed that most of them were mixtures of oligosaccharides of different

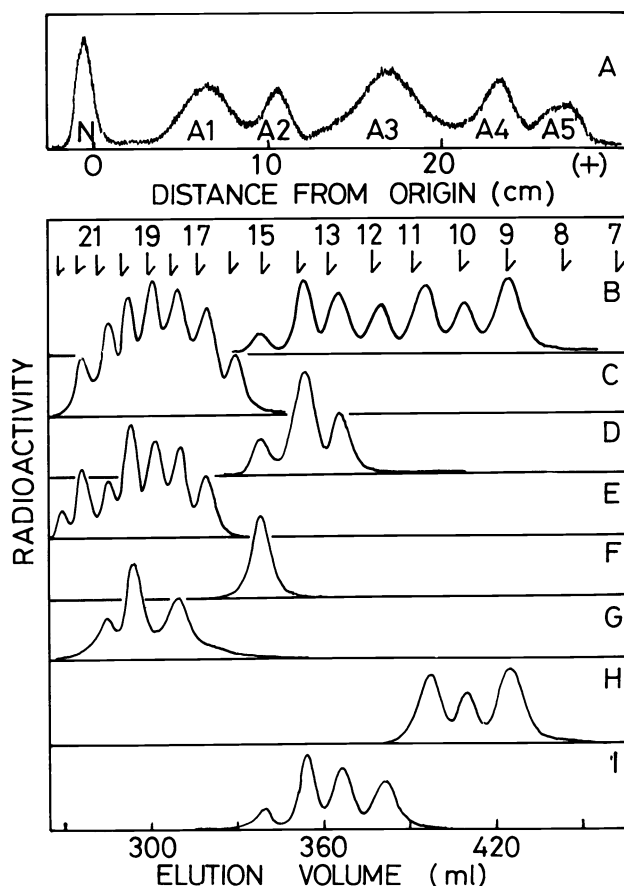


Fig. 1. Electrophoretogram of the radioactive oligosaccharides liberated from rat kidney γ -GTP by hydrazinolysis (A), and gel permeation chromatography of neutral oligosaccharide fractions (B to I). Paper electrophoresis was performed at pH 5.4 and gel permeation chromatography was performed as reported previously (35). The black arrows indicate the eluting positions of glucose oligomers (numbers indicate the glucose units). B, fraction N in (A); C to G, the neutral oligosaccharide fractions obtained by sialidase digestion of fractions A1 to A5 in (A), respectively; H and I, fraction N obtained from the heavy and the light subunits of rat kidney γ -GTP, respectively.

sizes (Fig. 1B-I). These results indicated that the carbohydrate moieties of rat and bovine kidney γ -GTPs are almost identical but are extremely heterogeneous. Structural studies of each fraction in Fig. 1 revealed that they are mixtures of neutral and acidic oligosaccharides as summarized in Fig. 2. Two characteristic features of the sugar chains of both enzymes became apparent by these studies: the one is that the sugar chains are enriched with nonreducing terminal β -N-acetylglucosamine residues and the other is the presence of bisecting N-acetylglucosamine in more than 85% of the sugar chains.

COMPARATIVE STUDIES OF THE SUGAR CHAIN PATTERNS OF TWO SUBUNITS AND OF ISOZYMIC FORMS OF RAT KIDNEY γ -GTP (22)

By comparative study of the sugar chains of the α - and β -subunits of human chorionic gonadotropin, we found that structurally different sugar chains are linked at the four asparagine-loci of this glyco-hormone (23). Since γ -GTP is composed of two glycosylated subunits, it is important to know whether the two subunits have different sets of sugar chains shown in Fig. 1 or not. When the heavy and the light subunits of rat kidney γ -GTP purified by preparative SDS-polyacrylamide gel electrophoresis were subjected to hydrazinolysis, 2 and 3 moles of oligosaccharides were released from 1 mole of each subunit, respectively. Since 5 mole of oligosaccharides were

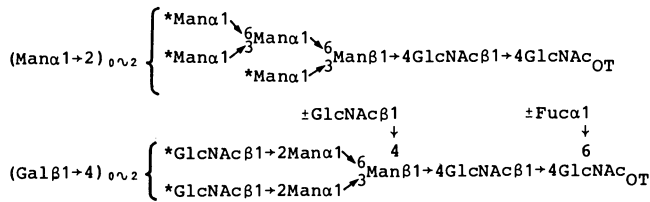
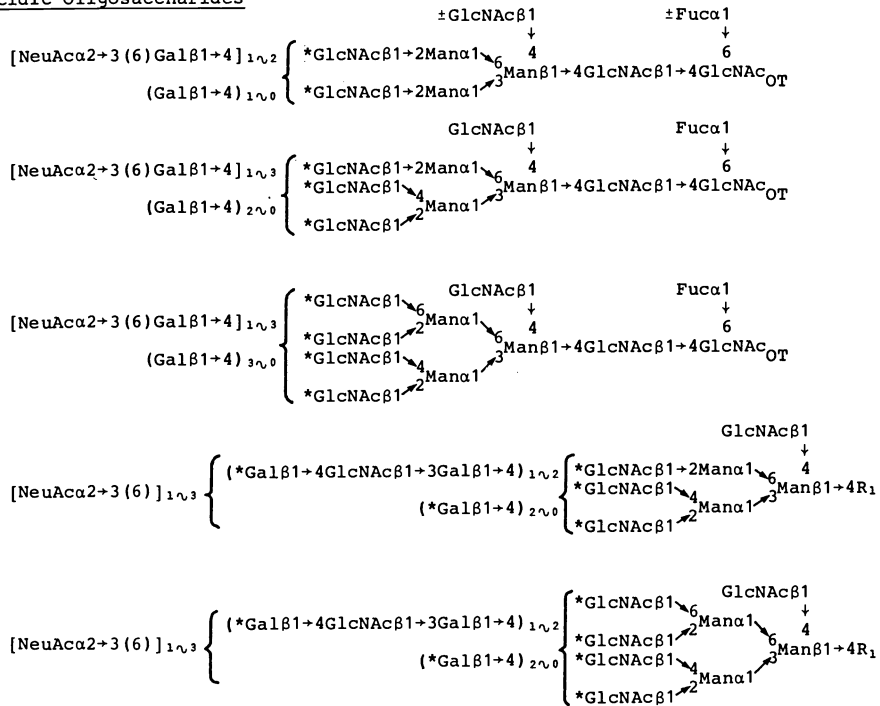
Neutral OligosaccharidesAcidic Oligosaccharides

Fig. 2. Structures of all oligosaccharides liberated from rat kidney γ -GTP by hydrazinolysis. $\text{R}_1 = \text{GlcNac}\beta 1+4(\text{Fuc}\alpha 1+6)\text{-GlcNac}_{\text{OT}}$. The asterisks indicate the locations where outer sugars are linked.

released from 1 mole of whole γ -GTP, the data indicated that quantitative release of the sugar chains was performed. Paper electrophoresis of the radioactive oligosaccharide mixtures obtained from the two subunits were almost the same as shown in Fig. 1A for the whole enzyme. Analysis of the two neutral oligosaccharide fractions by gel permeation chromatography, however, gave completely different elution patterns as shown in Fig. 1H and I. Structural study of each peak in Fig. 1H and I revealed that all high mannose-type sugar chains of γ -GTP are included only in the heavy subunit while non-sialylated biantennary complex-type sugar chains are found only in the light subunit. Such specific distributions of different sugar chains at different asparagine-loci of the glycoprotein molecule were also found in immunoglobulin M (24), porcine pancreatic ribonuclease (25) and bovine blood coagulation factors II and IX (26) together with human chorionic gonadotropin other than -Asn-X-Ser/Thr- sequence influences the processing of the high mannose-type sugar and the formation of the complex-type sugar chain structures. Since the heavy subunit of γ -GTP is attached by its N-terminus to the plasma membrane, a part of its high mannose-type sugar chains might be restricted from further processing by steric effects. As described in section 2, many isozymic forms were found in rat kidney γ -GTP, and the sugar moiety of this enzyme is the most likely cause of the range of pI values. In order to prove this possibility, four isozymic forms of rat kidney γ -GTP with different isoelectric points were subjected to hydrazinolysis. When the radioactive oligosaccharide fractions obtained

TABLE I. Numbers of sugar chains in one molecule of the four isozymic forms of the rat kidney γ -GTP (22).

Isozymic fraction	pI	Total sugar chains	Fractions				Sialic acid
			N ^a	A1+A2 ^a	A3+A4 ^a	A5 ^a	
I	7.5	3.20	2.06	0.89	0.19	0.06	1.39
II	7.0	3.54	2.01	1.17	0.32	0.04	1.89
III	6.7	3.90	1.91	1.42	0.51	0.06	2.56
IV	5.3	5.79	1.97	2.02	1.51	0.29	5.62

The values are expressed in total sugar and in classification by their sialic acid contents. Numbers of sialic acid residues in each isozymic form were calculated from the experimental values.

^a N, A1+A2, A3+A4, and A5 indicate neutral, monosialyl, disialyl, and trisialyl oligosaccharide fractions, respectively. The fraction names are the same as shown in Fig. 1A.

from the four samples were subjected to paper electrophoresis, all of them gave five peaks as shown in Fig. 1A. However, the ratios of the five peaks were different in the four samples. Based on the radioactivity in each peak, the numbers of sugar chains in each peak liberated from one molecule of the four isozymic forms were calculated and summarized in Table I. An interesting result was that all four isozymic forms contain 2 moles of neutral sugar chains per molecule. On the contrary, the number of acidic sugar chains in one molecule of each isozymic form was quite different. In Table I, isoelectric points of the four enzymes are also included. Based on the sialic acid content in each acidic sugar chain, the total number of sialic acid residues in each enzyme was calculated as shown in Table I. The numbers showed a reciprocal relationship to the isoelectric points of isoenzymic forms: an increase of 1 mole of sialic acid corresponding to a decrease of 0.5 in the value of isoelectric point. Therefore, the isozymic forms of rat kidney γ -GTP contain different amounts of sialic acid residues as the result of different contents of acidic sugar chains. It is well established that in N-glycosidically linked glycoproteins, the asparagine residue bearing the sugar chain is invariably part of the tripeptide sequence -Asn-X-Ser- or -Asn-X-Thr-, where X represents one of 20 amino acids except proline and aspartic acid (27). However, asparagine in this sequence is not always glycosylated (28) and one possible explanation for this is that posttranslational folding of the polypeptide chain may inhibit the transfer of the oligosaccharide from the lipid intermediate to the asparagine residue (29). Possibly, the difference in the relative rate of translation and glycosylation is the reason for the difference in the numbers of sugar chains found in the isozymic forms of γ -GTP.

ORGAN-SPECIFIC AND SPECIES-SPECIFIC DIFFERENCES IN THE SUGAR CHAINS OF γ -GTP

Based on the structural similarity of the sugar chains of rat and bovine kidney γ -GTP, we at first considered that the sugar chains of this enzyme might not show species differences. However, comparative studies of the sugar chains of mouse kidney and mouse liver γ -GTPs performed to investigate the organ-specific differences of the sugar chains of the enzyme revealed that the story is not that simple (30). As shown in Fig. 3A and B, the fractionation patterns of the radioactive oligosaccharides obtained from the mouse kidney enzyme and from the liver enzyme were completely different: all oligosaccharides from the liver enzyme contain 1 to 4 sialic acids, while more than 70% of those from the kidney enzyme were neutral. Comparative study of the desialylated oligosaccharides by gel permeation chromatography also indicated that the oligosaccharides from the two enzymes were different (Fig. 3C and D). Preliminary studies by exoglycosidase digestion indicated that the peaks named as bi, tri, and tetra in Fig. 3C and D were bi-, tri-, and tetraantennary complex-type sugar chains, respectively. More detailed structural study of the dotted peak in Fig. 3C revealed that the major oligosaccharides from the mouse liver is a mixture of oligosaccharides having Man α 1 \rightarrow 6 (Man α 1 \rightarrow 3)Man β 1 \rightarrow 4GlcNAc β 1 \rightarrow 4GlcNAc as their core and either

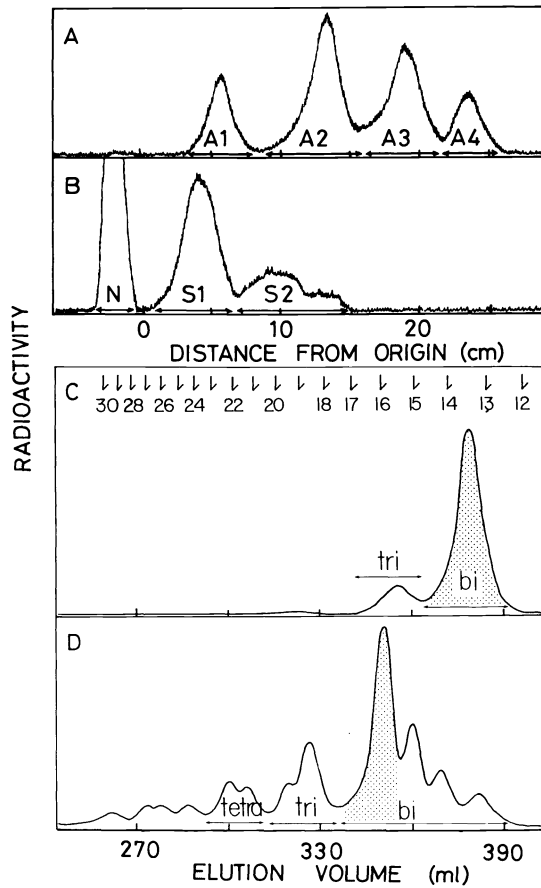
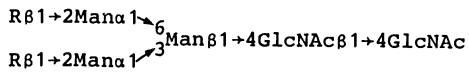


Fig. 3. Electrophoretograms of the radioactive oligosaccharides liberated from mouse liver (A) and kidney (B) γ -GTP by hydrazinolysis, and their gel permeation chromatograms after sialidase digestion (C and D), respectively. Conditions of fractionations and black arrows are the same as described in Fig. 1.

Mouse liver



R represents either $\text{Sia}\alpha 2 \rightarrow 6\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$
 or $\text{Sia}\alpha 2 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3(\text{Sia}\alpha 2 \rightarrow 6)\text{GlcNAc}$.

Mouse kidney

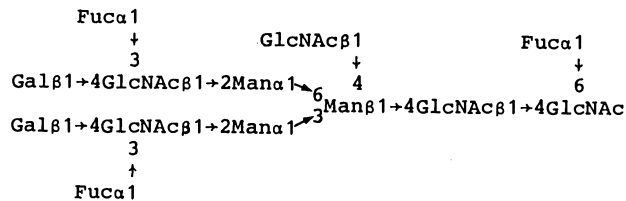


Fig. 4. Structures of the major sugar chains of mouse liver and kidney γ -GTP.

Gal β 1 \rightarrow 4GlcNAc or Gal β 1 \rightarrow 3GlcNAc as their outer chains. It was also found that the more highly sialylated fraction in Fig. 3A contains more Gal β 1 \rightarrow 3GlcNAc outer chain, and the desialylated A-4 contains this outer chain only. These results indicated that the major sugar chains of the mouse liver γ -GTP have structures shown in Fig. 4. The dotted peak in Fig. 3D was found to have the structure as shown in Fig. 4. Since this biantennary peak was found as the major component of the neutral oligosaccharide fraction of the mouse kidney γ -GTP (fraction N in Fig. 3B), it should occur as a non-sialylated form in the enzyme.

These studies indicated that there is a prominent organ-specificity in the structures of sugar chains of γ -GTP. In addition, comparison of the sugar chain structure of the mouse kidney enzyme with those of the rat and bovine kidney enzyme indicated that species-specific differences also exist in the sugar chains of γ -GTP.

STRUCTURAL CHANGES OF THE SUGAR CHAINS OF γ -GTP BY MALIGNANT TRANSFORMATION

As described in the introduction, γ -GTP of malignant tissue behaves differently from its physiological counterpart. Since evidence indicating that the transformational change of γ -GTP is mainly induced in the carbohydrate moiety of the enzyme molecule was reported, comparative studies of the sugar chains of γ -GTPs of normal and malignant tissues are interesting. Such investigations, however, must be performed by using the same tissue because organ-specific differences in the sugar chains of γ -GTP were found. So we carried out comparative study of the sugar chains of γ -GTPs purified from rat liver and from rat ascites AH-66 hepatoma (31).

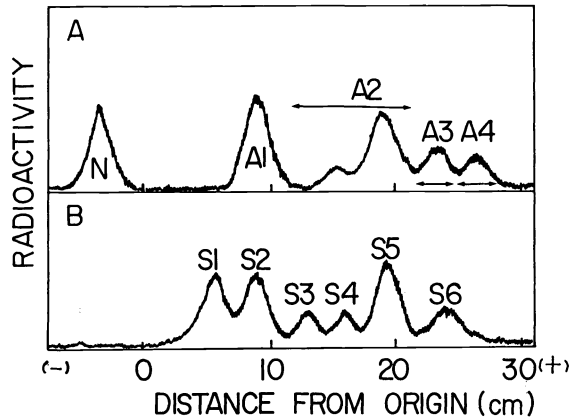


Fig. 5. Electrophoretograms of the radioactive oligosaccharides liberated from AH-66 γ -GTP (A) and normal rat liver γ -GTP (B) by hydrazinolysis. Conditions of paper electrophoresis were the same as in Fig. 1.

When the radioactive oligosaccharide fractions released from the two enzymes by hydrazinolysis were subjected to paper electrophoresis, they gave quite distinct fractionation patterns. The oligosaccharide fraction from AH-66 γ -GTP was separated into a neutral (N) and four acidic (A1 to A4) fractions (Fig. 5A), while that from rat liver enzyme was separated into six acidic (S1 to S6) fractions (Fig. 5B). Since the mouse liver γ -GTP does not contain any neutral sugar chains (Fig. 3A), complete absence of neutral sugar chains is considered as one of the characteristic features of the liver γ -GTP. The acidic oligosaccharides released from the two enzymes were completely converted to neutral oligosaccharides upon exhaustive sialidase

digestion, indicating that the acidic nature of these oligosaccharides can be totally ascribed to their sialic acid residues. The neutral oligosaccharide fraction N from the AH-66 hepatoma enzyme and the neutral oligosaccharide mixture obtained from each acidic fraction from the AH-66 hepatoma and the rat liver enzymes were separated into several oligosaccharide peaks by gel permeation chromatography (data not shown). Based on the structures of the neutral oligosaccharides, the numbers of sialic acid residues in each acidic fraction, and the comparison of the methylation data of acidic fractions before and after sialidase digestion, the whole structures of the sugar chains of the AH-66 γ -GTP and of the liver γ -GTP were elucidated as summarized in Fig. 6.

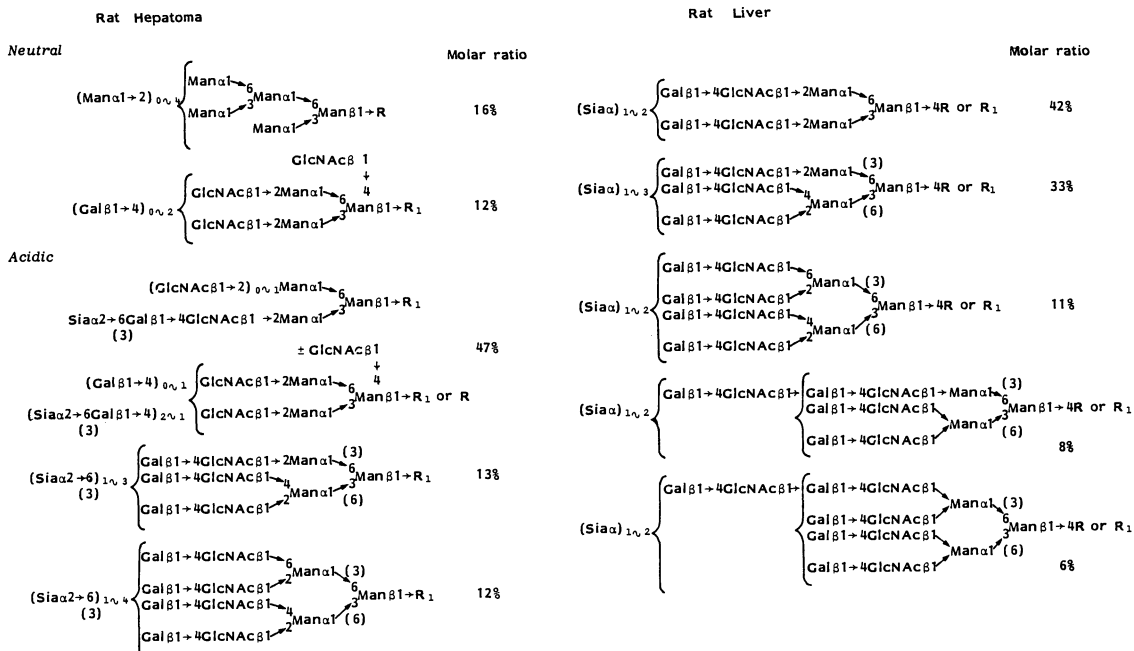


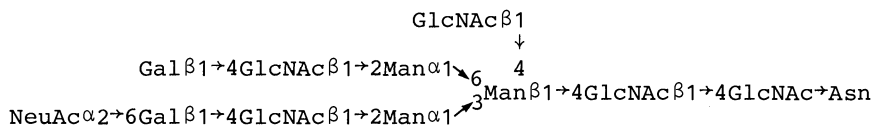
Fig. 6. Structures of sugar chains of γ -GTPs purified from AH-66 cells and from normal rat liver.

More prominent structural differences among the sugar chains of the two enzymes have become evident by this study. Although heterogeneity was observed in the number of sialic acid and fucose residues per molecule, the structures of acidic sugar chains of liver γ -GTP indicated that they are complete bi-, tri-, and tetraantennary complex-type sugar chains. On the contrary, many of the complex-type sugar chains of the hepatoma γ -GTP have incomplete outer chains. Another important finding is that bisecting *N*-acetylglucosamine residues were detected in more than 40% of the total sugar chains of the hepatoma γ -GTP. As already shown, the residue was found in the sugar chains of γ -GTP purified from rat, bovine and mouse kidney. However, it cannot be found in the sugar chains of the mouse and rat liver γ -GTP. Furthermore, none of the glycoproteins synthesized in the liver studied thus far has sugar chains with bisecting *N*-acetylglucosamine residues. Therefore, it is most probable that hepatocytes of most mammals do not contain the *N*-acetylglucosaminyl transferase responsible for the addition of bisecting *N*-acetylglucosamine residues. Therefore, detection of this residue in as much as 39% of the total sugar chains of the AH-66 γ -GTP is important because malignant transformation induces an enzyme which is suppressed in the normal liver tissue. Another important fact is that the total number of asparagine-linked sugar chains in one molecule of the AH-66 γ -GTP was approximately four times higher than that in one molecule of the rat liver γ -GTP. It is improbable that the increase in sugar chains was induced by the structural change of the polypeptide moiety of the γ -GTP, because the enzyme from various tumor and normal tissues showed the same amino acid composition (32) and the same immunogenicity. As already described in a previous section, isozymic forms of γ -GTP with different numbers of sugar chains were found. Therefore, the increase in the number of sugar chains per molecule of the hepatoma γ -GTP might be induced by a change in the biosynthetic process of the γ -GTP molecule. The structural change described here is not restricted to the AH-66

hepatoma, but is distributed widely in hepatoma because the γ -GTP purified from the azo-dye induced hepatoma also have bisected complex-type asparagine-linked sugar chains.

APPLICATION OF THE TRANSFORMATIONAL CHANGE OF γ -GTP SUGAR CHAINS TO THE DIAGNOSIS OF HEPATOMA

As described in the INTRODUCTION, γ -GTP has been expected to be a useful marker for the diagnosis of hepatoma. The γ -GTP level in serum reflects the enzyme level in the liver, and the level is actually elevated in hepatoma. However, because the level of γ -GTP in serum is also elevated in many noncancerous hepatic diseases such as alcoholic hepatitis and biliary obstruction, a high incidence of false-positives is one of the biggest problems in using this enzyme as a diagnostic marker of hepatoma. Cummings and Kornfeld (33) recently reported that E-PHA, a lectin obtained from red kidney bean (*Phaseolus vulgaris*) binds specifically to the following biantennary complex-type sugar chain.



The presence of bisecting *N*-acetylglucosamine residues is essential because non-bisected biantennary oligosaccharides did not show any interaction with

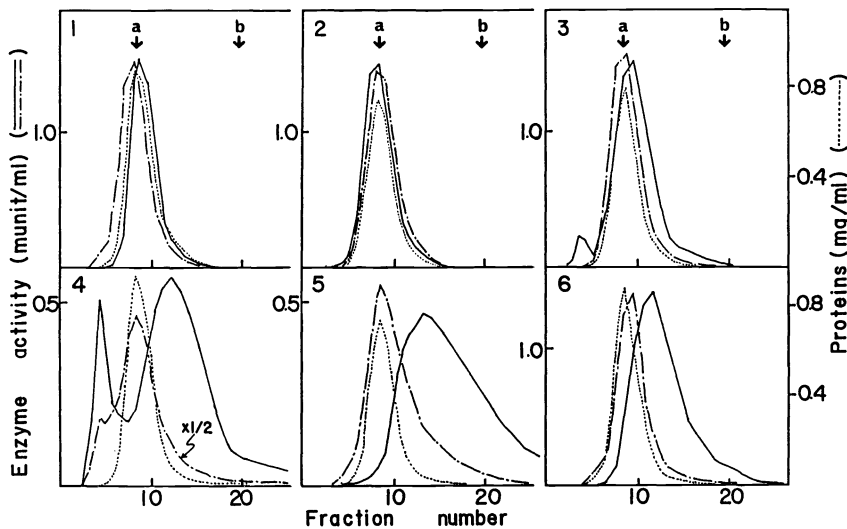
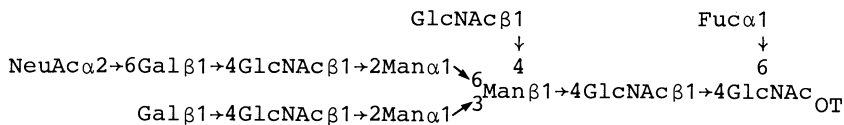


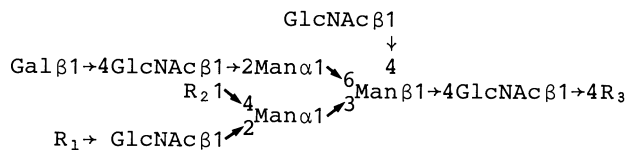
Fig. 7. Chromatography of serum samples on E-PHA agarose column (36). E-PHA agarose purchased from E-Y Laboratories Inc., San Mateo, was packed to make a column of 0.6 cm diameter and 18 cm height and washed thoroughly with phosphate buffered saline, pH 7.4. Serum samples were applied to the column and eluted with the phosphate buffered saline. Fractions (0.53 ml) were collected at 12 ml/h and the protein (— · — · —) and γ -GTP activity (serum samples, -----; desialylated serum samples, —) in each fraction were determined by the method of Lowry et al (37) and of Orlowsky and Meister (38), respectively. Arrows at the top of figures are the eluting positions of authentic oligosaccharides: a, NeuAc $\alpha 2 \rightarrow 6$ Gal $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 2$ Man $\alpha 1 \rightarrow 6$ (GlcNAc $\beta 1 \rightarrow 4$)(Gal $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 2$ Man $\alpha 1 \rightarrow 3$)Man $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 4$ (Fuc $\alpha 1 \rightarrow 6$)GlcNAc_{OT}; b, Gal $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 2$ Man $\alpha 1 \rightarrow 6$ (GlcNAc $\beta 1 \rightarrow 4$)(Gal $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 2$ Man $\alpha 1 \rightarrow 3$)Man $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 4$ (Fuc $\alpha 1 \rightarrow 6$)GlcNAc_{OT}. 1 to 3, sera from non-hepatoma patients with high γ -GTP levels; 4 to 6, sera from hepatoma patients.

the lectin. They also reported that removal of the sialic acid has no effect on the interaction but the two galactoses must be present as parts of the determinant for the lectin-oligosaccharide interaction. Since the binding specificity of the E-PHA is exactly what appeared in hepatic γ -GTP by malignant transformation, we examined the behavior of serum γ -GTP obtained from three hepatoma patients and three non-hepatoma patients with high serum γ -GTP level on an E-PHA-agarose column. When the six serum samples were applied to the E-PHA column, almost all γ -GTP activities were eluted together with the major protein peak, indicating that no retardation was observed in the case of the enzyme from hepatoma patients (Fig. 7, dotted lines).

These results could indicate that the human hepatoma γ -GTP has no bisected complex-type sugar chains which were found in the rat hepatoma γ -GTP. However, while performing the above experiment, we found that the following oligosaccharide isolated from a Bence-Jones protein showed no interaction with the E-PHA-agarose column although it was bound to the column after sialidase digestion (arrows in Fig. 7).



Accordingly, we reinvestigated in detail the behavior of various oligosaccharides with bisecting *N*-acetylglucosamine residues and confirmed that the minimal structural unit required for E-PHA binding is as follows, in which R_1 and R_2 represent either hydrogen or sugars and R_3 represents either $\text{GlcNAc} \rightarrow \text{Asn}$ or $(\text{Fuc}\alpha 1 \rightarrow 6)\text{GlcNAc}_{\text{OT}}$ (34).



Therefore, even if the human hepatoma γ -GTP has bisected complex-type sugar chains, it cannot bind to E-PHA agarose column so long as the galactose residue of the $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 2\text{Man}\alpha 1 \rightarrow 6$ group of the sugar chains is sialylated.

Based on this information, γ -GTPs in the six sera were first desialylated by sialidase digestion and then passed through the E-PHA agarose column. As shown by solid line in Fig. 7, γ -GTP activities in the sera of hepatoma patients were all retarded on the column, while those in the sera of non-hepatoma patients were eluted at the same positions as those before sialidase treatment. These results indicated that the human hepatoma γ -GTP has sialylated complex-type sugar chains with bisecting *N*-acetylglucosamine residues and can be discriminated by its behavior on E-PHA agarose columns from the human liver γ -GTP which lacks the bisecting sugar chain.

CONCLUDING REMARKS

Since abnormalities induced in gene regulation are not simple, the structural changes raised in the sugar chains of a variety of glycoproteins are by no means the same. However, the change does not occur randomly to all glycosidic linkages. Possibly, there are several key glycosyl transferases which play roles in differentiation and are therefore more susceptible to the transformational change of the cells.

As is well documented in the case of γ -GTP, such transformational changes can only be picked up by study of the whole sugar chains of a glycoprotein. Establishment of hydrazinolysis (19), a chemical method for releasing quantitatively the asparagine-linked sugar chains of glycoproteins as oligosaccharides has opened the way to perform such analysis. By using this method, we have recently found that human chorionic gonadotropin produced in choriocarcinoma has very unusual sugar chains which have never been found in normal glycoproteins (39). Careful analysis of the sugar chains of other glycoproteins produced by a variety of tumors may reveal innumerable abnor-

mal structures in the near future. These tumor-specific changes in the sugar chains of glycoproteins will no doubt provide us with new ways for the diagnosis of cancer and its immunological treatment.

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