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Abnormal Isoelectric Focusing Patterns of Serum Galactosyltransferase Activity in Patients with Liver Neoplasia (41759)

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Abstract. Serum galactosyltransferase activity (GT) has been studied using isoelectric focusing chromatography in normal subjects, in patients with non-neoplastic liver diseases, and patients with liver neoplastic diseases (hepatoma and liver metastases) using isoelectric focusing chromatography. Freshly obtained serum was applied to an isoelectric focusing column containing Ampholine (pH 4–6) and the fractions were assayed using asialo-agalactofetuin as the galactose acceptor. Three peaks of GT activity were found in sera from normal subjects at pH 4.8, 4.95, and 5.1. In contrast, sera from patients with hepatoma or with gastrointestinal adenocarcinoma with liver metastases contained only two peaks of GT activity (p*I* 4.75 and 4.95). Sera from patients with non-neoplastic liver diseases had similar GT isoelectricfocusing patterns as those from normal subjects. These results suggest that the isoelectric focusing patterns of serum GT activity may be useful in characterizing human liver neoplasia.

Galactosyltransferase (GT) activity has been detected in various extracellular fluids including serum (1), milk (2), amniotic fluid (3), and malignant effusions (4). In normal human serum, two different types of GT enzymes were identified by their substrate specificity (5). One of the GT species incorporated galactose from uridine diphosphate galactose (UDP-Gal) into sialic acid-free ovine submaxillary mucin, whereas the other GT transferred galactose from UDP-Gal to free *N*-acetylglucosamine or *N*-acetylglucosamine-containing glycoproteins. Elevations of total serum GT activity have been reported in patients with cancer (6–8), but these elevations were neither consistent nor specific. An isoenzyme of serum GT, with slower mobility in polyacrylamide gel electrophoresis, has been described as being specifically related to tumor cells (9). This study, however, has not been confirmed, mainly due to difficulties in reproducing the gel electrophoresis technique because of the very low yield of this slow moving GT activity. We have previously found that GTs from culture media of human hepatoma cells and Chang cells (derived from normal human liver) were different in their elution patterns on a concanavalin A–Sepharose column (10). The present report describes studies on the isoelectric focusing elution profiles of GT activities using sera obtained from normal adults, and patients with non-neoplastic liver diseases, hepatoma, or gastrointestinal adenocarcinoma with or without liver metastases. Isoelectric focusing patterns of GT from human milk, amniotic fluid, and malignant ascites (11) and from human ovarian cyst fluids and serum (12) have been studied. Recently Kessel *et al.* (13) have reported abnormal isoelectric focusing patterns of plasma fucosyltransferase activity in chronic granulocytic leukemia patients.

Materials and Methods. Fetuin (prepared by the Spiro Method) was purchased from Grand Island Biological Co., Grand Island, New York. UDP-galactose was obtained from Sigma, St. Louis, Missouri. UDP-[^{3}H]galactose was from New England Nuclear, Boston, Massachusetts. Ampholine, pH 4–6, was from LKB, Rockville, Maryland. All of the serum samples used for these experiments were freshly drawn, prior to treatment with any chemotherapeutic drugs. Blood was collected in Vacutainers without anticoagulant. After centrifugation, the serum sample was placed in ice briefly and then layered on an isoelectric focusing column.

Isoelectric focusing studies of serum GT activities were performed using 1.5 ml serum

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on an LKB Model 8100 Ampholine column with a capacity of 110 ml and a cooling jacket that maintained a temperature of 4°C. Ampholytes (1% ampholine) with a pH range of 4–6 were stabilized with sucrose in a linear gradient of 5 to 50% (wt/vol). Isoelectric focusing was started with an initial voltage of 400 V and an initial power output of 2 W. Focusing was completed within 20 hr at which time the column was emptied and 2-ml fractions were collected. The pH was determined on each fraction with a Corning pH meter (Model 10).

GT activity assay. The assay method for GT activity has been described (10). The assay mixture for GT activity contained: 10 μl of 0.75 *M* sodium cacodylate, pH 7.0, containing 0.1 *M* $MnCl_2$, 10 μl of 50 mg/ml fetuin free of sialic acid and galactose (SGF-fetuin) (14), 6.5 nmole of UDP-[^{3}H]galactose (24.6 cpm/pmole), and 50 μl of sample. Final volumes were brought to 80 μl with water. Incubations were carried out at 37°C for 1 or 2 hr. The reactions were stopped by adding 1 ml of 10% trichloroacetic acid, and the mixtures were passed through Metrical Membrane filters (Gelman Sciences Inc., Ann Arbor, Mich.). After the filters were washed three times with 1 ml of 10% trichloroacetic acid, they were put into vials with 7.5 ml of Aquasol II (New England Nuclear, Boston, Mass.) and counted in a Beckman LS-250 liquid scintillation spectrophotometer.

Results. Ampholines with a pH range between 3 and 10 were used first and the GT activities obtained from normal and patients' sera after isoelectric focusing were shown to be located between pH 4 and 6 (data not shown). In order to increase the resolution, a pH 4–6 Ampholine was subsequently used in all experiments. Serum obtained from four normal adults was studied (Table 1). A typical pattern of the isoelectric focusing profiles of GT activity from normal serum is illustrated in Fig. 1A. Peaks with GT activity were found at approximately pH 4.80, 4.95, and 5.10. Serum from one of the normal adults was taken on four different days and tested. The isoelectric focusing patterns of the GT activity were shown to be reproducible (data not

TABLE I. SERUM GALACTOSYLTRANSFERASE ACTIVITY AND ITS ISOELECTRIC POINTS

Name	Sex	Disease	GT activity[a] (cpm/hr/10 μl)	GT isoelectric point		
				I[d]	II[d]	III[d]
HE	M	None	1119 ± 79[b]	4.80	4.93	5.08
LI	M	None	1425 ± 121	4.85	4.94	5.15
QI	M	None	1379 ± 118	4.84	4.98	5.12
WA	M	None	1482 ± 93	4.81	4.97	5.10
YU	M	Hepatoma	1160 ± 58	4.85	4.92	—
KA	F	Gastric-liver[c]	1841 ± 106	4.74	4.90	—
SA	M	Colon-liver	1284 ± 97	4.67	4.90	—
CR	M	Colon-liver	1750 ± 134	4.75	4.85	—
AC	M	Colon-liver	1605 ± 54	4.85	5.02	—
HA	M	Colon-liver	1893 ± 84	4.78	4.95	—
NE	F	Colon-liver	1409 ± 10	4.74	4.93	—
FE	F	Pancreas-liver	1885 ± 107	4.77	4.93	—
RD	M	Pancreas-liver	1560 ± 131	4.61	4.89	—
RA	M	Pancreas-liver	1858 ± 115	4.71	4.90	5.18
KU	M	Hepatic cirrhosis	1818 ± 116	4.82	4.95	5.13
DE	F	Hepatic cirrhosis	1446 ± 95	4.76	4.93	5.04
FD	M	Alcoholic hepatitis	1110 ± 78	4.83	4.97	5.10
LE	F	Alcoholic hepatitis	1783 ± 102	4.81	4.96	5.08
DG	F	Alcoholic hepatitis	1361 ± 84	4.85	4.94	5.04

[a] Activity determined in triplicate before isoelectricfocusing.
[b] Means ± SD.
[c] Hyphen indicates metastases from the indicated carcinoma to the liver.
[d] I = approximately p*I* 4.80, II = p*I* 4.95, III = p*I* 5.10.

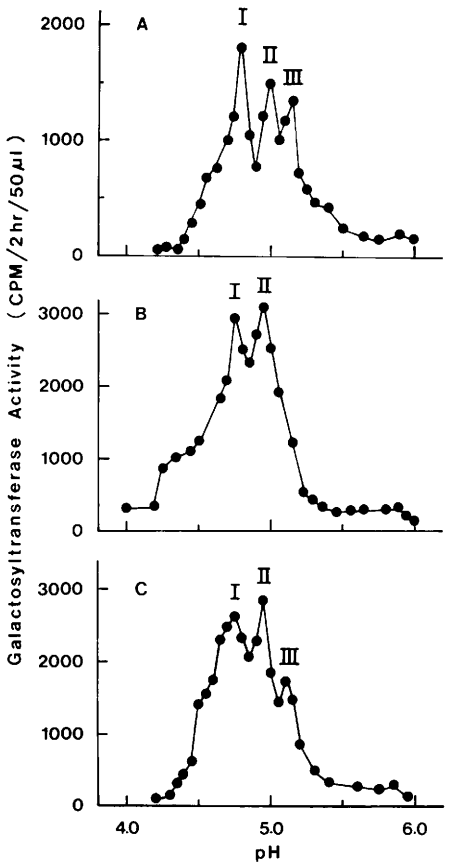

FIG. 1. Representative isoelectric focusing patterns of serum GT activity in (A) a normal adult, (B) a patient with colonic carcinoma with liver metastases, and (C) a patient with alcoholic hepatitis.

shown). However, serum studied after freezing and thawing showed an additional GT activity peak at a lower pH (approximately pH 4.6). Therefore, only freshly drawn blood was used in subsequent experiments. Ampholine and sucrose in the fractions did not affect the GT activity assay since mixtures of 1% Ampholine and different concentrations of sucrose (5, 20, and 30%) showed little inhibitory effect on GT activity (data not shown). The yield of GT activity recovered after isoelectric focusing was at least 85%.

Serum samples from 10 patients with either liver metastases or hepatoma were analyzed (Table 1). Two GT activity peaks, located at approximately pH 4.75 and 4.95, were found in nine of these samples. Only one serum sample from a patient with liver metastases exhibited three peaks of GT activity with pH locations similar to those seen in serum from normal subjects. A typical pattern of serum GT activity from a patient with colon carcinoma with liver metastases is shown in Fig. 1B. Mixing experiments that combined aliquots from the pH 4.75 or 4.95 peaks with aliquots from the pH 5.10 peak did not indicate the presence of a GT inhibitor in the pH 5.10 fraction from serum of a patient with colonic carcinoma with liver metastases (data not shown).

Serum from patients with liver diseases other than malignancies, including three cases of alcoholic hepatitis and two cases of liver cirrhosis, were used as another control group. The results showed that the isoelectric focusing patterns of GT activity from this group (see Fig. 1C for a representative pattern) were similar to that found in normal adults (Fig. 1A). One sample obtained from a patient with colonic cancer, but without liver metastases, also showed an isoelectric focusing pattern of GT activity similar to that found in sera from normal subjects. These results are summarized in Table I.

The range for unfractionated serum GT activity from normal adults with SGF-fetuin as a substrate was 1100–1500 cpm/hr/10 μl (Table 1). The range of GT activity in sera from liver cancer patients was slightly higher (1100–1900 cpm/hr/10 μl) than that from sera of normal adults, and was comparable to that in sera from patients with liver diseases but without liver neoplasia (1100–1800 cpm/hr/10 μl). Serum GT activity was also detected and showed similar isoelectric focusing pattern by using two other substrates, ovalbumin and asialo-mucin, but the activities were less than those assayed using SGF-fetuin (data not shown).

Discussion. Although only a small number of samples were studied, the results suggest that the isoelectric focusing patterns of GT activity in serum of patients with liver cancer are different from those in serum of normal subjects or in serum from patients with non-neoplastic liver diseases. Topping and Watkins (12) found only one peak of GT activity upon isoelectric focusing of human serum. However, their samples were 30–40% ammonium sulfate fractions of serum and the pH range used was 3–10. Even though only one single

peak was apparent in their results, the peak was broad and the p*I*s of individual samples ranged from 4.6 to 5.2.

Compared with the gel electrophoresis technique for studying serum GT isoenzymes (9), the isoelectric focusing method used here is more time-consuming, but the results show that with isoelectric focusing, the total recovery of GT activity is generally greater than 85% and that the GT activity in each fraction is high enough to be assayed easily and reproducibly.

There are still other advantages of this isoelectric focusing method. Enough GT activity can be collected in each fraction that the enzymatic specificities (substrate and type of linkage catalyzed) as well as differences in the specificities among different fractions can be studied. This will allow for the subsequent development of simplified assays. For example, the GT activity of the pH 5.10 peak in normal serum, which is missing in serum from patients with liver cancer, can be studied for its substrate specificity and for the type of linkage that it catalyzes. Specific substrates, such as *N*-acetylglucosamine and *N*-acetylgalactosamine, or specific inhibitors, such as β(1–4)galactosidase from *Streptococcus pneumoniae* (15), may be used to provide a specific assay method for the pH 5.10 GT activity in unfractionated serum.

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