Increase of Fucosylated Serum Cholinesterase in Relation to High Risk Groups for Hepatocellular Carcinomas

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ABSTRACT

Serum cholinesterase (ChE) (E.C. 3.1.1.8) is a glycoprotein which has 36 potential sites of asparagine-N-linked sugar chains. The structures of oligosaccharides released from ChE on hydrazinolysis were studied by serial lectin affinity column chromatography, exoglycosidase digestion, and methylation analysis. Seventy-three percent of the sugar chains occurred as bi-antennary oligosaccharides and the remainder as C-2 and C-2,4/C-2,6 branched tri- and tetra-antennary oligosaccharides. Several percentages of the Lewis A antigenic determinant and fucosylated mannose core were linked to them, and their sialic acid residues were linked to nonreducing terminal galactose residues at the C-3 and C-6 positions.

Aleuria aurantia lectin-reactive ChE with the Lewis A antigenic determinant increased in hepatocellular carcinomas and liver cirrhosis compared with chronic hepatitis; on the other hand, Aleuria aurantia lectin-reactive ChE did not change significantly after transcatheter embolization and was not related to the serum levels of α-fetoprotein and carcinoembryonic antigen in patients with hepatocellular carcinomas. Accordingly, the analysis of Aleuria aurantia lectin-reactive ChE is clinically useful for differentiating liver cirrhosis from chronic hepatitis and to identify high risk groups for hepatocellular carcinomas, i.e., cirrhotic patients in Child's A grade.

INTRODUCTION

Approximately 80% of patients with HCC* in Japan have associated, underlying LC (1), and recently two-thirds of cirrhotic patients were reported to die of an associated HCC (2). Kobayashi et al. (3) examined the following serum risk factors for the development of HCC in patients with LC, i.e., age, sex, Child's classification (4), hepatitis B virus markers, alcohol intake, history of blood transfusion, and family history of chronic liver diseases, and found that Child's A grade is the most significant factor. Thus, the early discrimination of LC from CH is very important. Once the diagnosis of LC is made, the patient is thought to be in a precancerous state and thus must be carefully followed to detect small-sized HCC as early as possible. But thus far, few biochemical markers are available for discriminating LC from CH.

ChE (E.C. 3.1.1.8) formed in the liver is widely measured as a liver function test that shows protein synthetic activity. Although the activity of serum ChE has been reported to decrease in LC or HCC (5), it is impossible to discriminate LC from CH on the basis of serum ChE activity. Since the electrophoretic patterns of serum ChE in patients with LC and HCC to an extent are different from those of serum ChE in patients with CH (6, 7), but the difference disappeared with sialidase digestion (7), it is suggested that the sugar chains of ChE might be altered in patients with LC.

If the structural change of serum ChE in patients with LC can be easily and constantly detected, it may be useful for discriminating LC from CH and for identifying high risk groups for HCC. Because serum ChE consists of four subunits linked through sulphide bonds and each subunit has nine potential asparagine-N-linked sugar chain sites (8), alteration of the sugar chains should be magnified. In the present study, the sugar chain structures of serum ChE and the binding of serum ChE in patients with LC and HCC to an Aleuria aurantia lectin column will be described.

MATERIALS AND METHODS

Lectins, Chemicals, and Antibodies. RCA-I-agarose (4 mg/ml gel), LCA-agarose (4 mg/ml gel), L-PhA, E-PhA, and MAL were purchased from Hohnen Oil Corp. (Tokyo, Japan). ConA-Sepharose was from Pharmacia Biotechnology, Inc. (Upssala, Sweden). AAL-Sepharose (7 mg/ml gel), DSA-Sepharose (3 mg/ml gel), TJA-1-Sepharose (3 mg/ml gel), L-2,4-PhA-Sepharose (9.8 mg/ml gel), E-2,4-PhA-Sepharose (4.8 mg/ml gel), and MSA-Sepharose (10.7 mg/ml gel) were prepared according to the CNBr-method (9).

Received 6/17/93; accepted 10/29/93.

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This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and Special Funds from the Science and Technology Agency of the Japanese Government.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: HCC, hepatocellular carcinoma; LC, liver cirrhosis; CH, chronic hepatitis; CH, cholinesterase; RCA-I, Ricinus communis agglutinin-I; LCA, lentil lectin; L-PhA, phytohemagglutinin-L; E-PhA, phytohemagglutinin-E; MAL, Mucous amurensis lectin; ConA, concanavalin A; AAL, Aleuria aurantia lectin; DSA, Datura stramonium agglutinin; TJA-I, Trichosanthes japonica agglutinin-I; i.d., inside diameter; GlcNAc, N-acetylglucosamine; Man, mannose; Neu, neuraminic acid.

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Purification of Serum/Plasma ChE. Ammonium sulfate fractionation of 500 ml of plasma was performed, and the fraction that precipitated between 50 and 65% saturation was resuspended in 100 ml of 0.1 M phosphate buffer (pH 6.7) and then dialyzed against the same buffer. One-fifth of the enzyme solution was applied to a Blue-Sepharose CL-6B column (3.5 i.d. x 15 cm long) equilibrated with 0.1 M phosphate buffer (pH 6.7). The resulting pass-through fraction containing ChE was then applied to a ConA-Sepharose column (1.5 i.d. x 30 cm long) equilibrated with 0.1 M phosphate buffer (pH 6.7) containing 0.15 M NaCl. The eluate with 0.4 M methyl-α-d-mannopyranoside was concentrated and then dialyzed against 25 mM histidine-HCl buffer (pH
6.3). The dialyzed sample was applied to a chromatofocusing column of PBE 94 gel (1.6 i.d. x 50 cm long) equilibrated with the above buffer, and then the column was eluted isocratically with 8 times-diluted polybuffer 74 (pH 3.0). The ChE eluted at pH 3.8 was concentrated and applied to a TSK G4000 SW column (0.75 i.d. x 30 cm long) equilibrated with 0.1 M phosphate buffer (pH 6.7) containing 0.15 M NaCl. The enzyme fraction was pooled, concentrated, and then stored at -20°C. The reduced purified ChE gave a single band at M, 80,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The results of purification are summarized in Table 1.

Release of Asparagine-N-linked Sugar Chains from Serum ChE. Serum ChE (2 mg) was subjected to hydrazinolysis at 100°C for 8 h as reported previously (15). After N-acetylation, one half of the released oligosaccharides was reduced with NaB3H4 and the remaining half was reduced with NaB2H4 for methylation analysis. The total yield of radioactive oligosaccharides was 1.1 × 106 cpm.

Affinity Chromatography of Radioactive Oligosaccharides or Serum ChE on Immobilized Lectin Columns. Columns containing 1 ml of RCA-1-agarose, TJA-I-Sepharose, ConA-Sepharose, E4-PHA-Sepharose, L4-PHA-Sepharose, DSA-Sepharose, AAL-Sepharose, MAL-Sepharose, and LCA-agarose, TJA-I-Sepharose, ConA-Sepharose, E4-PHA-Sepharose, L4-PHA-Sepharose, DSA-Sepharose, AAL-Sepharose, MAL-Sepharose, and LCA-agarose were equilibrated with 10 ml Tris-HCl (pH 7.4) containing 0.02% NaN3 and 0.15 M NaCl, respectively. Tryptically labeled oligosaccharides or serum ChE dissolved in 100 μl of Tris-HCl were applied to a column, which was then kept at 4°C for 15 min. Elution was started with 4-10 ml of Tris-HCl at 4°C, followed by 5 ml of Tris-HCl containing 10 mM lactose (RCA-I column), 0.1 M lactose (TJA-I column), 0.4 mM lactose (MAL column), 5 mM methyl-α-D-glucopyranoside (ConA column), 1% N-acetylglucosamine oligomers (DSA column), 5 mM 1-fucose (AAL column), and 0.2 mM methyl-α-D-mannopyranoside (LCA column), respectively, at 20°C.

Glycosidase Digestion. Radioactive oligosaccharides were digested in one of the following ways at 37°C for 17 h except for Salmonella sialidase: Salmonella sialidase digestion, 10 milliunits of enzyme in 0.2 M citrate-phosphate buffer, pH 6.0 (20 μl) for 2 h; Arthrobacter, 100 milliunits of enzyme in sodium acetate buffer, pH 5.0 (40 μl); digestion with a mixture of diplococcal β-galactosidase and jack bean β-N-acetylhexosaminidase, 2 milliunits of β-galactosidase and 1 unit of β-N-acetylhexosaminidase in 0.2 M citrate-phosphate buffer, pH 5.5 (20 μl); and digestion with almond α-L-fucosidase, 40 micromunits of α-L-fucosidase in 0.2 M citrate-phosphate buffer, pH 6.0 (10 μl). The other enzyme digestions were performed according to the procedures described previously (14).

Analytical Methods. Methylation analysis of oligosaccharides was performed as reported previously (16). Analysis of partially O-methylated hexitols and N-acetylglucosaminol, which was performed with a gas chromatograph-mass spectrometer (Model GC-MS JMS-SX 102; Japan Electron Optics Laboratory, Tokyo) equipped with a fused silica capillary column coated with cross-linked SPB-35 (0.25 mm i.d. x 30 m long) (17). High-voltage paper electrophoresis was performed with pyridine-acetate buffer, pH 5.4 (pyridine/acetic acid/water, 1:3:187) at a potential of 73 V/cm for 90 min. Radiochromatography was performed with a Raytest radiochromatogram scanner (Model RITA-90).

Bio-Gel P-4 (<45 μm) column chromatography (2 cm i.d. x 1.25 m long) was performed as reported previously (18). Radioactivity was determined with a Beckman liquid scintillation spectrometer (Model LS-6000 LL).

Serum Samples. Serum samples were obtained from 50 patients with HCC or control patients. The serum samples were stored at -30°C until used. The diagnoses of liver diseases were made on the bases of the results of liver function tests, ultrasonography, computed tomography, angiography, and histological examination of liver specimens obtained at biopsy or operation. Serum ChE activity was measured with ChE B-test Wako (Wako Pure Chemical Co., Osaka, Japan). One IU was taken as the amount of enzyme which hydrolyzed 1 μmol of benzoylcholine chloride in 1 min at 37°C. The concentrations of α-fetoprotein and carcinoembryonic antigen in the samples were determined by an ELISA.

RESULTS

Structural Studies of Oligosaccharides Released from Serum ChE

Paper Electrophoresis of Tritium-labeled Oligosaccharides Released from Serum ChE. When the tritium-labeled oligosaccharides released from serum ChE were subjected to paper electrophoresis, they were separated into five acidic fractions (A1, A2, A3, A4, and A5) as shown in Fig. 1A. The percentage molar ratio of A1, A2, A3, A4, and A5 was calculated to be 39:14:24:11:12 from the radioactivities. A part of these acidic oligosaccharides was adsorbed to a TJA-I column, which specifically interacts with Neu5Acα2→6Galβ1→4GlcNAc group (Ref. 19; Fig. 1B). The remaining acidic oligosaccharides were digested with a mixture of diplococcal β-galactosidase, almond α-L-fucosidase and jack bean β-N-acetylhexosaminidase, which specifically hydrolyzes Galβ1→4GlcNAc and Galβ1→4(Fucα1→3)GlcNAc groups, the radioactive neutral fractions were converted to two radioactive oligosaccharides.

<table>
<thead>
<tr>
<th>Table 1 Purification of cholinesterase from human plasma</th>
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<tr>
<td><strong>Step</strong></td>
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<tr>
<td>Plasma</td>
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<tr>
<td>(NH4)2SO4 (50-65%)</td>
</tr>
<tr>
<td>Blue-Sepharose</td>
</tr>
<tr>
<td>Con A-Sepharose</td>
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<tr>
<td>Chromatofocusing</td>
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<td>G 4000 SW</td>
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*Based on the hydrolysis of benzoylcholine chloride (Cholinesterase B-test Wako). One IU was taken as the amount of enzyme which cleaved 1 mol of substrate in 1 min at 37°C.
Mannitol as 1.0.

were further confirmed to be Man\[^{\beta1\rightarrow6}\text{Man}\]\[^{\alpha3\rightarrow3}\text{Man}\] and Man\[^{\alpha1\rightarrow6}\text{Man}\]\[^{\alpha3\rightarrow3}\text{Man}\] as judged on analysis of digested oligosaccharides. These results indicated that the oligosaccharides comprise complex type sugar chains with a fucosylated or nonfucosylated mannose core and contain different numbers of Gal\[^{\beta1\rightarrow4}\text{GlcNAc}\] or Gal\[^{\beta1\rightarrow4}\text{Fuc}\] residues substituted at the C-2, C-2 and C-4, and C-2 and C-6 positions, respectively, and all galactose residues occur as nonreducing termini, indicating that the Gal\[^{\beta1\rightarrow4}\text{GlcNAc}\beta1\rightarrow3\text{Gal}]

Methylation analysis of desialylated oligosaccharides (AN) obtained from serum ChE. The tritium-labeled sugars as described in "Materials and Methods." The resultant fractions were then subjected to Bio-Gel P-4 column chromatography. These neutral oligosaccharides were separated into a pass-through fraction (ConA\(^{-}\)), and a fraction that bound to the column and was eluted with 5 mM methyl-\(\alpha\)-glucopyranoside (ConA\(^{+}\)) (Fig. 2B). The ConA\(^{+}\) fraction passed through a DSA-Sepharose column (Fig. 2C) and was retarded on an E\(_4\)-PHA-Sepharose column (Fig. 2D). On an AAL-Sepharose column, the ConA\(^{+}\) fraction was separated into a pass-through fraction (AAL\(^{-}\)), and a fraction that bound to the column and was eluted with 5 mM l-fucose (AAL\(^{+}\)) (Fig. 2E). These fractions were named fractions I and II, respectively (Fig. 2E). The ConA\(^{+}\) fraction was separated into three fractions on a DSA-Sepharose column chromatography, which were named the DSA\(^{-}\), DSA\(^{+}\) and DSA\(^{+}\) fractions, respectively (Fig. 2F). The DSA\(^{+}\) fraction was retarded on an L\(_4\)-PHA-Sepharose column (Fig. 2G) and passed through an AAL-Sepharose column (Fig. 2H). The DSA\(^{+}\) fraction was only retarded on an E\(_4\)-PHA-Sepharose column (Fig. 2I) passing through an AAL-Sepharose column (Fig. 2J). These DSA\(^{-}\) and DSA\(^{+}\) fractions were named fractions III and IV, respectively. The DSA\(^{+}\) fraction in Fig. 2F was retarded on an AAL-Sepharose column (Fig. 2K) and passed through an L\(_4\)-PHA-Sepharose column (Fig. 2L). On an E\(_4\)-PHA-Sepharose column, the L\(_4\)-PHA\(^{+}\) fraction in Fig. 2L was separated into a pass-through fraction and a retarded fraction, which were named fractions V and VI, respectively (Fig. 2M). Fractions V and VI were digested with almond \(\alpha\)-fucosidase I, which specifically hydrolyzes Fuc\[^{\alpha1\rightarrow3}\text{GlcNAc}\] or Fuc\[^{\alpha1\rightarrow3}\text{GlcNAc}\] (21), and then were analyzed on a DSA-Sepharose column. Fraction V bound to the column and was eluted with 1% N-acetylglucosamine oligomers and was named fraction V' (Fig. 2N). Fraction VI was retarded on the column (Fig. 2O) and was named fraction VI'.

When the six fractions (I–VI; Fig. 2) were analyzed by Bio-Gel P-4 column chromatography (18), fractions I, II, IV, V, and VI gave symmetrical single peaks, and fraction III was separated into two components, IIIa and IIIb, as summarized in Table 3.

### Structures of the Neutral Oligosaccharides

Structural analysis revealed that fractions I–VI contained one oligosaccharide, respectively. Therefore, they will be called oligosaccharides I, II, IIIa, IIIb, IVa, IVb, V, and VI. The structures of the neutral oligosaccharides are shown in Table 3.
### Table 3 Behavior on several lectin columns, glucose units on a Bio-Gel P-4 column, numbers of released N-acetyllactosamine residues, and proposed structures of oligosaccharides I–VI

<table>
<thead>
<tr>
<th>Oligosaccharides</th>
<th>Behavior on lectin columns&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Glucose units&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Numbers of released N-acetyllactosamine residues&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Proposed structures</th>
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<tbody>
<tr>
<td></td>
<td>ConA</td>
<td>DSA</td>
<td>E&lt;sub&gt;4&lt;/sub&gt;-PHA</td>
<td>L&lt;sub&gt;4&lt;/sub&gt;-PHA</td>
</tr>
<tr>
<td>I (67.5)</td>
<td>+</td>
<td>-</td>
<td>r</td>
<td>-</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>II (5.5)</td>
<td>+</td>
<td>-</td>
<td>r</td>
<td>-</td>
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<td></td>
<td></td>
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<tr>
<td>IIIa (3.8)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>r</td>
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<tr>
<td>IIIb (V') (2.1)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>r</td>
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<td></td>
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<tr>
<td>IV (VI') (14.9)</td>
<td>-</td>
<td>r</td>
<td>r</td>
<td>-</td>
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<tr>
<td>V (2.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>r</td>
</tr>
<tr>
<td>VI (3.9)</td>
<td>-</td>
<td>-</td>
<td>r</td>
<td>r</td>
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<sup>a</sup> +, −, r indicate bound, passed through, and retarded on the respective lectin columns, respectively.

<sup>b</sup> Glucose units indicate the effective sizes of oligosaccharides on a Bio-Gel P-4 column.

<sup>c</sup> Numbers of released N-acetyllactosamine residues on digestion with a mixture of diplococcal β-galactosidase and jack bean β-N-acetylhexosaminidase.

<sup>d</sup> R<sub>1</sub> indicates 4GlcNAcβ1→4GlcNAc<sub>α</sub>.

<sup>e</sup> R<sub>2</sub> indicates 4GlcNAcβ1→4(Fucα1→6)GlcNAc<sub>α</sub>.

<sup>f</sup> Oligosaccharides V and VI digested with almond α-L-fucosidase were used.

IV, V, and VI, respectively. On digestion with a mixture of diplococcal β-galactosidase and jack bean β-N-acetylhexosaminidase, different numbers of N-acetyllactosamine residues were released from oligosaccharides I, II, IIIa, IIIb, and IV, and α-L-fucosidase digested oligosaccharides V and VI (V' and VI'). By comparing the mobility of each oligosaccharide before and after these glycosidase digestions, the numbers of N-acetyllactosamine residues released from oligosaccharides I, II, IIIa, IIIb, IV, V', and VI' were calculated to be 2, 2, 4, 3, 3, and 3, respectively (Table 2), and then these enzyme-digested oligosaccharides I, IIIa, IIIb, IV, V', and VI' were converted to tri-mannosyl N,N'-diacetyltobiotol; and oligosaccharide II was converted to fucosylated tri-mannosyl N,N'-diacetyltobiotol (data not shown). The glycosidic linkages and locations of the outer chains in these seven oligosaccharides were determined by methylation analysis and from the behavior of the oligosaccharides on immobilized Con<sub>A</sub>, DSA, E<sub>4</sub>-PHA, L<sub>4</sub>-PHA, and AAL columns (Table 3). Oligosaccharides containing the Galβ1→4GlcNAcβ1→2Man01→6-Manβ1→4GlcNAcβ1→4GlcNAc<sub>α</sub> group are retarded on an L<sub>4</sub>-PHA column and adsorbed to a DSA column and eluted with 1% N-acetylgalactosamine oligomers (22).
Sugar Chain Structural Changes of Serum Cholinesterase in Patients with Hepatocellular Carcinomas and Benign Liver Diseases

The activity of serum ChE has been reported to decrease in LC; however, it is impossible to discriminate LC from CH on the basis of serum ChE activity. We preliminarily investigated, by means of several lectin column chromatographies, whether the sugar chain structures of serum ChE are altered in HCC and benign liver diseases. All the serum ChE in patients with HCC, LC, CH, and in the healthy individuals bound to RCA, MAL, ConA, TJA-I, and DSA columns (data not shown). Interestingly, the rate of binding of serum ChE to an AAL-Sepharose column greatly increased in HCC and LC compared to that of CH and healthy individuals. Because fucosylation of the sugar chains is the molecular basis for AAL-reactive variation of serum ChE, in an attempt to increase the efficiency of discriminating CH from LC, ChE was studied by means of AAL-Sepharose column chromatography.

AAL-binding Serum ChE in Patients with HCC and Benign Liver Diseases. The percentages of AAL-binding serum ChE in patients with HCC and benign liver diseases are shown in Fig. 3. The sera studied were from 50 patients with HCC, 47 with LC, and 40 with CH. The mean percentages of AAL-binding serum ChE were 60.1 ± 17.2% (SD) in HCC, 57.3 ± 17.5% in LC, 21.1 ± 8.0% in CH, and 16.9 ± 5.4% in healthy individuals (Fig. 3), and the percentage of fucosylated ChE was significantly higher in LC and HCC than in CH and healthy individuals (P < 0.01). The percentage of AAL-binding ChE in HCC was the same as in LC. Because 80% of HCC patients (42 of 50) had cirrhotic lesions in their livers, AAL-binding ChE may

Oligosaccharides containing the Galβ1→4GlcNAcβ1→2Manβ1→6Glcβ1→4GlcNAcβ1→4GlcNAcOT group are retarded on a DSA column (24); oligosaccharides with two C-2 substituted α-mannosyl residues bind to a ConA-Sepharose column and can be eluted with 5 mM methyl-α-glucopyranoside (22); oligosaccharides with a Lewis X antigenic determinant are retarded on an AAL column (25); and oligosaccharides with fucosylated proximal N-acetylgalactosaminol at the C-6 position bind to an AAL column (25). Accordingly, ConA-Eα-PHA′ oligosaccharide I should be a bi-antennary component with a nonfucosylated mannose core; ConA-Eα-PHA′AAL+ oligosaccharide II a bi-antennary component with a fucosylated mannose core; DSA′-Lα-PHA′ oligosaccharide IIIa a C-2,6 and C-2,4 branched tetra-antennary component; DSA′-Lα-PHA′ oligosaccharide IIIb a C-2,6 and C-2 branched tri-antennary component; and DSA′Eα-PHA′ oligosaccharide IV a C-2,4 and C-2 branched tri-antennary component. AAL+ oligosaccharide V was converted to a DSA′ component (fraction V′; Fig. 2N) on almond α-L-fucosidase I digestion, indicating that it is a C-2,6 and C-2 branched tri-antennary component with a Lewis X antigenic determinant. From the results so far described, the structures of oligosaccharides I–VI are proposed to be as shown in Table 3.
be produced in liver cirrhotic tissue and may be indicative of a predisposition to HCC. This hypothesis was supported by the finding that the percentage of AAL-binding ChE was not correlated with the level of serum α-fetoprotein or carcinoembyonic antigen (data not shown). When the changes in tissue AAL-binding ChE percentage in 10 patients with HCC were examined before and after transcatheter arterial embolization treatment, the percentage of AAL-binding ChE did not change with the treatment except in one case (data not shown).

**DISCUSSION**

This report deals with the structures of N-linked sugar chains of serum ChE and the increase of AAL-reactive serum ChE in relation to high risk groups for HCC.

The sugar chains of serum ChE were determined to be sialylated bi-, tri-, and tetraantennary oligosaccharides containing a trace amount of a Lewis X antigenic determinant and a fucosylated mannose core, although it could not be determined in this study whether the Lewis X antigenic determinant is sialylated. These structures are generally observed in serum glycoproteins, including ceruloplasmin, α1-antitrypsin, orosomucoid, etc., which are produced in parenchymal cells of human liver and secreted (27). Furthermore, it was elucidated in this study that AAL-reactive serum ChE increases in LC and HCC with LC and that the measurement of AAL-binding serum ChE is useful not only for discriminating LC from CH but also for identifying high risk groups for HCC according to the results of Kobayashi et al. (3).

An increase of sugar chains containing Lewis X antigenic determinants had already been found in serum transferrin from patients with HCC developing after LC (28). Because fucosylated transferrin is also produced in cirrhotic liver cells and Galβ1→4(Fucα1→3)GlcNAc residue group (Lewis X antigenic determinant) and the Fucα1→4GlcNAc group are present in the sugar chains of serum ChE (see Table 3), the type of fucosyl residues in serum ChE which increases in high risk groups for HCC should be determined. LCA specifically interacts with →2(→6)Manα1→6(→2Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc→Asn (26). Accordingly, the problem should be resolved by AAL and LCA serial lectin column chromatographies. After the intact serum ChE in patients with HCC, LC, CH, and in a healthy individual had been separated on an AAL-Sepharose column, the respective AAL+ ChE was sequentially applied to an LCA-agarose column. As shown in Fig. 4, most AAL+ ChE in a patient with CH and in a healthy individual was bound to an LCA-agarose column and eluted with 0.2 M methyl-α-mannopyranoside; on the other hand, the AAL+ ChE in patients with HCC and LC was separated into LCA+ and LCA− fractions, indicating that AAL "LCA+ ChE should have oligosaccharides with a fucosylated mannose core (oligosaccharide II; Table 3), and AAL−LCA+ ChE should have oligosaccharides containing a Lewis X antigenic determinant (oligosaccharides V and VI; Table 3). The percentage of AAL-reactive ChE in patients with HCC, LC, and CH did not alter by desialylation compared to that of intact ChE (data not shown). However, it could not be determined whether the Lewis X antigenic determinants were sialylated because we have not yet analyzed whether neutral oligosaccharides and sialylated oligosaccharides differ in their affinity to AAL column by using standards.

The serum concentrations of total ChE, AAL+LCA+ ChE, AAL+LCA− ChE, and AAL−LCA− ChE in patients with HCC and LC (n = 14), LC (n = 21), CH (n = 17), and in healthy individuals (n = 16) were investigated. As shown in Fig. 5 and Table 4, AAL+LCA+ ChE containing a fucosylated mannose core did not change or rather decreased in HCC and LC, and total serum ChE greatly decreased; on the other hand, AAL+LCA− ChE containing a Lewis X antigenic determinant increased about 5 times in HCC and LC, in comparison with CH and healthy individuals. These results indicate that the analysis of AAL-reactive ChE is clinically useful for differentiating LC from CH and for identifying high risk groups for HCC.

**Table 4** Mean ± SD serum activity (IU/liter) toward AAL+LCA+, and AAL−LCA− ChE in hepatic diseases

<table>
<thead>
<tr>
<th>Hepatic diseases</th>
<th>Total ChE</th>
<th>AAL+LCA+ ChE</th>
<th>AAL+LCA− ChE</th>
<th>AAL−LCA− ChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC (n = 14)</td>
<td>683.6 ± 235.6</td>
<td>251.2 ± 57.0</td>
<td>145.0 ± 38.8</td>
<td>287.4 ± 181.3</td>
</tr>
<tr>
<td>LC (n = 21)</td>
<td>647.4 ± 282.2</td>
<td>212.3 ± 93.2</td>
<td>134.7 ± 51.8</td>
<td>300.4 ± 214.9</td>
</tr>
<tr>
<td>CH (n = 17)</td>
<td>1421.6 ± 391.0</td>
<td>44.0 ± 36.3</td>
<td>230.5 ± 82.9</td>
<td>1147.1 ± 344.4</td>
</tr>
<tr>
<td>NC (n = 16)</td>
<td>1320.6 ± 334.0</td>
<td>41.4 ± 44.0</td>
<td>199.4 ± 38.8</td>
<td>1320.6 ± 334.0</td>
</tr>
</tbody>
</table>

4 Manuscript in preparation.
teins produced in cirrhotic tissues may be similar to that of the stage-specific embryonic antigen, which is related to cell compaction in the morula (32, 33).

REFERENCES


Increase of Fucosylated Serum Cholinesterase in Relation to High Risk Groups for Hepatocellular Carcinomas

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