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A novel method for determination of α1,6fucosyltransferase activity using a reducing oligosaccharide from egg yolk as a specific acceptor

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A new method for determination of α1,6fucosyltransferase activity has been described. Recently, the disialyl-biantennary undecasaccharide was prepared in high yield from egg yolk [(1996), *Carbohydr Lett* 2: 137–42]. By treatment of this oligosaccharide with neuraminidase and β-galactosidase, we readily obtained an asialo-agalacto-biantennary heptasaccharide (GlcNAcβ 1,2Manα1,6[GlcNAcβ1,2Manα1,3]Manβ1,4GlcNAcβ1,4GlcNAc). Using this asialo-agalacto-oligosaccharide as an acceptor, fucosyltransferases from human plasma and extracts of various human hepatoma cell lines were assayed in the presence of GDP-[^{3}H]fucose. The reaction mixture was applied to a column of GlcNAc-binding, *Psathyrella velutina* lectin coupled gel. All the fucosylated acceptor were bound to the column which was eluted with 50 mM GlcNAc. Structural analyses revealed that only the innermost GlcNAc residue of the acceptor was fucosylated through an α1,6-linkage, and the oligosaccharide prepared could be used as a specific acceptor for α1,6fucosyltransferase. The present method was used to screen plasma α1,6fucosyltransferase in several patient groups, and significantly elevated activities were found in samples from patients with liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma.

Keywords: **α1,6fucosyltransferase, GlcNAc-binding lectin, Fuc-binding lectin, egg yolk oligosaccharide**

Abbreviations: **GlcNAc, *N*-acetyl-D-glucosamine; Man, D-mannose; Fuc, L-fucose; Gal, D-galactose; NeuAc, *N*-acetylneuraminic acid; PVL, *Psathyrella velutina* lectin; AAL, *Aleuria aurantia* lectin; LCA, *Lens culinaris* agglutinin; ASAG, asialo-agalacto; YDS, yolk di-sialyloligosaccharide; DEY, delipidated egg yolk; AFP, alpha-fetoprotein; PBS, phosphate buffered saline; TBS, Tris-HCl buffered saline; GDP, guanosine diphosphate; PA, 2-aminopyridine; EDTA, ethylenediaminetetraacetic acid; NEM, *N*-ethylemaleimide; HPLC, high-performance liquid chromatography**

Introduction

The fucosyl residues are common in mammalian glycoproteins and glycolipids where they are bound to Gal and GlcNAc residues in an α-linkage to C-2 position in Gal and C-3, -4, and -6 positions in GlcNAc. Most of the fucosyl residues involved in the peripheral structures of glycoconjugates show both blood group specificities and related antigen activities [1]. Recently, much attention has been focused on the fucosylated glycoconjugates because some have been reported to be expressed and accumulate on the cell surface during cell development, differentiation, and adhesion, as well as during progression and metastasis of tumors [2, 3]. All the fucosyl residues are synthesized by the action of corresponding α-fucosyltransferases in the presence of GDP-fucose and the acceptor substrates. To understand the biosynthetic pathways of each fucosylated glycoconjugates, it is important to specify and characterize fucosyltransferases which are involved in the syntheses of their fucosyl linkages. It is, therefore, essential to develop assay methods for individual fucosyltransferases.

In our previous studies [4-10], simple and specific assay methods for α1,2, α1,3 and α1,4fucosyltransferase as well as a few glycosyltransferases were developed using chemically synthesized compounds as acceptors. All the acceptors used in these studies were demonstrated to be specific for individual α-fucosyltransferases because there was only one link-

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age to be fucosylated in such acceptors. However, α1,6fucosyltransferase, which transfers fucose to innermost core GlcNAc residue, has not been conveniently quantified. The enzyme activity has been commonly assayed using asialo-agalacto-fetuin or -transferrin as an acceptor [11, 12] whose sugar chains are difficult to prepare homogeneously and to locate the fine structure of the acceptor site(s). Recently, a fluorescent assay method for α1,6fucosyltransferase activity has been reported using a 4-(2-pyridyl-amino)butylamine coupled glycopeptide from bovine γ-globulin as an acceptor [13]. It seemed to be applicable for the assay and the purification of α1,6fucosyltransferase.

The presence of α1,6fucosyl residues attached to the core GlcNAc residues of glycoproteins has been demonstrated in *N*-glycosidic sugar chains in human and, in particular, in oncofetal antigens such as α-fetoprotein [14]. A correlation between the α1,6fucosylation of the core GlcNAc residues and carcinogenesis has also been demonstrated in hepatocellular carcinomas and benign liver diseases [15, 16] and, therefore, the activity of α1,6fucosyltransferase is expected to be higher in these conditions.

In this study, we describe a novel assay method for determination of α1,6fucosyltransferase activity which involves preparation of a reducing oligosaccharide as a specific acceptor and isolation of the fucosylated product by affinity chromatography on a GlcNAc-binding lectin column [17]. With the present method, significantly elevated activities of α1,6fucosyltransferase were demonstrated in plasma samples from patients with liver diseases.

Materials and methods

Materials

GDP-L-[^{3}H]fucose (85.1 GBq/mmol) and Aquasol-2 were obtained from New England Nuclear (Boston, Massachusetts). GDP-Fucose and α-fucosidase (bovine kidney) were purchased from Sigma Chemicals (St. Louis, Missouri). α1, 3/4Fucosidase (FUCase I) and α1,6fucosidase (FUCase III) were from GLYKO (Novato, California). α1,2Fucosidase was prepared and purified from *Bacillus fulminans* [18]. Sialidase (*Arthrobactor ureafaciens*) was obtained from Nakalai Tesque, Kyoto, Japan. β-Galactosidase (*Streptococcus* 4664K) and PA-labeled oligosaccharides, (GlcNAcβ1,2Man)$_2$α1,6(3)Manβ1,4GlcNAcβ1,4GlcNAc-PA, (GlcNAcβ1,2Man)$_2$α1,6(3)Manβ1,4GlcNAcβ1,4[Fucα1,6]GlcNAc-PA, (Galβ1,4GlcNAcβ1,2Man)$_2$α1,6(3)Manβ1,4GlcNAcβ1,4GlcNAc-PA and (Galβ1,4GlcNAcβ1,2Man)$_2$α1,6(3)Manβ1,4GlcNAcβ1,4[Fucα1,6]GlcNAc-PA were from Seikagaku, Tokyo, Japan. TSK-gel (ODS-80TM) was purchased from Tosoh, Tokyo, Japan. HiLoad Superdex pg, Superdex Peptide HR columns, Sephadex G-25 (fine grade) and lentil lectin-Sepharose were from Pharmacia Biotech, Uppsala, Sweden. All other materials used were of the highest grade commercially available. Hepatoma cell lines, HuH-7, KIM-1, HepG2, and huH-1 were cultured as previously described [19]. Plasma samples were obtained from patients with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma and healthy volunteers and were stored at −80°C until use. Protein concentrations were determined with DC Protein Assay Kit (Bio-Rad, California),

Isolation and purification of the acceptor

Yolk di-sialyloligosaccharide (YDS) was isolated and purified from hen's egg yolk as previously described [20]. Twenty mg of YDS was treated with 400 μl of 0.2 M sodium acetate buffer (pH 5.0) and 0.2 U of sialidase. The mixture was incubated for 48 h at 37°C and then diluted with the same volume of distilled water. Subsequently, 800 μl of 0.05 M of sodium acetate buffer (pH 6.0) and 5 mU of β-galactosidase were added, and the mixture was incubated for 48 h at 37°C. The resulting enzyme-digested oligosaccharides were applied to a HiLoad Superdex pg column and monitored by measuring the test absorption at 200 nm and refractive index (Shodex RI, Shimadzu, Model SE-61, Tokyo, Japan). The undecasaccharide-containing fractions were pooled, lyophilized, and rechromatographed using a Superdex Peptide HR column. The asialo-agalacto (ASAG)-YDS (8 mg) was obtained after lyophilization of the corresponding major peak. A fraction of the mixture treated only with sialidase was also applied to the same columns mentioned above and the asialo-YDS (AS-YDS) was obtained (data not shown).

Preparation of affinity gels

Psathyrella velutina lectin (PVL) [21] and *Aleuria aurantia* lectin (AAL) [22], purified as previously described, were coupled to Affi-Gel 10 [21–25] using the method recommended by the manufacturer (Bio-Rad, Boston, Massachusetts). The coupling reaction was allowed to proceed for 4 h at 4°C in 0.1 M carbonate buffer, pH 8.5 containing PVL (20 mg/ml), and 10 mM D-GlcNAc or AAL (10 mg/ml) and 10mM L-fucose. The amount of PVL and AAL coupled to the gel was calculated to be 10.0 and 4.6 mg/ml gel, respectively. Both PVL- and AAL-coupled Affi-Gel 10 gels were packed in a plastic column (0.9 × 7.9 cm). The PVL-Affi-Gel10 gel was also packed in a K9/15 column (Pharmacia Biotech) and the affinity chromatography was carried out using an FPLC system (Pharmacia Biotech) for assaying a large number of samples.

Enzyme preparations and reaction mixture

Plasma samples were obtained from healthy volunteers and patients with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma who were admitted to the First Department of Surgery, Gunma University Hospital, and Third Department of Internal Medicine, Hyogo College of Medicine Hospital. Various cell lines from human hepatoma, HuH7, KIM-1, huH-1, and HepG2, grown in the

appropriate medium and to confluence, were harvested and washed with 0.02M phosphate buffered saline, pH 7.0 (PBS). The packed cells were then dissolved in a small amount of PBS containing 2% Triton X-100 and extracted following sonication. After centrifugation at 10,000 rpm for 30 min, the supernatant was used for the assays. The incubation mixture in a total volume of 50 µl containing 10 nmol GDP-[^{3}H]fucose (92,400 dpm), 4 µmol HEPES-NaOH buffer, pH 7.0, 10 nmol acceptor substrate (ASAG-YDS, see below), and 20 µl of plasma or 10 µl of cell extract was incubated at 37°C for 4 h. One µg of Triton X-100 (Sigma Chemical Co., St. Louis, MO) was added when the enzyme from the cell extract was assayed. After incubation, an equal volume of absolute ethanol was added, and the mixture was centrifuged at 10,000 g for 10 min. The supernatant was used for assaying α1,6fucosyltransferase activity as described below.

Structural analysis of the acceptor substrate and the fucosylated product

^{1}H-NMR spectra of oligosaccharides were obtained with a JNM-A 500 (500 MHz) instrument (JEOL, Tokyo, Japan). Lyophilized samples were dissolved in D_2O. Spectra were recorded at 300K, and the resonance of each component was indicated relative to acetone (2.216 ppm). Oligosaccharides (0.05 − 1 nmol) in water were dried in a glass tube and were labeled with 2-aminopyridine (PA) as described by Kondo *et al.* [26]. PA-labeled oligosaccharides were purified by passing the reaction mixture through Sephadex G-25 (fine grade) column (1 × 48 cm) and eluted with 0.012 M ammonium bicarbonate. Analyses of PA-oligosaccharides were performed by HPLC on an ODS-silica column (Tohso, Tokyo, Japan) using LC-6A (Shimadzu, Tokyo, Japan) equipped with a fluorescence spectro monitor (RF535). The initial mobile phase was 0.1 M acetic acid-diethylamine buffer (pH 4.0) and the sample was eluted by a linear gradient of 0.5% n-buthanol in the same buffer from 5 to 100% for 60 min at a flow rate of 1 ml/min.

Results

Characterization of acceptor substrate, ASAG-YDS

The sialylglycopeptides having NeuAcα2,6Galβ1,4GlcNAc moiety have been previously isolated from delipidated egg yolk (DEY) [20], and the YDS (NeuAcα2,6Galβ1,4GlcNAcβ1,2Man)$_2$α1,6(3)Manβ1,4GlcNAcβ1, 4GlcNAc) has been prepared in a large scale from the DEY after treatment with glycopeptidase from apricot seed [20]. Figure 1 shows the chromatographic profile of the YDS after treatment with neuraminidase and β-galactosidase, successively (asialo-agalacto-YDS, ASAG-YDS). The YDS, eluted between 40 to 50 ml as a single peak (Fr. I in Figure 1) were detected by both a refractometer and absorption at 200 nm (data not shown) on a HiLoad Superdex pg column. They

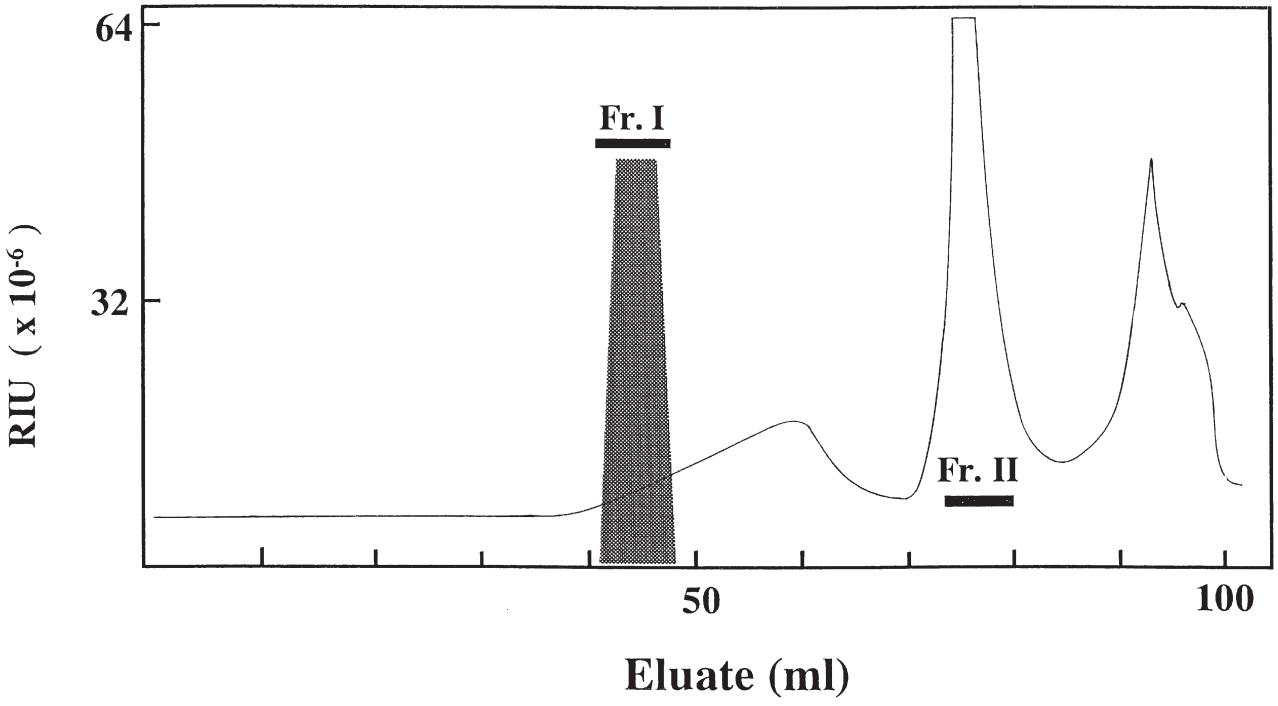

Figure 1. Elution profiles of YDS on a HiLoad Superdex pg column after treated with neuraminidase and β-galactosidase. Oligosaccharides were monitored by a refractive index monitor, and the fractions containing the appropriate heptasaccharide (dashed line) were pooled as ASAG-YDS. Fr. I and II show the position of the fractions containing YDS and ASAG-YDS, respectively.

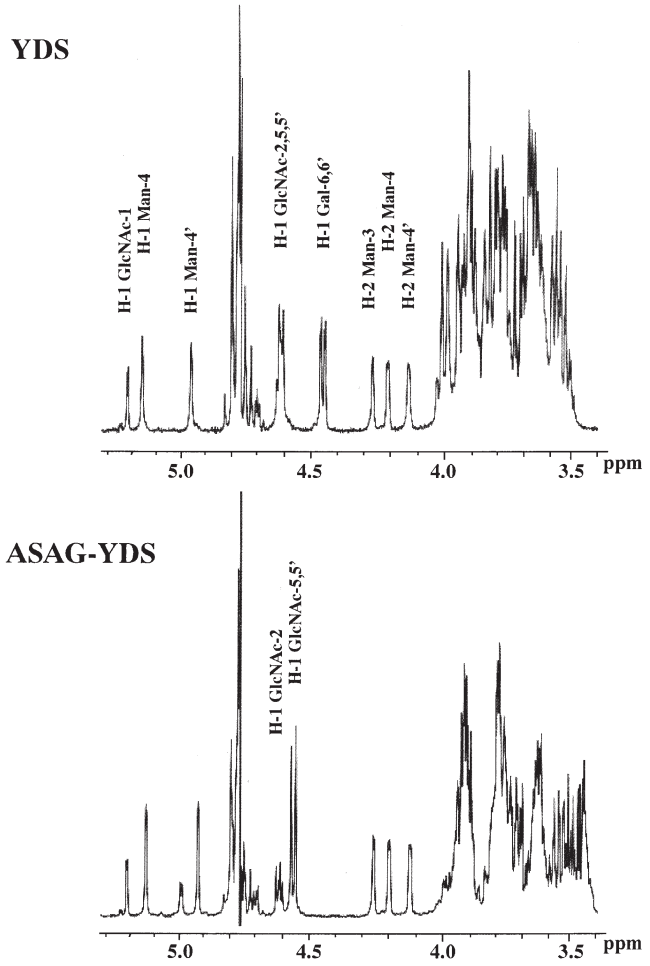

Figure 2. 500MHz ^{1}H-NMR spectrum of YDS and ASAG-YDS.

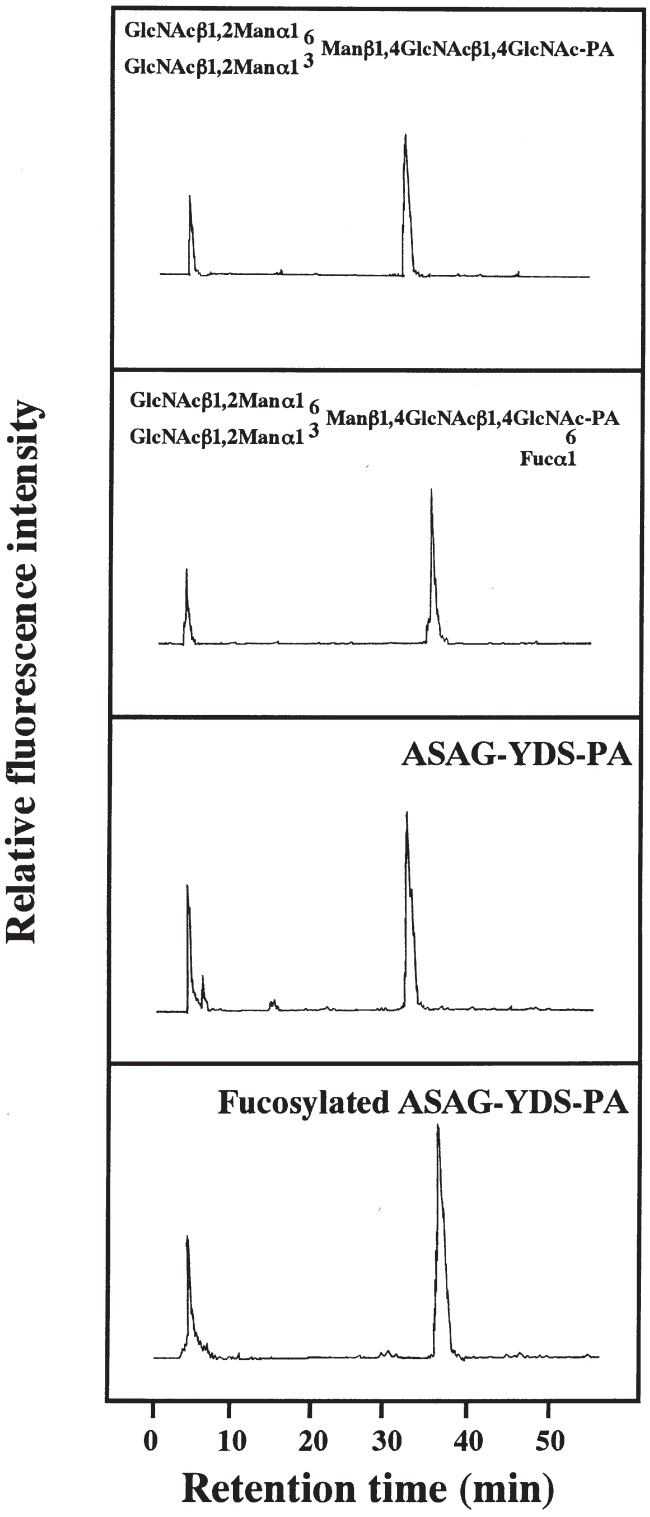

Figure 3. HPLC profile of the pyridylaminated (PA) oligosaccharides.

were cleaved by treatment of neuraminidase and β-galactosidase, and the fractions coincided with the eluted position of MW1000-1500 (Fr. II in Figure 1) were pooled and lyophilized. These fractions were rechromatographed on a Superdex Peptide HR column and recovered as a single peak described above (data not shown).

The ^{1}H-NMR spectra of YDS and ASAG-YDS are shown in Figure 2. Each structure was assigned as reported previously [20]. The anomeric protons of β-Gal residues were not observed on the spectrum of the ASAG-YDS. This indicates that Gal as well as NeuAc residues linked to the non-reducing end of the YDS were eliminated by the sequential treatment with neuraminidase and β-galactosidase. The structure of ASAG-YDS was also confirmed by the HPLC elution pattern of PA-labeled ASAG-YDS in comparison with the authentic PA-oligosaccharide (Figure 3).

Detection of α1,6fucosyltransferase

The reaction mixture containing 20 μl of plasma sample was applied on a PVL-Affi-Gel 10 column equilibrated with 0.02M Tris-HCl buffered saline, pH 7.0 containing 5 mM $CaCl_2$ (Ca-TBS), and allowed to stand at room temperature for 30 min. The column was washed with 15 ml of the same buffer, followed by the elution buffer contain-

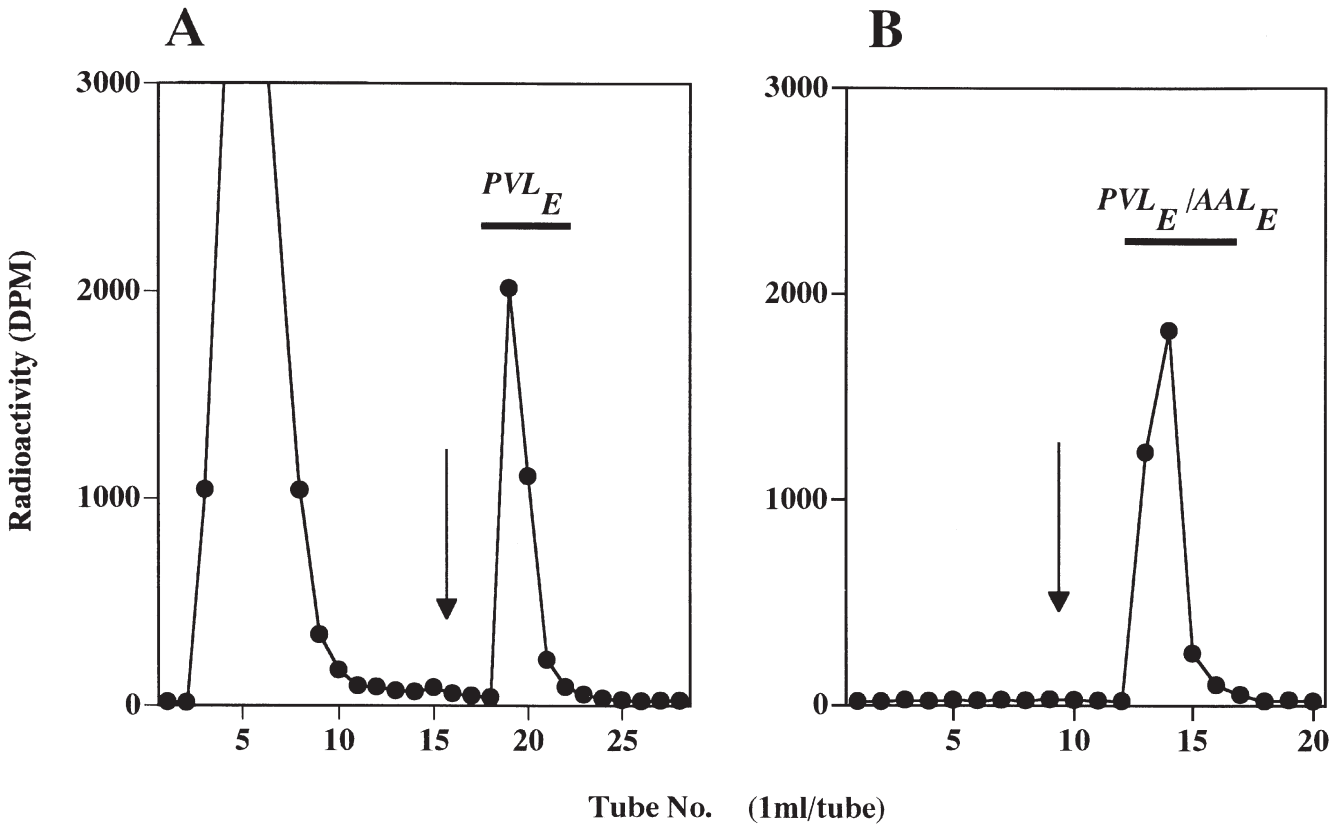

Figure 4. Serial lectin affinity chromatography of [^{3}H]fucosylated ASAG-YDS. Reaction mixture containing 20 μl of plasma sample was applied to a column of PVL-Affi-Gel 10 (A). The eluted fractions (PVL_E) were then applied to a column of AAL-Affi-Gel 10 (B). The eluted fractions (PVL_E/AAL_E) were pooled and counted as a fraction of fucosylated ASAG-YDS. Each value was calculated as fucose incorporated under the standard assay.

ing 50 mM of GlcNAc. Each fraction of 1 ml was collected, and the radioactivity was determined by a liquid scintillation spectrometer equipped with an Aquasol-2 scintillator (Figure 4A). The radioactivity of the washed fractions was found to consist of unreacted GDP-[^{3}H]fucose and free [^{3}H]fucose. No radioactivity was detected in the eluted fractions when ASAG-YDS was not added to the reaction mixture as control (data not shown). On the other hand, the radioactivity of the eluted fractions (PVL_E) was attributable to fucosylated ASAG-YDS as characterized below. All the radioactivity was recovered from the column. The eluted fraction, PVL_E was then applied to an AAL-Affi-Gel 10 column (0.9 × 7.9 cm) equilibrated with TBS. The column was washed with 10 ml of the same buffer, followed by the elution buffer containing 20 mM Fuc. All the radioactivity was bound to the column and eluted as the PVL_E/AAL_E fraction with the addition of fucose into the column (Figure 4B). It was also determined that the radioactivity in the PVL_E fraction was exactly the same as that in the PVL_E/AAL_E fraction. When the PVL_E was applied to a lentil lectin-Sepharose column, which was reported to bind glycopeptide having Fucα1,6GlcNAc-Asn [12], some of the radioactivity was eluted before starting the specific elution with methyl α-mannoside (data not shown).

Characterization of the fucosylated-ASAG-YDS

The fucosylated product, the PVL_E/AAL_E fraction was treated with α-L-fucosidase preparations. All the [^{3}H]fucose was released after treatment with bovine kidney α-fucosidase, but no free [^{3}H]fucose was observed when α1,2- and/or α1,3/4fucosidase preparation was treated. However, the treatment with α1,6fucosidase resulted in the cleavage of [^{3}H]fucose from the product (data not shown). The fucosylated product was also prepared as the PA-labeled form described in Materials and Methods and analyzed on HPLC with authentic PA-labeled sugars. As shown in Figure 3, the retention time of PA-labeled fucosylated ASAG-YDS was found to be represented by a single peak which coincided with that of Fucα1,6fucosylated ASAG-YDS. When the AS-YDS was used as an acceptor in place of the ASAG-YDS, the fucosylated product was also recovered in the reaction mixture. However, the profile of the PA-labeled products on the HPLC indicated that no α1,6 linkage was present in the fucosyl residues (data not shown). These

results indicated that the Fuc residue is only transferred into the innermost GlcNAc residue through the α1,6 linkage when the Gal residue was removed from the nonreducing terminal of YDS and that the ASAG-YDS could be a specific acceptor for α1,6 fucosyltransferase. From the profiles of serial lectin affinity chromatographies and the structural analyses of fucosylated ASAG-YDS, it was clear that the activity of α1,6fucosyltransferase could be simply determined as the radioactivity in the PVL_E fraction.

Requirement for plasma α1,6fucosyltransferase

The incorporation of fucose increased lineally with substrate concentration up to approximately 10 nmol (Figure 5A) and with incubation time during 12 h (Figure 5B), respectively. The activity of α1,6fucosyltransferase was found to have a pH optimum between 6 and 7 (Figure 5C). Among the cations tested, Cu^{++} was the only inhibitor for plasma α1,6fucosyltransferase, but addition of other cations such as Mn^{++}, Mg^{++} or Ca^{++} seemed to have a weak effect on the enzyme activity (Table 1). Ethylenediaminetetraacetic acid (EDTA) and *N*-ethylmaleimide (NEM) did not show significant inhibition of the enzyme activity.

α1,6Fucosyltransferase activity in plasma of patients with liver disease and in cell extracts

Plasma samples from patients with chronic hepatitis (n=20), liver cirrhosis (n=17), and hepatocellular carcinoma (n=27), and healthy controls (n=25) were assayed for α1,6fucosyltransferase activity using an FPLC system consisting of PVL-Affi-Gel 10 (Table 2). All the patients with liver disease showed high levels of the enzyme activity compared with that from healthy controls ($P < .001$).

The enzyme activities were also detected in cell lines from human hepatoma, HuH7, KIM-1, HepG2, and huH-1. The incorporation of fucose was also found to increase linearly with protein concentration in HuH7 cells, up to 100 μg under standard conditions.

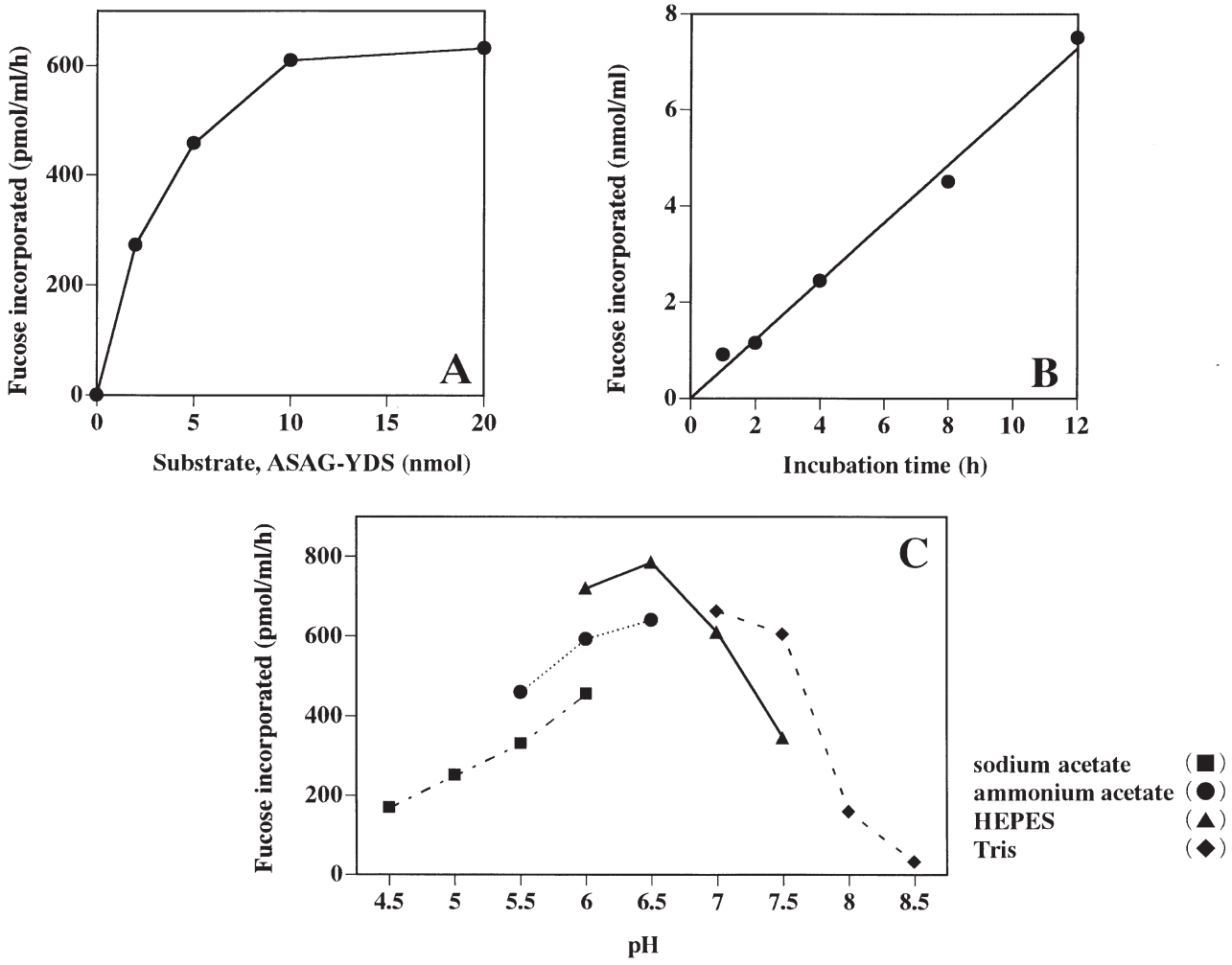

Figure 5. Dependency of plasma α1,6fucosyltransferase activity on concentrations of substrate (A) and incubation time (B) and effect of pH on the activity (C).

Table 1. Requirements for plasma α1,6fucosyltransferase

Components of incubation mixture*	*Fuc incorporated, pmol/ml/h*	*%*
Standard	609	100.0
Plus $MnCl_2$	491	80.6
Plus $MgCl_2$	743	122.0
Plus $CaCl_2$	730	119.9
Plus $CuSO_4$	132	21.7
Plus EDTA	557	91.5
Plus NEM	548	90.0

*Amounts of 1.0 μmol of each element were used.

Discussion

α-1,6Fucosyltransferase, which catalyzes the transfer of fucose from GDP-fucose to the innermost GlcNAc residue of *N*-glycosidic sugar chains, has been reported to be present in HeLa cells [27], rat liver [28], porcine liver [29], human skin fibroblasts [30, 31], human serum, platelets [32], plasma [16, 32] and liver tissue [16]. To determine the enzyme activity, *N*-glycosidic glycoproteins such as fetuin and transferrin were used as acceptors after removal of sialyl and galactosyl residues from their nonreducing ends. They were prepared with the enzymatic cleavage of both residues from the intact glycoproteins with heterogeneous sugar chains. It is, therefore, difficult to obtain specific acceptors with fine structures, and it also has a limitation for assaying a large number of samples simultaneously since the isolation and characterization of the fucosylated products is complicated and time-consuming.

As previously reported [29], α1,6-fucosyltransferase requires some of the structural features of *N*-glycosidic sugar chains as an acceptor substrate and acts on the innermost GlcNAc residue of the oligosaccharide with $GlcNAc_2Man_3GlcNAc_2$ structure. Recently, a glycopeptide from bovine γ-globulin, coupled with 4-(2-pyridylamino)butylamine, was prepared and α1,6fucosyltrasnferase activities in the rat tissues and in cell extracts from hepatoma, and gastric cancer cell lines were assayed by measuring the fluorescence intensity of the fucosylated sugar chains with uncleaved GlcNAc residue at the reducing end, which is essential for its role as an acceptor [13]. We have previously [4–7, 31–41] shown that chemically synthesized oligosaccharides were available for specifically assaying a series of glycosyltransferases, including α1,2-, α1,3-, and α1,4fucosyltransferases, and enabled us to measure a large number of samples at one time and, in particular, for typing of blood groups and diagnosis. It is necessary for this purpose and for the development of simple and reliable assay methods to prepare a monospecific acceptor substrate for a single enzyme.

Previously, a series of sialyloligosaccharides could be obtained with high yields from the hen's egg yolk [42, 43]. More recently, the disialyl biantennary undecasaccharide (YDS) could be prepared efficiently from the egg yolk glycopeptide after treatment with apricot glycopeptidase [20]. It has been demonstrated that YDS is the main constituent of the sialyloligosaccharides found in hen's egg yolk, and this, in addition to being cheap, has merit for readily preparing the acceptor substrate from such a source. In this study, the asialo-agalacto-YDS was prepared and purified conveniently as described in Materials and Methods. This substrate has β-GlcNAc residues at the nonreducing end of the oligosaccharide on which α1,3 and/or α1,4fucosyltransferase could act to produce Fuc α1,3GlcNAcβ and/or Fuc α1,4GlcNAcβ linkage. However, no such fucosylation could be observed in this study using enzyme preparations containing α1,3- and α1,4-, as well as α1,6fucosyltransferases. Therefore, ASAG-YDS could be used as a specific acceptor for α1,6fucosyltransferase even though other fucosyltrans-

Table 2. α1,6Fucosyltransferase activities

Sample		*α1,6Fucosyltransferase activity*	
Human plasma		mean ± S.D. (pmol/ml/h)	
Healthy control	(n=25)	586.3 ± 176.6	* * *
Chronic hepatitis	(n=20)	1,605.3 ± 690.8	* * *
Liver cirrhosis	(n=17)	1,191.1 ± 433.0	* *
Hepatoma	(n=15)	1,258.8 ± 240.6	*
Cell lines from human hepatoma		pmol/mg/h	
HuH7		313	
KIM-1		908	
HepG2		364	
huH-1		633	

*Difference between groups was analyzed statistically by the Student's *t*-test. $P < .001$*
P values between Healthy controls vs. Chronic hepatitis, Healthy controls vs. Liver cirrhosis, and Healthy controls vs. Hepatoma were less than 0.001

ferases coexist in samples tested. It has been shown that removal of the GlcNAc residue from or addition of the Gal residue to the terminal *N*-glycosidic sugar chains abolishes the activity of the acceptor for α1,6fucosyltransferase [31]. Consistent with this assertion, asialo-YDS could not serve as a substrate for α1,6fucosyltransferase but could serve certain other fucosyltransferase(s) which might be α1,2 and/or α1,3fucosyltransferase. However, the precise order of fucosylation, including α1,2, α1,3, α1,4, and α1,6 ones in the *N*-glycosidic sugar chains, awaits further determination.

The principle of the isolation of fucosylated product in this study is based on the highly specific binding activities of the two lectins against sugar structures. Previous investigations on the binding specificity of PVL with various complex oligosaccharides [21, 22] showed that the lectin reacts with the nonreducing terminal GlcNAc residue of the asialo-agalocto-YDS structure but do not show any affinity for the sialyl-galactosyl or asialo-galactosyl structures. As described above, all the fucosylated ASAG-YDS, as well as unreacted ASAG-YDS, were bound to PVL-column and eluted with the addition of GlcNAc. In contrast, AAL, which binds to α-fucosyl linkages, shows high affinity for Fuc α1,6GlcNAc linkage at the reducing end of the *N*-glycosidic sugar chain [25]. The fucosylated product was recovered from the PVL-column as well as from the AAL-column whose fucosylated linkage was confirmed to be only α1,6.

A quantitative method for α1,6fucosyltransferase activity has also been reported with asialo-agalacto-transferrin glycopeptide as an acceptor and *Lens culinaris* agglutinin (LCA)-immobilized gel to isolate the α1,6fucosylated product [12]. LCA has been widely used for the detection of α1,6fucosylated glycoproteins and glycopeptides, and the presence of α1,6fucosyl residues attached to the asparagine-linked GlcNAc residue was demonstrated to be essential for binding to the LCA-column [44]. Previously [31], a LCA-column was also used for the isolation of α1,6fucosylated biantennary oligosaccharide whose structure is similar to that of ASAG-YDS. In our study, however, the AAL-column could not be replaced by the LCA-agarose column since a part of the α1,6fucosylated ASAG-YDS did not bind to the column tightly and was retarded in the column.

Plasma α1,6fucosyltransferase detected with the present assay method showed different requirements for elements compared with other previously known human fucosyltransferases. α-Fucosyltransferase, such as α1,2-, α1,3- and α1,4fucosyltransferases (Fuc-TI- VII), require cations for their activities and are sensitive to the cystein-selective reagent NEM except Fuc-TIV and Fuc-TVII [45–47]. α1,6Fucosyltransferase in plasma did not require cations (Mn^{++}, Mg^{++}, Ca^{++}) and seemed to be resistant to NEM. As demonstrated before [13], α1,6fucosyltransferase from other sources showed different requirements and optimum pH suggesting that there could be more than two different species of α1,6fucosyltransferases.

Levels of plasma α1,6fucosyltransferase activities were found to be significantly elevated in patients with hepatoma compared with healthy controls. The elevated activities was also found not only in plasma samples but also in liver tissues from patients with hepatoma when assayed using asialo-agalacto-fetuin as an acceptor [16]. However, the requirements for metal ions and pH optimum of the enzyme were quite different from that described in the present investigation. On the contrary, no requirement for metal ions and similar pH optimum was recently demonstrated in α1,6fucosyltransferase purified from porcine brain [13].

Expression of aberrant fucosylations of glycoproteins and glycolipids have often been reported to be associated with malignancy. Similarly, elevated activities of fucosyltransferases, including a new candidate involved in tumor-associated fucosylations of glycoconjugates, were demonstrated in our previous studies on α1,2 α1,3, and α1,4fucosyltransferases [6, 33–37, 39, 41]. α1,6Fucosyl residue, which is common in the *N*-glycosidic sugar chains of glycoproteins, has been reported to increase in the sugar chains of serum proteins and α-fetoprotein (AFP) [14, 15,46]. It is likely that the elevated activities of α1,6fucosyltransferase found in plasma from patients with hepatoma have a role in the increase in α1,6fucosylation of AFP. AFP has been considered the most valuable marker for diagnosing hepatoma, and analyses of sugar chains of AFP showed that the amount of LCA-reactive AFP could be used to detect early hepatoma, which may develop as a consequence of liver cirrhosis [15]. Therefore, as we have emphasized for the diagnosis of cancer [6, 34–37, 39], attention should be focused on the change in the levels of α1,6fucosyltransferase activity, because such changes may occur prior to the expression of tumor-associated fucosyl-glycoconjugates. The present method of assaying α1,6fucosyltransferase has clinical relevance; the measurement of α1,6fucosyltransferase could be taken as a tumor marker in liver diseases. Clinical evaluation of this enzyme is in progress using a large number of samples from patients with liver diseases and other sources of the enzyme together with the aid of the PVL-gel and FPLC system developed in this study.

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