J. Biochem. 120, 385-392 (1996)

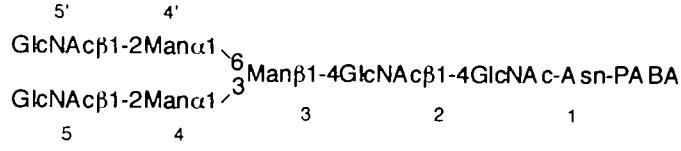
A Fluorescent Assay Method for GDP-L-Fuc:N-Acetyl-β-D-Glucosaminide α1-6Fucosyltransferase Activity, Involving High Performance Liquid Chromatography[1]

Naofumi Uozumi,* Tadashi Teshima,† Toshihiro Yamamoto,† Atsushi Nishikawa,* Yu-E Gao,* Eiji Miyoshi,* Cong-Xiao Gao,* Katsuhisa Noda,* Kazi Nazrul Islam,* Yoshito Ihara,* Shigeru Fujii,‡ Tetsuo Shiba,† and Naoyuki Taniguchi*,[2]

*Department of Biochemistry, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565; †Peptide Institute, Protein Research Foundation, 4-1-2 Ina, Minoh, Osaka 562; and ‡Laboratory of Chemistry, Kansai Medical University, Hirakata, Osaka 573

Received for publication, April 11, 1996

An assay method for GDP-L-Fuc:N-acetyl-β-D-glucosaminide α1-6fucosyltransferase (α1-6FucT; EC 2.4.1.68) activity has been developed, involving a fluorescent pyridylaminated substrate. A glycopeptide derived from bovine γ-globulin was coupled with 4-(2-pyridylamino)butylamine (PABA) through the peptide bond, and the following substrate was obtained.



The substrate and guanosine diphospho-fucopyranoside (GDP-Fuc) were incubated with a crude enzyme extract for 2 h, and then the enzymatic product was separated by reversed phase HPLC. Quantitation of the product involved measurement of the fluorescence intensity of the fucosylated pyridylaminated sugar. The structures of both synthesized GnGn-bi-Asn-PABA (substrate), and synthesized GnGnF-bi-Asn-PABA (product) were analyzed by ^{1}H NMR. The enzymatic product was also analyzed by ^{1}H NMR and was found to have α1-6fucose at the reducing end GlcNAc. This method is highly specific for α1-6FucT and is applicable for various experiments, including purification and cell culture ones.

Key words: carbohydrate structure, fluorescent labeling, α1-6fucosyltransferase, glycosyltransferase activity, HPLC.

GDP-L-Fuc:N-acetyl-β-D-glucosaminide α1-6fucosyltransferase (α1-6FucT) catalyzes the transfer of fucose from GDP-fucose to the reducing end GlcNAc of complex N-linked glycans *via* α1-6linkage (Fig. 1). Some properties of α1-6FucTs in different enzyme sources have been reported. α1-6FucT from porcine liver was first characterized (*1, 2*). Interestingly, Koscielak *et al.* reported that the enzyme was released from platelets during blood clotting (*3, 4*). Voynow *et al.* first purified and characterized α1-6FucT from a human fibroblast cell line (*5, 6*). Although this enzyme is presumed to act in the *cis* Golgi apparatus, where core fucosylation of N-glycan may affect a nascent glycoprotein, and therefore determine the further posttranslational processing and the function of glycopeptide, the biological function of this enzyme remains unknown (*7-9*).

From a clinical point of view, α1-6fucose attached to the reducing end GlcNAc has been reported to exhibit some relationship with hepatocellular carcinomas. Serum alpha-fetoproteins (AFP) of hepatomas and benign liver disease patients interact lentil lectin differently, and the lentil lectin binding variant contained fucosylated biantennary complex type N-linked oligosaccharides (*10*). Analyses of the carbohydrate structure of AFP from hepatoma cell lines indicated that most carbohydrates were α1-6fucosylated (*11, 12*). High activities were found in human hepatomas (*13*) and Ras protooncogene transfected cells (*14*). All these reports suggest that α1-6FucT is associated with malignant transformation, including hepatocarcinogenesis.

[1] This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Tel: +81-6-879-3421, Fax: +81-6-879-3429

Abbreviations: α1-6FucT, GDP-L-Fuc:N-acetyl-β-D-glucosaminide α1-6 fucosyltransferase; PA, 2-aminopyridine; PABA, 4-(2-pyridylamino)butyl amine; GDP-Fuc, guanosinediphospho-fucopyranoside; GlcNAc, N-acetylglucosamine; Pht, phthaloyl; TFA, trifluoroacetic acid; DMF, dimethylformamide; HOBt, 1-hydoxybenzotriazole; HPLC, high performance liquid chromatography; AFP, alpha-fetoprotein; ^{1}H NMR, proton nuclear magnetic resonance; Aloc-, allylchloroformyl-; GnGn-bi-Asn-PABA, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-2)Manβ1-4GlcNAcβ1-4GlcNAc-Asn-PABA; GnGnF-bi-Asn-PABA, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-2)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc-Asn-PABA; WSC, water soluble carbodimide; THF, tetrahydrofuran.

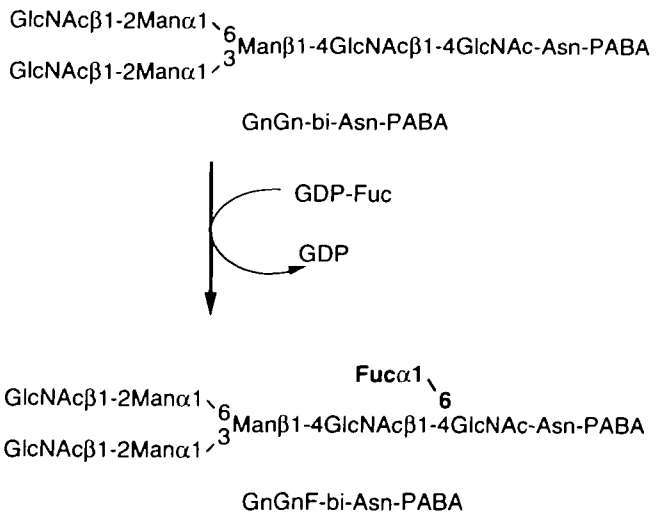


Fig. 1. **The oligasaccharide structure, GnGnF-bi-Asn-PABA, produced through the action of α1-6FucT with a fluorescence-labeled oligosaccharide, GnGn-bi-Asn-PABA.**

Most of the glycosyltransferase activities have been determined by the product separation method (*15*). Voynow *et al.* established an assay method for α1-6FucT using lectin chromatography to separate enzymatic products (*5*). These methods, however, need a radio-labeled substrate and usually involve tedious and time-consuming procedures. In previous studies, we developed an assay to separate and quantitate an enzymatic product of *N*-acetylglucosaminyltransferases using a relatively simple reverse-phase HPLC method (*16-18*). This method is based on the pyridylamination of sugar chain as reported by Hase *et al.* (*19*). In this study, we have developed a simple assay method for α1-6FucT, in which a 4-(2-pyridylamino)butylamine (PABA) labeled oligosaccharide was used as an acceptor substrate for α1-6FucT. The present method involving PABA-labeled oligosaccharide provides a powerful tool for the purification and cell culture experiments of α1-6FucT.

MATERIALS AND METHODS

2-Amino pyridine and 4-aminobutylaldehydediethylacetal were obtained from Wako Pure Chemicals, Osaka. β-Galactosidase (*Aspergillus* sp.) was obtained from Toyobo, Osaka. TSK-gel ODS-80TM and Amide-80 columns were purchased from Tosoh, Tokyo. Bovine γ-globulin and pronase (*Streptomyces griseus*) were purchased from Sigma Chemical and Seikagaku Kogyo, Tokyo, respectively. *N*-Ethylcarbonylphthalimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride were purchased from Nacalai Tesque. Fucosidase (bovine kidney) was purchased from Sigma Chemical.

Preparation of 4-(2-Pyridylamino)Butylamine—4-(2-Pyridylamino)butylamine (PABA) is an aminopyridine derivative, whose amino and 2-aminopyridine residues are bonded by an alcan group, as shown in Fig. 2. *N*-Ethylcarbonylphthalimide (8.04 g, 36.7 mmol) was dissolved in 40 ml THF, and then to the mixture, 4-aminobutylaldehydediethylacetal (5.63 g, 34.9 mmol) and triethylamine (3.89 g, 34.4 mmol) were added with stirring. After 2 h, the

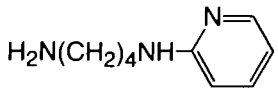


Fig. 2. **The structure of the C-terminal fluorescent reagent, PABA.**

reaction mixture was evaporated to dryness. The organic layer was extracted with ethylacetate and then washed with H_2O. The compound I-containing ethylacetate layer was dried using $MgSO_4$, and then evaporated (Fig. 3A). The yield of compound I (9.41 g, 32.3 mmol) was 92.5%.

Compound I (9.41 g, 32.3 mmol) in Fig. 3A was dissolved in acetone (93 ml) and then to the mixture, 1 M HCl (63 ml) was added with stirring. The reaction was performed under reflux for 15 min. The compound II-containing fraction was evaporated, and the organic layer was extracted with ether and then washed with H_2O. Ether was dried with $MgSO_4$ and then evaporated. The yield of compound II (7.5 g, 34 mmol) was 97.4%.

Compound II (7.5 g, 34 mmol) in Fig. 3A was dissolved in methanol (308 ml), and then 2-aminopyridine (3.706 g, 34 mmol) and acetic acid (13.86 ml) were added with stirring. After reaction for 4 h, boranedimethylamine complex (2.39 g) and acetic acid (12.2 ml) were added. An aliquot was applied to a silica gel column, and compound III was eluted with a mixture of toluene and acetone (1:1). The yield of compound III (5.31 g, 17.01 mmol) was 48.7%.

Compound III (5.31 g, 17.01 mmol) in Fig. 3A was dissolved in 104 ml of ethanol, and then to the mixture, hydrazinehydrate (2.52 g, 49.9 mmol) was added with stirring, followed by incubation for 2 h under reflux to remove amino residue protectors. After evaporation, compound IV in Fig. 3A was extracted with ethylacetate and then filtrated to remove detached phthaloyl residues. The extracted layer was evaporated until hydrazine was completely removed. The yield of compound IV (2.28 g, 13.7 mmol) was 39.2%.

Preparation of an Aloc-Asn-Oligosaccharide (V)—Oligosaccharide-Asn-OH (16 mg, 10.1 μmol) obtained from γ-globulin, which was digested by pronase according to the general method (*20*), as shown in Fig. 3B, was dissolved in a mixture of diethylether (1 ml), triethylamine (6.08 μl), and H_2O (1 ml). To the solution, allylchroloformate (3.63 μl) was added with stirring. After stirring for 6 h, an aliquot was dried up and dissolved in H_2O, and then applied to a TSK gel Amide-80 column (21.5 mm × 30 cm). After washing the column with 80% acetonitrile, sugar chains were eluted with 0.1% TFA in a linear gradient manner. The eluted fractions containing sugars detected with phenol-sulfuric acid compound V in Fig. 3B were collected. The yield of compound V (4.2 mg, 2.5 μmol) was 24.7%.

Preparation of a PABA-Labeled Glycopeptide (Compound VII)—1-Ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride (Water-soluble carbodimide, WSC) (0.47 mg, 2.51 μmol) was added to a mixture of DMF (1 ml), compound V in Fig. 3B (4.2 mg, 2.5 μmol), 1-hydroxybenzotriazole (HOBt) (1.01 mg, 7.5 μmol), and compound IV in Fig. 3A; PABA (3.75 μmol) with stirring. After reaction for 6 h at room temperature, an aliquot was applied to a TSK gel Amide-80 column (21.5 mm × 30 cm). After washing with 80% acetonitrile, sugar chain was eluted with 0.1% TFA in a linear gradient manner. The eluted

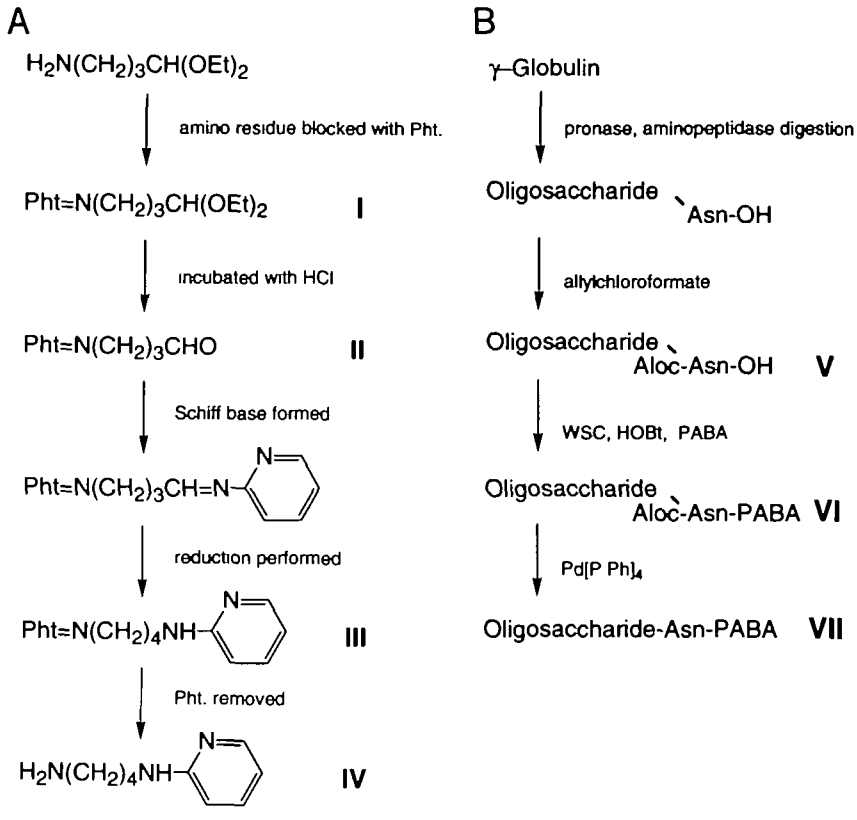


Fig. 3. **A: Preparation procedures of PABA.** After blocking amino residues, an aldehyde group was reacted with amino pyridine to form a Schiff base. After the reduction, amino blocker (Pht) was removed. The procedures are described in detail under "MATERIALS AND METHODS." **B: Preparation of oligosaccharide-Asn-PABA.** After the amide residues of oligosaccharide-Asn had been blocked with allylchloroformate, PABA was coupled, using water-soluble carbodimide. Then the aloc residue was removed by treatment with Pd[P Ph]$_4$. The procedures are described in detail under "MATERIALS AND METHODS."

fractions containing compound **VI** in Fig. 3B were dried up. Compound **VI** was dissolved in a mixture of DMF (1 ml) and diethylamine (100 μl), and then Pd[P Ph]$_4$ (20 mg) was added to remove allylformyl residues. After 30 min an aliquot was evaporated, the residue was dissolved in H_2O and then applied to a TSK gel Amide-80 column (21.5 mm × 30 cm). After washing the column with 80% acetonitrile, component **VII** in Fig. 3B were eluted with 0.1% TFA in a linear gradient manner. The yield of component **VII** (1.95 mg, 1.13 μmol) was 11.2% (Fig. 3B).

Preparation of GnGn-bi-Asn-PABA—Compound **VII** in Fig. 3B was applied to a TSK gel ODS 80TM column (4.6 mm × 150 mm) and was eluted with 20 mM ammonium acetate pH 4.0 containing 0.1% butanol in an isocratic manner to purify GnGnF-bi-Asn-PABA in Fig. 1. GnGn-bi-Asn-PABA, which can be used as a substrate for α1-6FucT (Fig. 1), was obtained from GnGnF-bi-Asn-PABA by digestion with bovine kidney fucosidase for 7 days. The final step of purification of GnGn-bi-Asn-PABA was performed by HPLC on an ODS 80TM column (4.6 mm × 150 mm) to eliminate fucose, fucosidase, and undigested sample. GnGn-bi-Asn-PABA was eluted with 20 mM ammonium acetate pH 4.0 containing 0.1% butanol in an isocratic manner.

Structural Analysis of Synthesized GnGn-bi-Asn-PABA and Synthesized GnGnF-bi-Asn-PABA—Synthesized GnGn-bi-Asn-PABA and synthesized GnGnF-bi-Asn-PABA were lyophilized against 99.80% D_2O twice, and then dissolved in 99.96% D_2O. ^{1}H-NMR spectroscopy (400 MHz) was carried out on each component with a Varian Unity 400 spectrometer at 35°C, using methanol as a standard.

Preparation of a Crude Enzyme Extract of Porcine Liver—Porcine livers were homogenized in 4 volumes of 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose with an Ultra Turrax homogenizer (Ika-werk, FRG). After centrifugation of 900 × *g* for 10 min, the supernatant was collected. Then an aliquot was centrifuged at 105,000 × *g* for 2 h, and the pellet was resuspended in the homogenation buffer.

Preparation of Crude Enzyme Extracts of Various Rat Tissues and Cell Lines—Various rat tissues and cells were homogenized in 4 volumes of 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose with an Ultra Turrax homogenizer (Ika-werk). After centrifugation of 900 × *g* for 10 min, the supernatant was collected and used as a crude enzyme preparation.

α1-6FucT Assay—The standard incubation mixture for the α1-6FucT assay contained the following components in a final volume of 50 μl: 200 mM MES-NaOH buffer (various pHs), 1% Triton X-100, 50 μM GnGn-Asn-PABA, and 500 μM GDP-Fuc. After a mixture had been incubated at 37°C for 2 h, the reaction was stopped by heating at 100°C for 1 min. The sample was then centrifuged at 15,000 × *g* for 10 min, and 10 μl of the supernatant were used for analyses. The product was separated by HPLC on a TSK-gel ODS-80TM column (4.6 mm × 150 mm). Elution was performed at 55°C with 20 mM acetate buffer, pH 4.0, containing 0.1% butanol in an isocratic manner. The fluorescence of the column elute was detected with a fluorescence photometer (Shimadzu, model RF 535, Tokyo), excitation and emission wavelengths of 320 and 400 nm, respectively. The amount of the product was estimated from the fluorescence intensity. The activity of the enzyme was expressed as pmol of fucose transferred/h.

A

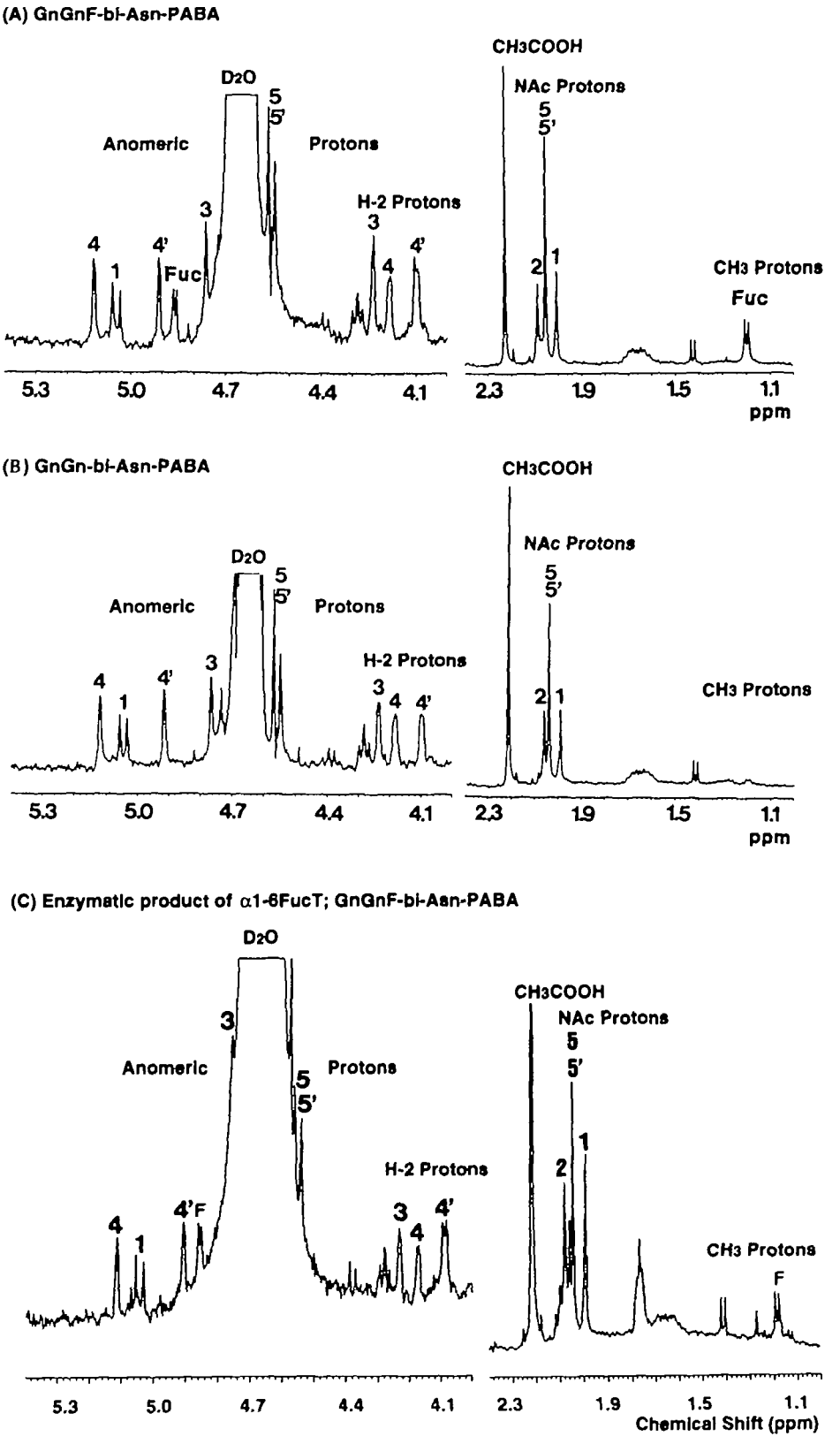


Fig. 4. **A: The ^{1}H-NMR spectra of synthesized GnGnF-bi-Asn-PABA (A) (upper panel) and synthesized GnGn-bi-Asn-PABA (B) (middle panel).** An enzymatic product (C) of porcine brain α1-6FucT showed the same value as synthesized GnGnF-bi-Asn-PABA (lower panel). The experimental details are given under "MATERIALS AND METHODS." The chemical shift values of the proton (H-1, H-2) and methyl proton (NAc, CH_3) signals are summarized in Table I. **B: Structural characterization of GnGn-bi-Asn-PABA.** Synthesized GnGn-bi-Asn-PABA was analyzed by ion splay mass spectrometry. Presumable molecular weight of GnGn-bi-Asn-PABA was 1,579.

Protein was determined with a Bio-Rad protein assay kit (BCA kit), using bovine serum albumin as a standard.

Column Chromatography of α1-6FucT—Concentrated human serum was applied to a GDP-hexanolamine column 4B (5 mm × 50 mm) at room temperature, and then eluted with 20 mM phosphate buffer, pH 7.0, containing 3 M KCl in a linear gradient manner. Each fraction was examined for the activity of α1-6FucT, as described above.

RESULTS

Structural Analysis of Synthesized GnGn-bi-Asn-PABA and Synthesized GnGnF-bi-Asn-PABA—The spectra of synthesized GnGn-bi-Asn-PABA and synthesized GnGnF-bi-Asn-PABA are shown in Fig. 4A. The chemical shift values of their amomeric proton and methyl proton signals are summarized in Table I. In comparison with the signal of GnGn-bi-Asn-PABA, typical signals of Fuc at $\delta = 4.865$ ppm (H-1) and $\delta = 1.195$ (CH_3) were observed in the analysis of GnGnF-bi-Asn-PABA. The proton signal of GlcNAc-2 shifted from $\delta = 2.069$ ppm to $\delta = 2.086$ ppm on the addition of Fuc at the C-6 position of GlcNAc-1. Mass spectrometry yielded a major molecular ion signal at 790.3 mass units for GnGn-bi-Asn-PABA (M.W. 1,579) (Fig. 4B).

Structural Analysis of Enzymatic Product of Porcine Brain α1-6FucT—To confirm the structure of the product of α1-6FucT from porcine brain, the reaction was performed in a large scale, and the enzymatic product was analyzed with a ^1H NMR spectrophotometer. This indicated that α1-6FucT of porcine brain catalyzes the transfer of 1 mol of fucose to 1 mol of GnGn-bi-Asn-PABA. The chemical shift value of the enzymatic product on ^1H NMR analysis was the same as that of synthesized GnGnF-bi-Asn-PABA (Fig. 4A). The enzymatic product gave two typical signals of fucose at $\delta = 4.864$ ppm (H-1) and $\delta = 1.193$ ppm (CH_3).

α1-6FucT Assay—A typical elution pattern of the reaction product of α1-6FucT is shown in the upper panel of Fig. 5. The elution times of the substrate and product were 12.1 and 22.0 min, respectively. The elution pattern of the product is the same as that of authentic GnGnF-bi-Asn-PABA (lower panel of Fig. 5). Reaction mixtures without GDP-Fuc gave no enzymatic product (middle panel of Fig. 5). Treatment of the enzymatic product (α1-6FucT of

TABLE I. **Relevant ^1H-NMR parameters of structural reporter groups of constituent monosaccharides for GnGnF-bi-Asn-PABA and GnGn-bi-Asn-PABA.** Chemical shifts are given in ppm in D_2O (35°C) containing 0.1% TFA.

Reporter group	Residue	Chemical shift	
		GnGnF-bi-Asn-PABA	GnGn-bi-Asn-PABA
H-1	GlcNAc-1	5.052	5.045
	-2	N.D.	N.D.
	-5	4.570	4.567
	-5′	4.550	4.547
	Man-3	4.768	4.765
	-4	5.123	5.120
	-4′	4.917	4.915
	α1-6Fuc	**4.865**	—
H-2	Man-3	4.240	4.237
	-4	4.188	4.185
	-4′	4.099	4.098
CH_3	α1-6Fuc	**1.195**	—
NAc	GlcNAc-1	2.005	1.999
	-2	**2.086**	**2.069**
	-5-5′	2.053	2.050

N.D., not detectable.

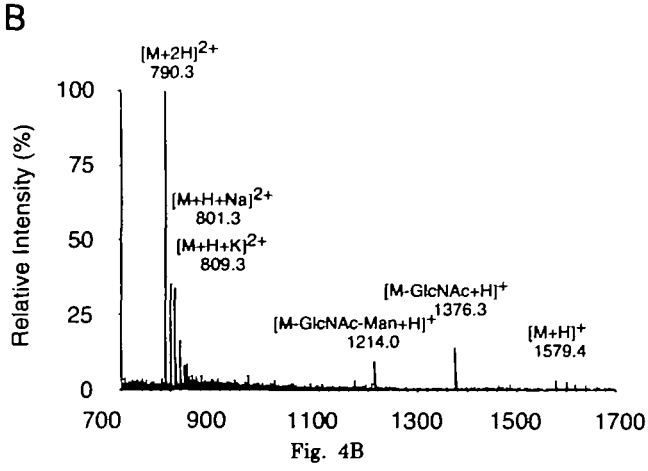


Fig. 4B

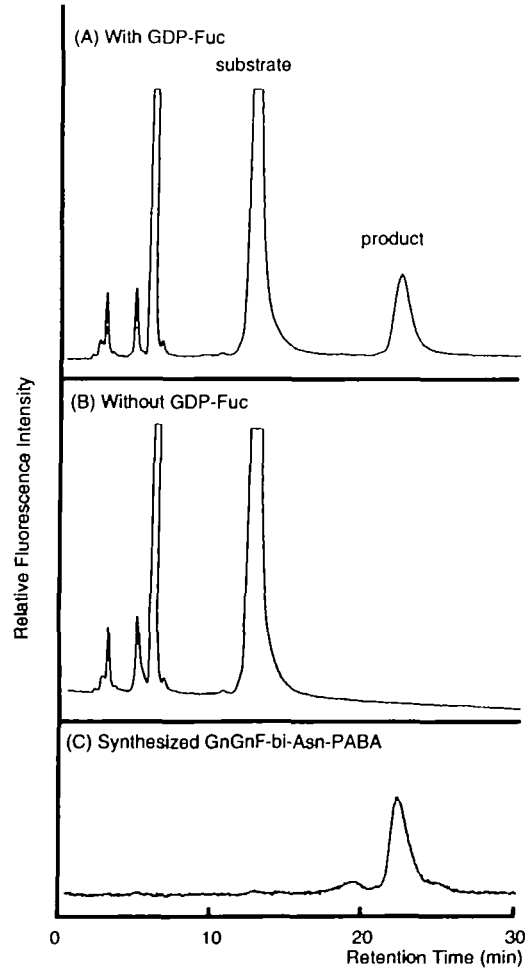


Fig. 5. **A typical HPLC elution pattern for enzymatic product on HPLC.** The PABA-labeled oligosaccharide and porcine microsomal fractions were reacted with GDP-Fuc (upper panel) or without GDP-Fuc (middle panel) for 2 h, and then subjected to HPLC. Synthetic GnGnF-bi-Asn-PABA was also shown in a lower panel. The procedures are described under "MATERIALS AND METHODS."

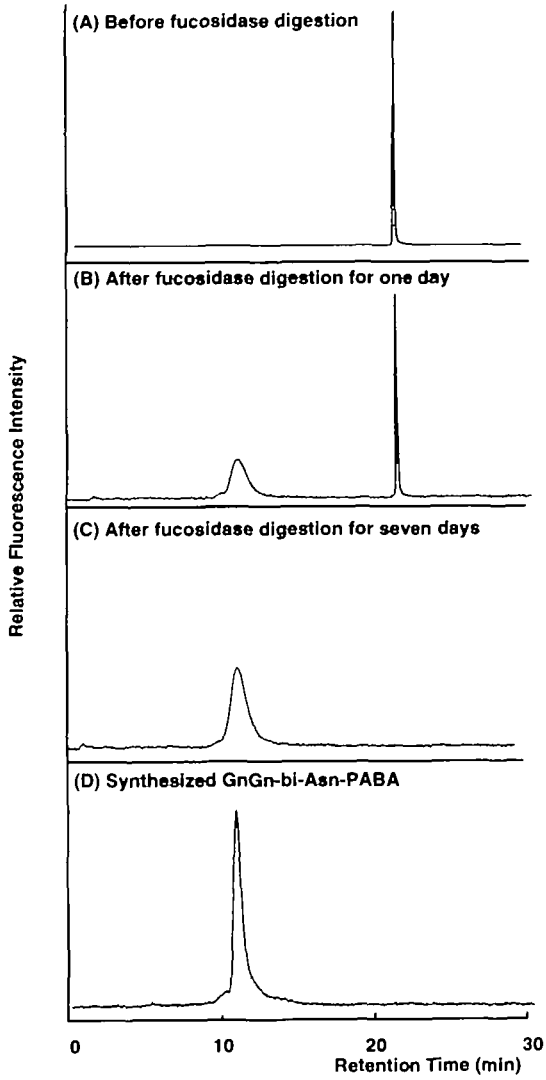


Fig. 6. **Effect of α-fucosidase on the product of α1-6FucT of porcine brain.** The product of α1-6FucT was collected and digested with bovine kidney α-fucosidase. Samples before (A) and after (B, C) α-fucosidase digestion were subjected to HPLC. After 7 days (C) digestion, the product of α1-6FucT was completely digested. The elution patterns of authentic GnGn-bi-Asn-PABA (D) are also shown.

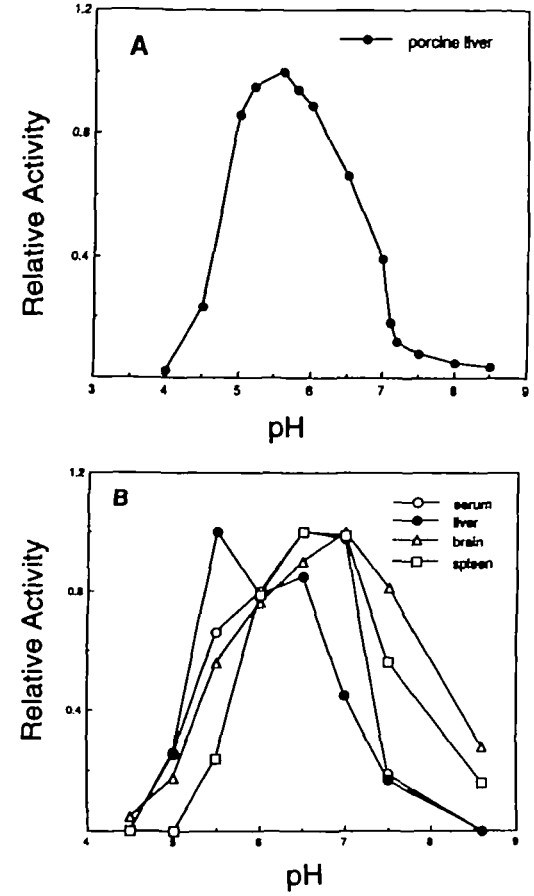


Fig. 7. **Effects of pH on the α1-6FucT activity of porcine liver (A), and various rat tissues (B).** Incubation was performed at 37°C for 2 h. The pH values were obtained in 250 mM acetate/NaOH (pH 4.0-5.2), Mes-NaOH (5.6-7.0), and Tris/HCl (7.1-8.5) buffer.

TABLE II. **Activities of α1-6FucT in rat liver, hepatomas, and ascites hepatoma cell lines.** The values represent mean±SD for 3-5 samples.

Tissues and cell lines	Activities (pmol/h/mg protein)
Rat liver	
Normal	15.6±4.5
Fetal liver (19 days)	10.2±3.9
DAB-induced hepatoma (21 weeks)	42.9±7.9
Hepatoma cell lines	
AH-66	22.1±2.3
AH-130	150.5±21.0

porcine brain) with α-fucosidase of bovine kidney altered the elution pattern on HPLC. In comparison with the retention time of the authentic standard, the additional component appearing with a concomitant decrease in the α1-6FucT product was identified as GnGn-bi-Asn-PABA (Fig. 6). The optimum pH of α1-6FucT in microsomal fractions of porcine liver was 5.6, those of α1-6 FucT in rat liver, brain, spleen, and serum being 5.5, 7.0, 6.5, 7.0, respectively (Fig. 7).

Suitable Assay Conditions for α1-6FucT of Liver—To determine suitable conditions for α1-6FucT activity of liver, the porcine liver microsomal fractions were incubated under various conditions, but the pH of the reaction mixture was fixed at 5.6. The enzyme reaction was linear with time for at least 120 min. The α1-6FucT activity was proportional to the enzyme protein concentration over the range tested. The velocity of the enzymatic reaction changed according to the concentration of the PABA labeled glycopeptide (Fig. 8). The apparent K_m values for GnGn-bi-PABA and GDP-Fuc were 17.4 and 46 μM, respectively. The α1-6FucT activities of various rat liver tissues were determined as described under "MATERIALS AND METHODS."

α1-6FucT Induced in Hepatoma Cells—Normal rat liver showed one of the lowest activities of α1-6FucT among various rat tissues (data not shown), and it is presumed that induction of α1-6FucT activity cause a remarkable change in the structure of the oligosaccharides on the cell surface. The activities of various rat liver tissues such as fetal liver, DAB induced hepatomas and ascites hepatoma cell lines AH-66 and AH-130 are shown in Table II. α1-6FucT activity was markedly elevated in all hepatoma tissues,

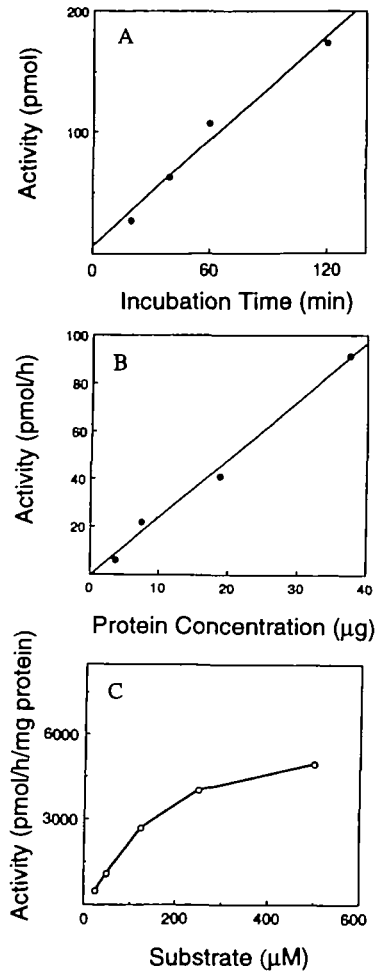


Fig. 8. **Kinetics of α1-6FucT of porcine liver.** The conditions for the enzyme reaction are as given in the text with the following modifications; A, effect of reaction time; B, effect of enzyme concentration; C, effect of substrate concentration.

especially in hepatoma cell line AH-130, as compared to that in normal adult liver (Table II).

Column Chromatography of Serum α1-6FucT—Using this assay, human serum α1-6FucT activity was detected in fractions from a GDP-hexanolamine Sepharose 4B column, as described under "MATERIALS AND METHODS." The elution profile revealed that α1-6FucT of human serum binds tightly to this column and the majority of total protein loaded was eluted in the pass-through fractions (Fig. 9). This step is effective for the purification of α1-6FucT, and the assay method described in this report was found to be a powerful tool for the purification of serum type α1-6FucT.

DISCUSSION

Fluorescence labeling of the reducing end of a sugar with 2-aminopyridine has become a useful method for carbohydrate structure analysis of glycoproteins, especially *N*-linked oligosaccharides (*20, 21*). This is because of the ease of both the detection and separation of PA-sugars in HPLC analysis. It has been reported that the PA-sugars reported by Hase *et al.* (*19, 22*) can also be used as substrates for glycosyltranferases, which catalyze *N*-linked type complex sugar chains (*16-18, 23, 24*). The PA-sugar, GnGn-bi-Asn-PABA, GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-2)Manβ1-4GlcNAcβ1-4GlcNAc-PA, was a powerful tool for purifying glycosyltransferases such as GnT-III and GnT-V (*25, 26*). But this PA-sugar cannot be used as a substrate for the enzymes which recognize its reducing end, because of the destroyed structure of the reducing end GlcNAc. In order to keep the reducing end GlcNAc intact, a new fluorescent component, 4-(2-pyridylamino)butylamine (PABA), was employed in the preparation of a fluorescent glycopeptide.

The difficulty in assaying the activity of α1-6FucT is due to the following reasons. First, an ordinary method requires the radio-labeled GDP-Fuc and lectin chromatography to isolate the enzymatic product (*5*), and the procedure is complicated and takes much more time than a procedure that involves a fluorescence labeled oligosaccharide. Sec-

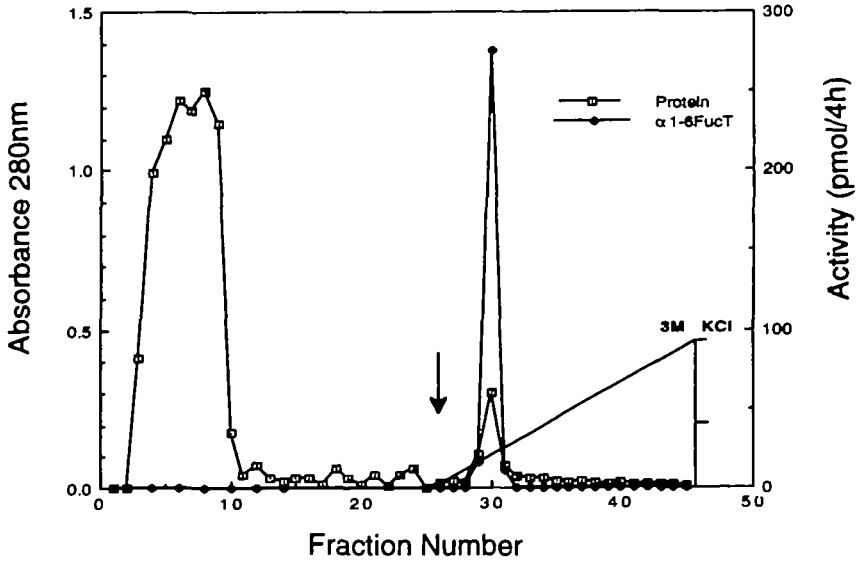


Fig. 9. **Elution profile of α1-6FucT of human serum on a GDP-hexanolamine column (5 mm×50 mm), linear gradient elution (the starting point is indicated by an arrow) with 20 mM phosphate potassium buffer, pH 7.0, containing 3.0 M KCl being performed.**

ond, although α1-6FucT requires the pyranose structure of the reducing end GlcNAc, an ordinary glycopeptide labeling method, such as the ^3H labeling method, requires the reduction of the oligosaccharides, and in the case of *N*-linked oligosaccharides, the reducing end GlcNAc structure is destroyed on reduction. Third, it is difficult to isolate fucose-containing oligosaccharides from non-containing ones except for the analysis of pyridylamination (*27, 28*).

Optimum pHs of α1-6FucTs of various rat tissues are different from each other (Fig. 7). α1-6FucT from a human fibroblast cell line required the Mg^{2+} cation, and did not bind to a GDP-hexanolamine column (*6*). However, α1-6FucT of human serum required no cations for its activity (data not shown), and bound tightly to a GDP-hexanolamine column (Fig. 9). α1-6FucT in rat liver has an optimum pH of 5.6. On the contrary α1-6FucT in rat brain has an optimum pH 7.0 (Fig. 7). These results suggest that at least two kinds of α1-6FucTs might exist and that α1-6FucT gene families may exist like the α1-3FucT and sialyltransferase families (*29, 30*). To solve this, cloning of the α1-6FucT gene is essential. The present method involving PABA-labeled oligosaccharide has become a powerful tool for the purification of α1-6FucT.

REFERENCES

1. Wilson, J.R., Willams, D., and Schachter, H. (1976) The control of glycoprotein synthesis: *N*-Acetylglucosamine linkage to a mannose residue as a signal of L-fucose to the asparagine-linked *N*-acetylglucosamine residue of glycopeptide from α1-acid glycoprotein. *Biochem. Biophys. Res. Commun.* **72**, 909-916
2. Longmore, G.D. and Schachter, H. (1982) Product-identification and substrate specificity studies of the GDP-fucose:2-acetamido-2-deoxy-β-D-glucoside (Fuc-linked GlcNAc) 6-α-L-fucosyltransferase in a Golgi-rich fraction from porcine liver. *Carbohydr. Res.* **100**, 365-392
3. Koscielak, J., Pacuszka, T., Kubin, J., and Zdziechowska, H. (1987) Serum α-6-L-fucosyltransferase is released from platelets during clotting of blood. *Glycoconjugate J.* **4**, 43-49
4. Koscielak, J., Antoniewicz-Papis, J., Zdebska, E., Maj, S., and Leszko, B. (1995) Activity of platelet α-6-fucosyltransferase is inversely related to blood platelet concentration. *Acta Biochim. Polon.* **42**, 35-39
5. Voynow, J.A., Scanlin, T.F., and Glick, M.C. (1988) A quantitative method for GDP-L-Fuc:*N*-acetyl-β-D-glucosaminide α1-6fucosyltransferase activity with lectin affinity chromatography. *Anal. Biochem.* **168**, 367-373
6. Voynow, J.A., Kaiser, R.S., Scanlin, T.F., and Glick, M.C. (1991) Purification and characterization of GDP-L-fucose-*N*-acetyl-β-D-glucosaminide α1-6 fucosyltransferase from culture human skin fibroblasts. *J. Biol. Chem.* **266**, 21572-21577
7. Hubbard, S.C. and Ivatt, R.J. (1981) Synthesis and processing of asparagine-linked oligosaccharide. *Annu. Rev. Biochem.* **50**, 555-583
8. Schachter, H., Narasimhan, S., Gleeson, P., and Vella, G. (1983) Control of branching during the biosynthesis of asparagine-linked oligosaccharides. *Can. J. Biochem. Cell. Biol.* **61**, 1049-1066
9. Kornfeld, R. and Kornfeld, S. (1985) Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54**, 631-664
10. Aoyagi, Y., Isemura, M., Yoshizawa, Z., Suzuki, Y., Sekine, C., Ono, T., and Ichida, F. (1985) Fucosylation of serum α-fetoprotein in patient with primary hepatocellular carcinoma. *Biochim. Biophys. Acta* **830**, 217-223
11. Aoyagi, Y., Suzuki, Y., Igarashi, A., Saitoh, M., Oguro, T., Yokota, S., Mori, T., Suda, T., Isemura, M., and Asakura, H. (1993) Carbohydrate structures of human α-fetoprotein of patients with hepatocellular carcinoma: Presence of fucosylated and non-fucosylated triantennary glycans. *Br. J. Cancer* **67**, 486-492
12. Ohno, N., Nishikawa, A., Kouketsu, M., Taga, H., Endo, Y., Hada, T., Higashino, K., and Taniguchi, N. (1992) Enzymatic basis of sugar structures of α-fetoprotein in hepatoma and hepatoblastoma cell lines: Correlation with α1-6 fucosyltransferase and *N*-acetylglucosaminyltranseferases III and V. *Int. J. Cancer* **51**, 315-317
13. Huchinson, W.L., Du, M.-Q., Johnson, P.J., and Williams, R. (1991) Fucosyltransferase: Differential plasma and tissue alteration in hepatocellular carcinoma and cirrhosis. *Hepatology* **13**, 683-688
14. Easton, E.W., Bolscher, J.G.M., and van den Eijnden, D.H. (1991) Enzymatic amplification involving glycosyltransferases forms the basis for the increase size of asparagine-linked glycans at the surface of NIH 3T3 cells expressing the N-ras protooncogene. *J. Biol. Chem.* **266**, 21674-21680
15. Palcic, M.M. (1994) Glycosyltransferases in glycobiology. *Methods Emzymol.* **230**, 300-316
16. Nishikawa, A., Fujii, S., Sugiyama, T., and Taniguchi, N. (1988) A method for the determination of *N*-acetylglucosaminyltransferase III activity in rat tissues involving HPLC. *Anal. Biochem.* **170**, 349-354
17. Nishikawa, A., Gu, J., Fujii, S., and Taniguchi, N. (1990) Determination of *N*-acetylglucosaminyltransferase III, IV and V in normal and hepatoma tissues of rats. *Biochem. Biophys. Res. Commun.* **152**, 107-112
18. Taniguchi, N., Nishikawa, A., Fujii, S., and Gu, J. (1989) Glycosyltransferase assay using pyridylaminated acceptor: *N*-Acetylglucosaminyl transferase III, IV, and V. *Methods Emzymol.* **179**, 397-409
19. Hase, S., Ibuki, T., and Ikenaka, T. (1984) Reexamination of the pyridylamination used for fluorescence labeling of oligosaccharides and its application to glycoproteins. *J. Biochem.* **95**, 197-203
20. Fujii, S., Nishiura, T., Nishikawa, A., Miura, R., and Taniguchi, N. (1990) Structural heterogeneity of sugar chains in immunoglobulin G. *J. Biol. Chem.* **265**, 6009-6018
21. Nishiura, T., Fujii, S., Kanayama, Y., Nishikawa, A., Tomiyama, Y., Iida, M., Karasuno, T., Nakao, H., Yonezawa, T., Taniguchi, N., and Tarui, S. (1990) Carbohydrate analysis of immunoglobulin G myeloma proteins by lectin and high performance liquid chromatography: Role of glycosyltransferases in the structures. *Cancer Res.* **50**, 5345-5350
22. Mega, T., Hamazume, Y., and Ikenaka, T. (1988) Synthesis of fluorescent amino acids and peptides with 2-aminopyridine at the carboxyl or amino terminus. *Bull. Chem. Soc. Jpn.* **61**, 4315-4321
23. Morita, N., Hase, S., Ikenaka, K., Mikoshiba, K., and Ikenaka, T. (1988) Pyridylamino sugar chain as an acceptor for galactosyltransferase. *J. Biochem.* **103**, 332-335
24. Tezuka, K., Hayashi, M., Ishihara, H., Akazawa, T., and Takahashi, N. (1992) Studies on synthetic pathway of xylose-containing N-linked oligosaccharides deduced from substrate specificity of the processing enzymes in sycamore cells (*Acer pseudoplatanus* L.) *Eur. J. Biochem.* **203**, 401-413
25. Nishikawa, A., Ihara, Y., Hatakeyama, M., Kangawa, K., and Taniguchi, N. (1992) Purification, cDNA cloning, and expression of UDP-*N*-acetylglucosaminide:β-D-mannoside β-1,4*N*-acetyltranseferase III from rat kidney *J. Biol. Chem.* **267**, 18199-18204
26. Gu, J., Nishikawa, A., Tsuruoka, N., Ohno, M., Yamaguchi, N., Kangawa, K., and Taniguchi, N. (1993) Purification and characterization of UDP-*N*-acetylglucosamine:α-6-D-mannoside β1-6*N*-acetyltransferase(*N*-acetyltransferase V) from a human lung cancer cell line. *J. Biochem.* **113**, 614-619
27. Staudacher, E., Altmann, F., Glossl, J., Marz, L., Schachter, H., Kamerling, J.P., Hard, K., and Vliegenthart, J.F.G. (1991) GDP-fucose:β-*N*-acetylglucosamine (Fuc to (Fucα1-6GlcNAc)-Asn-peptide)α1-3-fucosyltranseferase activity in haneybee (*Apis mellifica*) venom glands. *Eur. J. Biochem.* **199**, 745-751
28. Staudacher, E., Kubelka, V., and Marz, L. (1992) Distinct *N*-glycan fucosylation potentials of three lepidopteran cell lines. *Eur. J. Biochem.* **207**, 987-993
29. Natsuka, S., Gersten, K.M., Zenita, K., Kannagi, R., and Lowe, J.B. (1994) Molecular cloning of a cDNA encoding a novel human leukocyte α-1,3-fucosyltransferase capable of synthesizing the sialyl lewis X determinant. *J. Biol. Chem.* **269**, 16789-16794
30. Wen, D.X., Livingston, B.D., Medzihradsky, K., F., Kelm, S., Burlingame, A.L., and Paulson, J.C. (1992) Primary structure of Galβ1,3(4)GlcNAc α2,3-sialyltransferase determined by mass spectrometry sequence analysis and molecular cloning. *J. Biol. Chem.* **267**, 21011-21019