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Biosynthesis of a blood group H_1 antigen by α1,2-fucosyltransferase in PC12 cells

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We have examined the expression of GDP-fucose: glycosphingolipid fucosyltransferase activity in PC12 cells and PC12 sublines in relation to the neuronal differentiation induced by nerve growth factor (NGF) or dexamethasone. Transfer of fucose to paragloboside (nLc_4Cer) yielded a product which was determined to be a blood group H_1 antigen (Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer) by gas chromatography/mass spectrometry analysis and enzymatic hydrolysis, suggesting that PC12 cells have an α1,2-fucosyltransferase. Lactosylceramide was also fucosylated at a reduced rate. When the differentiation of PC12 cells and PC12 subline cells, PC12D and MR31, was induced by exposure to either NGF or dexamethasone, the fucosyltransferase activity for nLc_4Cer was found to decrease in both cell lines, suggesting the association with cell differentiation. This is the first report of the presence of an α1,2-fucosyltransferase in cultured neuronal cell lines which catalyses the *in vitro* biosynthesis from nLc_4Cer of a type-2 chain glycosphingolipid having the blood group H_1 determinant. The disaccharides, β-lactose and *N*-acetyllactosamine, were also fucosylated by PC12 cell enzyme, although the specificity for the carbohydrate structure was different from that for glycosphingolipids.

Keywords: α1,2-fucosyltransferase, blood group H_1, PC12 cells, glycosphingolipid, disaccharide

Abbreviations: Glc, D-glucose; Gal, D-galactose; GlcNAc, *N*-acetyl-D-glucosamine; Fuc, L-fucose; Cer, ceramide; nLc_4Cer, neolactotetraosylceramide (paragloboside); GDP, guanosine diphosphate; CDP, cytidine diphosphate; CTP, cytidine triphosphate; NGF, nerve growth factor; DX, dexamethasone; GC/MS, gas chromatography/mass spectrometry.

Introduction

Cell surface glycoconjugates play important roles in diverse cellular functions, and it is well known that their carbohydrate chains change dramatically during the differentiation of cultured cells or cellular oncogenic transformation (for reviews, see [1, 2]). Although one of these changes comprises changes in structure or the biosynthesis of fucose-containing glycosphingolipids [3], the significance of fucosylated glycoconjugates remains to be clarified. PC12 is a clonal cell line established from a rat pheochromocytoma, the neurite outgrowth and biochemical changes yielding neuron-like characteristics which are caused by exposure of the cells to nerve growth factor (NGF) [4]. The gangliosides and fucose-containing glycolipids in PC12 cells have been characterized [5–10]; and the activities of enzymes tranferring sugar residues to synthesize glycolipids have been reported to change in association with differentiation of the cells [11–13]. Recently, we established a new subline of PC12 cells, MR31, transfected with the dexamethasone-regulated *ras* oncogene [14], and found that polar components of neutral glycosphingolipids of MR31 cells are decreased compared with the parent PC12 cells, and that their tumorigenicity decreases in parallel with the changes in glycolipid composition [14, 15]. Considering that fucose-containing glycolipids are key substances modulating a variety of biological events, including differentiation and oncogenesis [1, 16], the above findings led us to examine the expression of GDP-fucose: glycosphingolipid fucosyltransferase activity in PC12 cells in relation to neuronal differentiation. We report here the presence of an α1,2-fucosyltransferase (EC 2.4.1.69) in PC12 cells, which catalyses the *in vitro* biosynthesis of a blood group antigen H_1 [17] from paragloboside (nLc_4Cer).

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Materials and methods

Materials

The glycosphingolipids, lactosylceramide (LacCer, Galβ1-4Glc-Cer), globotriaosylceramide (Gb_3Cer, Galα1-4Galβ1-4Glc-Cer), globoside (Gb_4Cer, GalNAcβ1-4Galα1-4Galβ1-4Glc-Cer) and paragloboside (nLc_4Cer, Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer), were isolated from human erythrocyte membranes, and digalactosylceramide (Gal_2Cer, Galα1-4Gal-Cer) from a kidney of a patient with Fabry's disease as described by Ando *et al.* [18]. The glycosphingolipids obtained were further purified by high performance liquid chromatography (HPLC) (Shimadzu SCL-10A system, Japan) on a column of GPG-010-7OH (4.6 × 250 mm; Nomura Chemical Co. Ltd, Japan), and proved to be sufficiently pure to determine fucosyltransferase activity by thin-layer chromatography (TLC). Galactosyl globotriaosylceramide ($GalGb_3$Cer, Galα1-3Galα1-4Galβ1-4Glc-Cer) from a PC12h tumour was kindly provided by Dr Ariga [9]. Phenyl-β-D-galactoside and disaccharide compounds, α-lactose (Galα1-4Glc), β-lactose (Galβ1-4Glc), *N*-acetyllactosamine (Galβ1-4GlcNAc), lacto-*N*-biose I (Galβ1-3GlcNAc) and galactosyl-*N*-acetylgalactosamine (Galβ1-3GalNAc), were purchased from Sigma (USA). SSEA-1 (Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer) used as standard for TLC radioautography was purchased from Iatron (Japan).

Cell culture

PC12 and MR31 cells [14] were grown on tissue culture plates in RPMI-1640 medium (Nissui Co. Ltd, Japan) supplemented with 10% fetal calf serum (HyClone, USA), 5% horse serum (Kohjin Co. Ltd, Japan), and 50 μg ml^{-1} kanamycin (Meiji Co. Ltd, Japan), under a humidified 5% CO_2 atmosphere at 37 °C. For neurite outgrowth experiments, the cells were cultured with 1% fetal calf serum in RPMI-1640 medium with N1 supplement, and 50 μg ml^{-1} kanamycin and 50 ng ml^{-1} nerve growth factor (NGF; Takara, Japan) or 1 μM dexamethasone (DX; Sigma). PC12D cells [19], provided by Drs Katoh-Semba and Sano, were usually grown in Dulbecco's modified Eagle medium (DME; Nissui, Japan) supplemented with 10% fetal calf serum, 5% horse serum and insulin (5 mg l^{-1}, Sigma). For neurite outgrowth experiments, the conditions were the same as those for PC12 and MR31 cells, except that DME was used.

Assay of the activity of GDP-fucose:nLc_4Cer fucosyltransferase

Cultured cells were washed with phosphate-buffered saline and then pelleted by centrifugation. The cell pellets were homogenized in five volumes of 50 mM HEPES buffer containing 0.5 M sucrose and 1 mM EDTA (pH 7.0) with a Sonifier cell disruptor 350 (Branson Sonic Power Co., USA). The cell homogenates were kept at −80 °C until used. The protein concentrations of the cell homogenates were determined by the method of Lowry *et al.* [20]. The activity of GDP-fucose: glycosphingolipid fucosyltransferase was determined according to the procedure of Holmes *et al.* [21] in a reaction mixture comprising 10 μg of substrate, 3 μmol of cacodylate buffer (pH 7.0), 0.3 mg of Triton X-100, 1 μmol of $MgCl_2$, 0.5 μmol of CDP-choline, 5 nmol of GDP-^{14}C-fucose (15 000 cpm nmol^{-1}; Du Pont/NEN Research Products, USA) and cell homogenate as an enzyme source (0.05–0.2 mg protein), in a total volume of 0.1 ml. The reaction mixture was incubated for 30 min at 37 °C, and the reaction was terminated by the addition of 5 μmol of EDTA. The reaction mixture was then applied onto a 1 ml Sep-Pak C18 cartridge and washed with 50 ml of H_2O. The reaction products of glycolipids were eluted with 2 ml of methanol and 12 ml of chloroform:methanol (2:1, by vol). The eluates were combined and concentrated to dryness. Half of the dried residue was used to count the radioactivity with a liquid scintillation counter (Aloka Co., USA), and the other half was subjected to TLC (for conditions, see below). An autoradiogram was generated by exposure of the TLC plate to X-ray film ('X-Omat' AR film, Kodak Co., USA). For measurement of GDP-fucose: disaccharide fucosyltransferase activity, the reaction mixture comprising 0.6 μmol of cacodylate buffer (pH 7.0), 2 μg of acceptor disaccharide, 0.2 μmol of $MgCl_2$, 0.1 μmol of CDP-choline, 1 nmol of GDP-^{14}C-fucose (15 000 cpm nmol^{-1}), and cell homogenate as an enzyme source (0.02–0.1 mg protein), in a total volume of 20 μl was incubated for 30 min at 37 °C. The reaction was stopped by the addition of 20 μl of ethanol, and then briefly centrifuged to remove protein. The resultant solution was applied onto a HPTLC-plate (Whatman, USA) and developed in a solvent of chloroform:methanol:water (60:40:9) containing 0.02% $CaCl_2$ as a final concentration, whereby unreacted GDP-^{14}C-fucose that stayed at the origin was removed. The bands containing the substrate and product were scraped off, and the radioactivity was counted by a liquid scintillation counter (LS-5801; Beckman, USA).

Separation of fucosylated products

To determine the structures of the fucosylated products of nLc_4Cer, three to four reaction tubes were used to perform the reaction on a large scale and with a prolonged incubation time. The reaction mixture in each tube consisted of 25 μg of nLc_4Cer, 6 μmol of cacodylate buffer (pH 7.0), 0.6 mg of Triton X-100, 2 μmol of $MgCl_2$, 1 μmol of CDP-choline, 10 nmol of GDP-fucose (non-radiolabelled, Sigma), and cell homogenate (0.5 mg protein), in a total volume of 0.2 ml. The reaction mixture was incubated for 24 h at 37 °C, 10 nmol of GDP-fucose and 0.2 mg of the cell homogenate being added at

intervals of 8 h. The reaction products were isolated on a Sep-Pak C18 cartridge as described above, and then separated by silica gel HPTLC (Merck, Germany) with a solvent system of chloroform:methanol:water (60:35:8). The lipids were visualized with iodine vapour, and the silica gel corresponding to the newly appeared band in the region lower than nLc_4Cer was scraped off (Fig. 2, shown by an asterisk) and extracted with chloroform:methanol: water (10:10:1). The product thus isolated was ascertained to comigrate with the radiolabelled product on TLC.

Carbohydrate analyses

The carbohydrate composition of the reaction product was determined by GLC of trimethylsilyl methyl glycosides [22]. To determine the carbohydrate structure, the materials (30–50 μg) were permethylated according to Anumula and Taylor [23]. Aliquots of the methylated samples were acetolysed with 0.5 N H_2SO_4 in 90% CH_3COOH at 80 °C for 18 h, reduced with $NaBH_4$, and then acetylated with acetic anhydride:pyridine (1:1) at 100 °C for 15 min according to Levery and Hakomori [24]. The partially methylated alditol acetates thus obtained were analysed by GC/MS (Shimadzu GCMS-QP1100EX) on a SPB-1 (0.32 mm × 30 m; Supelco, USA) or DB-225 (0.33 mm × 30 m; J & W Scientific, USA) fused-silica capillary column; the electron energy was 70 eV.

Hydrolysis of the fucosylated product with an α1,2-linkage specific fucosidase

The fucosylated product (about 5 μg) and 100 μg sodium cholate were suspended in 100 μl 10 mM citrate-phosphate buffer (pH 6.5). The reaction was carried out with 0.05 U *Bacillus* sp. K40T α-1,2-L-fucosidase (EC 3.2.1.63; Seikagaku Kogyo Co., Japan) at 37 °C for 16 h. The reaction mixture was desalted on a Sep-Pak C18 cartridge and then lipids were separated by HPTLC as described above.

Results

Characterization of the fucosyltransferase in PC12 cells

Fucosyltransferase activity was assessed with crude cell homogenates as the enzyme source. The maximal activity was observed at pH 7.0 in the presence of nLc_4Cer as a substrate, but the results indicated a rather broad pH optimum, *i.e.* from at least pH 6.0–7.5 (data not shown). Three detergents, Triton X-100, Triton CF-54 and sodium deoxycholate, were examined as to their effects on fucosyltransferase activity (Table 1). Triton X-100 gave the highest activity, the activity decreasing to 60 and 35% with Triton CF-54 and sodium deoxycholate, respectively. Other requirements for maximum fucosyltransferase activity are also shown in Table 1. The highest enzyme activity was attained in the presence of Mg^{2+}, whereas Mn^{2+} had much less stimulatory effect compared with Mg^{2+}. In order to prevent possible hydrolysis of GDP-fucose and reaction products by endogenous enzymes, 5 mM CDP-choline was added. Omitting CDP-choline from the reaction mixture caused a decrease in the fucosyltransferase activity of 60%. Under the optimal conditions, the fucosyltransferase activity was proportional to the protein concentration (0.05–0.3 mg) and linear synthesis of the fucosylated product was observed for 15–120 min.

Table 1. Requirements for the GDP-Fuc:nLc_4Cer fucosyltransferase reaction.

Conditions	*Specific activity* (pmol h^{-1} per mg protein)
Complete	426.9 ± 21.6
–nLc_4Cer	11.6 ± 0.7
–Mg^{2+}	143.2 ± 3.2
–Mg^{2+}, +Mn^{2+}	268.2 ± 11.2
–CDP choline	253.4 ± 7.4
–Triton X-100, +Na deoxycholate	151.4 ± 17.5
–Triton X-100, +Triton CF-54	251.2 ± 5.2

The complete reaction mixture is described under Materials and methods, and the incubation mixtures were modified as shown. The values are averages for three experiments and are expressed as means ± SEM.

Figure 1 shows autoradiography of the TLC-separated reaction products of catalysis by fucosyltransferase. Labelled products (double bands shown by an arrowhead) having a slightly lower mobility than nLc_4Cer were obtained, when nLc_4Cer was used as the substrate. In addition, lane 11 products were weak compared with lane 10 products, as discussed later (See Fig. 6). No incorporation of labelled fucose was observed when any other glycolipid was used as the substrate. The substrate specificity of the fucosyltransferase in PC12 cells, therefore, was examined in more detail (Table 2). The highest activity was observed for the synthetic substrate, phenyl-β-D-galactoside. The ability to transfer fucose to glycolipids was highest for nLc_4Cer; LacCer was also fucosylated at 60% relative to nLc_4Cer. Almost no incorporation of labelled fucose was observed into Gal_2Cer, Gb_3Cer, $GalGb_3Cer$ or Gb_4Cer. These findings suggest that the labelled product shown in Fig. 1B is fucosyl nLc_4Cer. Fucosyltransferase activity was also examined using disaccarides as the acceptor (Table 3). β-Lactose (Galβ1-4Glc) was the most potent substrate, with the specific activity being at the same level with that of LacCer, whereas *N*-acetyllactosamine (Galβ1-4GlcNAc) was a less effective substrate than β-lactose. The structures of fucosylated products were not determined in this study.

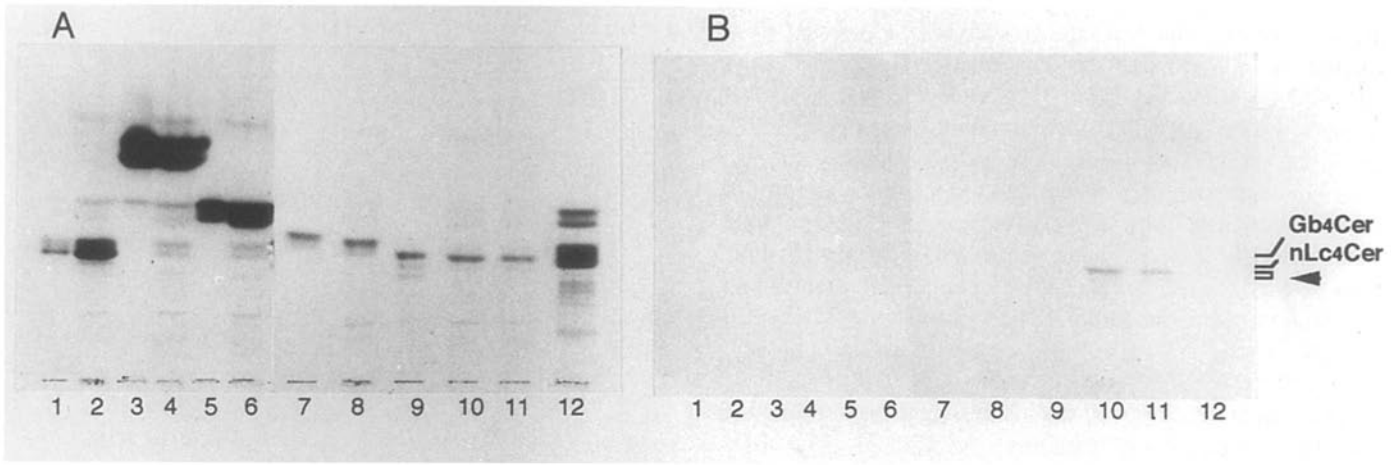


Figure 1. Thin-layer chromatography of products of transfer of ^{14}C-fucose to neutral glycolipids catalysed by PC12 cell homogenates. (A) staining with an orcinol spray; (B) autoradiography after TLC. Substrate and reaction products, respectively: lanes 1 and 2, $GalGb_3Cer$; lanes 3 and 4, Gal_2Cer; lanes 5 and 6, Gb_3Cer; lanes 7 and 8, Gb_4Cer; lane 9 and 10, nLc_4Cer; lane 11, products of lane 9 material catalysed by NGF-treated PC12 cells; lane 12, total neutral glycolipids of PC12 cells. An arrowhead indicates radiolabelled products (double bands).

Table 2. Glycolipid substrate specificity of PC12 cell fucosyltransferase.

Substrate	*Specific activity* (pmol h^{-1} per mg protein)
Phenyl-β-D-galactoside	38 655 ± 5107
Gal_2Cer	31.4 ± 4.7
LacCer	288.1 ± 36.6
$GalGb_3Cer$	22.0 ± 6.0
Gb_3Cer	21.4 ± 10.7
Gb_4Cer	48.1 ± 18.7
nLc_4Cer	476.9 ± 22.6

10 μg of each glycolipid or phenyl-β-galactoside were used as acceptor and assayed as described under Materials and methods.
The values are averages for three experiments and are expressed as means ± SD.

Table 3. Disaccharide substrate specificity of PC12 cell fucosyltransferase.

Substrate	*Specific activity* (pmol h^{-1} per mg protein)
Galα1-4Glc	79.8 ± 29.8
Galβ1-4Glc	252.0 ± 86.7
Galβ1-4GlcNAc	176.6 ± 26.5
Galβ1-3GlcNAc	95.7 ± 3.0
Galβ1-3GalNAc	48.1 ± 3.5

The reaction mixture contained 2 μg of the indicated disaccharide as acceptor and was incubated as described under Materials and methods.
The values are averages for three experiments and are expressed as means ± SD.

Determination of the structure of fucosyl nLc_4Cer by GLC and GC/MS

To determine the structure of fucosyl nLc_4Cer, a large scale experiment was carried out using purified nLc_4Cer (double bands on TLC) as the substrate. Figure 2 shows that a fucosylated product exhibiting double bands (asterisk) was newly generated, migrating between

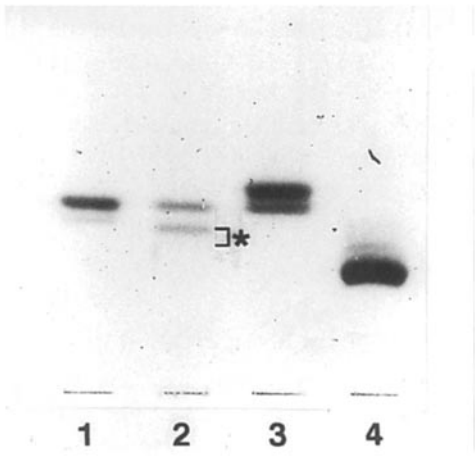


Figure 2. Thin-layer chromatography of the reaction products with GDP-Fuc:nLc_4Cer fucosyltransferase in PC12 cells on a large scale. Lane 1, nLc_4Cer as a substrate; lane 2, reaction products; lane 3, Gb_4Cer; lane 4, SSEA-1 antigen ($V^3Fuc\alpha$-nLc_6Cer). The glycolipids were separated with a solvent system of chloroform: methanol:water (60:35:8), and visualized with orcinol-H_2SO_4 reagent, followed by heating. nLc_4Cer as a substrate (lane 1) and reaction products (lane 2, asterisk) exhibit double bands, the upper band being more prominent, respectively.

nLc_4Cer and SSEA-1, and the materials in the bands were isolated by preparative HPTLC. The product was found to contain glucose, galactose, *N*-acetylglucosamine and fucose in the molar ratio of 1:2:1:1 by GLC analysis (Table 4). The partially methylated alditol acetates derived from nLc_4Cer and fucosyl nLc_4Cer were analysed by GLC on a SPB-1 column (Fig. 3). The total ion chromatograms show that the first peak of nLc_4Cer (Fig. 3A) was decreased and a new peak was generated after the reaction (Fig. 3B). The five peaks for fucosyl nLc_4Cer were identified (Fig. 3B), in order of retention time, as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol (Fuc_1-), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol (Gal_1-), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol ($-_4Glc_1$-), 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methygalactitol ($-_3Gal_1$-), and 1,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-deoxy-*N*-acetylmethyl-glucosaminitol ($-_4GlcNAc_1$-), respectively, by referring to the retention times and mass spectra obtained with an authentic mixture of methyl alditol acetates (BioCarb, Sweden). Since the fourth peak ($-_3Gal_1$-) exhibited a retention time very close to that of 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylgalactitol ($-_2Gal_1$-) on a SPB-1 column, a DB-225 column was used to separate them (Fig. 4). Three peaks (Gal_1-, $-_3Gal_1$-, and $-_4Glc_1$-) were obtained for nLc_4Cer (Fig. 4A). On the other hand, five peaks were obtained for fucosyl nLc_4Cer (Fig. 4B): the fourth peak

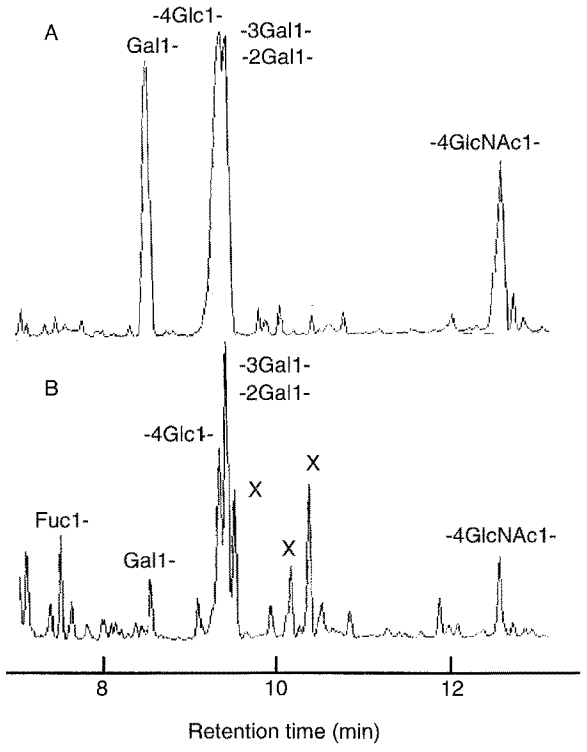


Figure 3. Total ion chromatography (SPB-1 column) of partially methylated alditol acetates derived from nLc_4Cer (A) and fucosyl nLc_4Cer (B). The analytical conditions are given under Materials and methods. X indicates non-hexitol contaminants.

Table 4. Compositions of carbohydrates and partially methylated alditol acetates derived from nLc_4Cer and its fucosylated products (Fuc-nLc_4Cer).

	Carbohydrate[a]				*Alditol acetates*					
	Fuc	*Gal*	*Glc*	*GlcNAc*	*1,5-O-Ac-2,3,4-O-Me-fucitol*	*1,5-O-Ac-2,3,4,6-O-Me-galactitiol*	*1,3,5-O-Ac-2,4,6-O-Me-galactitol*	*1,2,5-O-Ac-3,4,6-O-Me-galactitol*	*1,4,5-O-Ac-2,3,6-O-Me-glucitol*	*1,4,5-O-Ac-3,6-O-Me-2-N-Me-2-deoxyhexitol*
nLc_4Cer		1.9	1	0.8	–	+	+	–	+	+
Fuc-nLc_4Cer	0.7	1.7	1	0.9	+	±[b]	+	+	+	+

[a]Carbohydrate compositions were determined by GLC using TMS derivatives of methanolysates, and are expressed as molar ratios. The analytical conditions for TMS sugars and partially methylated alditoe acetates are given under Materials and methods.
[b]A contaminant peak derived from unreacted substrate.

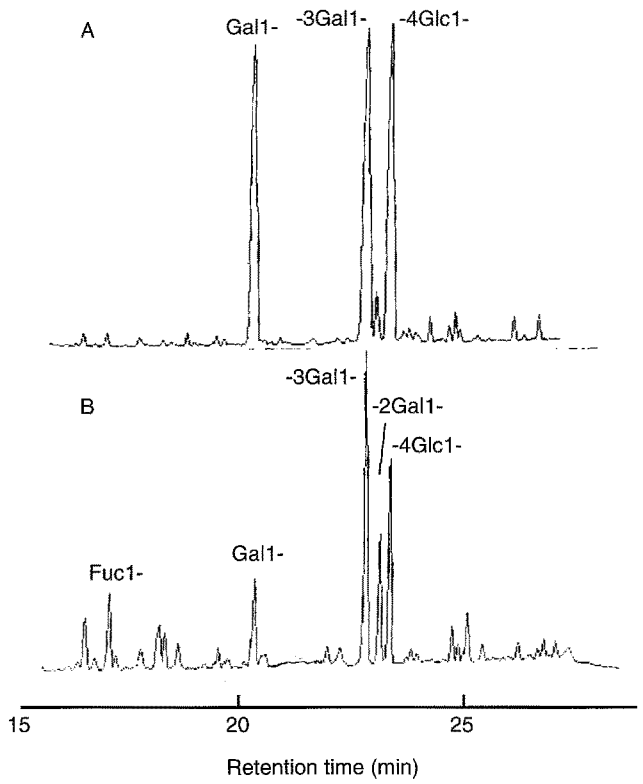


Figure 4. Total ion chromatography (DB-225 column) of partially methylated alditol acetates. A, nLc_4Cer; B, fucosyl nLc_4Cer. The analytical conditions are given under Materials and methods.

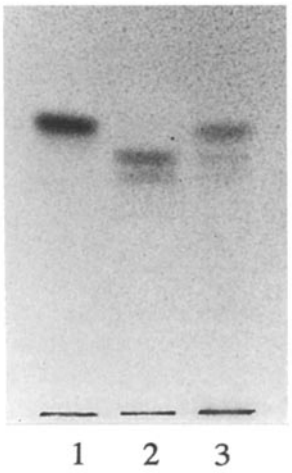


Figure 5. Thin-layer chromatography of α1,2-specific fucosidase-treated fucosyl nLc_4Cer. 1, nLc_4Cer; 2, fucosyl nLc_4Cer; 3, product on fucosidase digestion.

was determined to be 1,2,5-tri-*O*-acetyl-3,4,6,-tri-*O*-methylgalactitol, the mass spectrum being consistent with that reported by Jansson *et al.* [25]. The peak of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol (Gal_1-) was decreased, and 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol (Fuc_1-) and 1,2,5-tri-*O*-acetyl-3,4,6,-tri-*O*-methylgalactitol ($-_2Gal_1$-) were observed. A peak (Gal_1-) in Figs 3B and 4B was considered to be derived from unreacted nLc_4Cer as a contaminant. The overall results obtained on carbohydrate analysis of fucosyl nLc_4Cer are summarized in Table 4, which shows that a fucose residue is attached to the C2 position of the non-reducing end galactose. Based on these data, it was assumed that the structure of fucosyl nLc_4Cer is Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer.

α1,2-Linkage specific fucosidase digestion of fucosyl nLc_4Cer

To confirm the structure of fucosyl nLc_4Cer, hydrolysis of isolated fucosyl nLc_4Cer was carried out with an α1,2-linkage specific fucosidase. Fucosyl nLc_4Cer was converted to components which comigrated with nLc_4Cer on TLC after α1,2-linkage specific fucosidase digestion (Fig. 5). From these results together with those of carbohydrate analysis, it was concluded that fucosyl nLc_4Cer is a H_1 antigen glycosphingolipid consisting of type-2 chain (Galβ1-4GlcNAc-) with blood group H-specificity as reported in human erythrocyte membranes [17].

Changes in fucosyltransferase activity of differentiated PC12 and subline cells

Changes in the level of fucosyltransferase activity on neuronal differentiation induced by NGF or dexamethasone were investigated with PC12, MR31 and PC12D cells. MR31 [14] and PC12D [19] cells are considered to be at the early stage of neuronally differentiating PC12 cells. Figure 6 shows that the activity of GDP-fucose: nLc_4Cer α-1,2-fucosyltransferase in PC12 cells was about 1.5-fold greater than that in MR31 and PC12D cells.

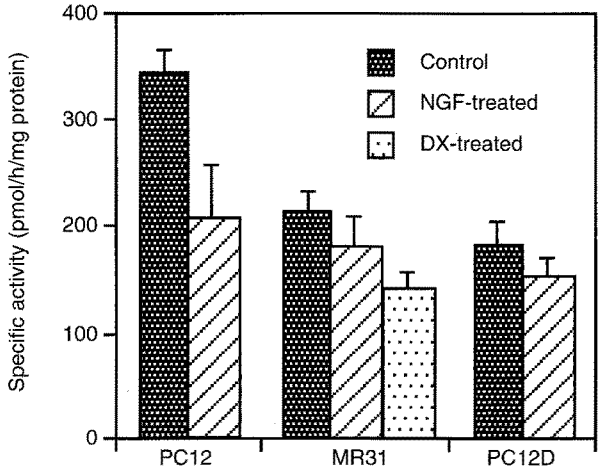
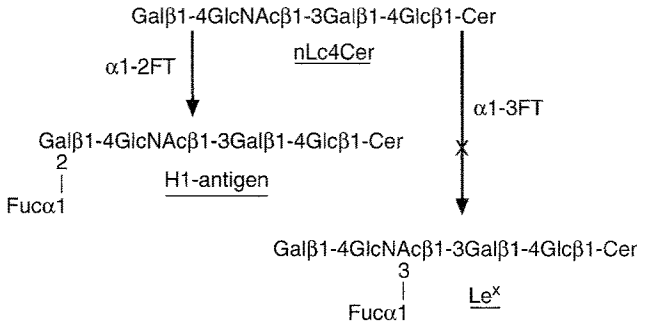


Figure 6. GDP-Fuc:nLc_4Cer α1,2-fucosyltransferase activity of differentiated PC12, MR31 and PC12D cells. Enzyme activity measurement and NGF or dexamethasone treatment were described under Materials and methods.

Moreover, the activity in NGF- or DX-treated cells was found to be reduced, compared with non-treated cells. In particular, the activity in NGF-treated PC12 cells was 60–65% that in the parent PC12 cells. These findings were consistent with those of an autoradiography study shown in Fig. 1B (Compare lane 10 with lane 11). Figure 7 shows the changes of fucosyltransferase activity in PC12 cells during differentiation after NGF treatment. The fucosyltransferase activity decreased to about 80% within 1 h, and continued to decrease gradually to almost 50% of that in the untreated cells on further incubation until 96 h.



Scheme 1. (FT, fucosyltransferase).

Discussion

The present study demonstrates that PC12 cells can transfer α-L-fucose from GDP-fucose to the nLc_4Cer acceptor to form a blood group H_1 antigen, Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer. Although the fucosyltransferase in PC12 cells was the most active toward the nLc_4Cer acceptor, reduced activity, *i.e.* 60% relative to nLc_4Cer, was also found when LacCer was used as the substrate. No other neutral glycosphingolipid tested was fucosylated. The disaccharide compounds, β-lactose and *N*-acetyllactosamine, were also found to be effectively fucosylated, the former being a more effective substrate than the latter, but different from the glycolipid substrates. The differences in the substrate specificities between glycolipids and disaccharides might be due to the presence or absence of ceramide moieties, or the presence of different types of fucosyltransferases in PC12 cells. Previously, Ariga *et al.* reported the presence of fucosyl $GalGb_3Cer$ in PC12h cells [9]. Although we preliminarily found that PC12 cells contained $GalGb_3Cer$ [15], $GalGb_3$-Cer was not a potent acceptor for α-L-fucose in our experimental system. The discrepancy may be due to the difference in subclonal cells, used, *i.e.* PC12 or PC12h cells. β-Galactoside α1,2-fucosyltransferase that synthesizes blood group H glycolipids has been characterized in various extraneural systems [26, 27]. nLc_4Cer is considered to be fucosylated through two pathways (Scheme 1), synthesizing a H_1 antigen or Le^x. Earlier, Basu *et al.* [28] reported α-fucosyltransferase activity in membrane preparations from bovine spleen, which catalyses the transfer of L-fucose to nLc_4Cer. In the present study, we demonstrated, by structural analysis of the reaction product, that a fucose residue was attached to a non-reducing terminal galactose through an α1-2 linkage. The possible synthesis of Le^x catalysed by α1,3-fucosyltransferase in PC12 cells was excluded by the findings that the fucosylated product was susceptible to a fucosidase specific to the α1-2 linkage, and that 1,3,4,5-tri-*O*-acetyl-6-*O*-methyl-2-deoxy-*N*-acetylmethylglucosaminitol was not detected on GC/MS. The efficient incorporation of α-L-fucose into phenyl-β-D-galactoside also supported an α1,2-type transferase [29]. To our knowledge, this is the first report showing the presence of an α1,2-fucosyltransferase synthesizing a blood group H_1 antigen in neuronal cells.

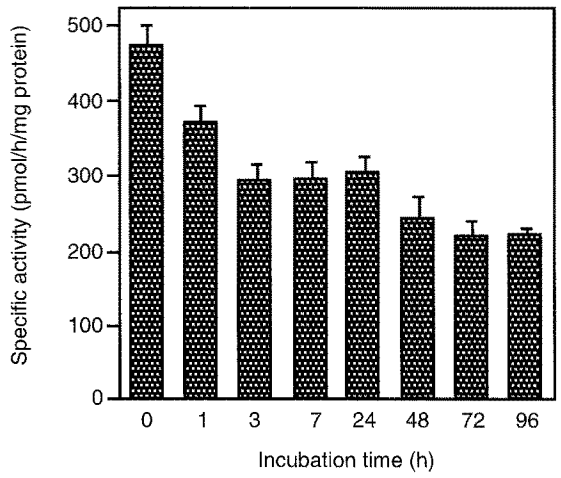


Figure 7. Changes in GDP-Fuc:nLc_4Cer α1,2-fucosyltransferase activity level in PC12 cells during differentiation induced by NGF. The incubation conditions are given under Materials and methods.

It should be noted that the α1,2-fucosyltransferase activity in PC12 cells decreased in association with the cell differentiation caused by NGF. MR31 [14] and PC12D [19] cells, which are considered to be more neuronally differentiated than the parent PC12 cells, also exhibited decreased α1,2-fucosyltransferase activity. In contrast to our finding that globoside (Gb_4Cer) was not fucosylated, Schwarting *et al.* reported that NGF enhanced the synthesis of fucosylgloboside and fucosylganglioside in PC12 cells [6], although the chemical structures of the reaction products were not determined precisely. Surface-expressed glycoconjugates containing terminal Fucα1-2Gal linkages have been reported to show striking temporal and spatial changes in their expression during human and murine embryogenesis [30–32]. Moreover, these linkages may be aberrantly expressed in association with oncogenic transformation [1–3]. Thus, the present findings showing decreased activity of an α1,2-fucosyltransferase in differentiated cells are considered to be consistent with increased activity of this

enzyme in undifferentiated cells, e.g. actively growing and transformed cells, as discussed above. Recently, molecular cloning and expression studies of β-galactoside α1,2-fucosyltransferase were reported [33–35]. Understanding of the role of H_1 antigen in the differentiation of PC12 cells will require further study.

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