Characteristics of Asparagine-Linked Sugar Chains of Sphingolipid Activator Protein 1 Purified from Normal Human Liver and GM1 Gangliosidosis (Type 1) Liver†

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ABSTRACT: Asparagine-linked sugar chains of sphingolipid activator protein 1 (SAP-1) purified from normal human liver and GM1 gangliosidosis (type 1) liver were comparatively investigated. Oligosaccharides released from the two SAP-1 samples by hydrazinolysis were fractionated by paper electrophoresis and by *Aleuria aurantia* lectin–Sepharose and Bio-Gel P-4 (under 400 mesh) column chromatography. Structures of oligosaccharides in each fraction were estimated from data on their effective molecular sizes, behavior on immobilized lectin columns with different carbohydrate-binding specificities, results of sequential digestion by exoglycosidases with different aglycon specificities, and methylation analysis. Sugar chains of SAP-1 purified from normal human liver and from GM1 gangliosidosis (type 1) liver were different from each other, although both of them were derived from complex-type sugar chains. The sugar chains of the former were the following eight degradation products from complex-type sugar chains by exoglycosidases in lysosomes: Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$, Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$, Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$, Manα1→6Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$, Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$, Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$, GlcNAcβ1→4GlcNAc$_{OT}$, and GlcNAc$_{OT}$. In contrast to these, the sugar chains of the latter were sialylated and nonsialylated mono- to tetraantennary complex-type sugar chains that were not fully degraded due to a metabolic defect in acid β-galactosidase activity.

The enzymatic degradation of a number of sphingolipids in the lysosomes is stimulated by small (8–13-kDa) glycoproteins named sphingolipid activator proteins SAP-1,[1] SAP-2, and SAP-3 (Wenger & Inui, 1984). These proteins were originally described by Mehl and Jatzkewitz (1964), Ho and O'Brien (1971), and Li and Li (1976).

SAP-1 activates the hydrolysis of cerebroside sulfate, GM1 ganglioside, and globotriaosylceramide by arylsulfatase A, acid β-galactosidase, and α-galactosidase, respectively (Vogel et al., 1987). The mechanism of activation appears to involve interaction with the sphingolipid substrates, solubilizing them for hydrolysis (Inui & Wenger, 1983). SAP-1 is a glycoprotein containing one asparagine-linked sugar chain, which is derived by proteolytic processing from a precursor encoded by a genetic locus on human chromosome 10 (Inui et al., 1985; O'Brien et al., 1988). The physiological significance of SAP-1 was demonstrated by the discovery of its deficiency in a variant form of metachromatic leukodystrophy (Li et al., 1985). Recently, Morimoto et al. (1988, 1989) proposed a new nomenclature for SAP-1 as saposin B, which reflected the domain of the precursor peptide.

Since SAP-1 is translocated into lysosomes after biosynthesis, its asparagine-linked sugar chain should act as a traffic signal to lysosomes, but no report has been found about the precise structure. In our preliminary experiments, structures of sugar chains of SAP-1 purified from normal human liver showed that they might be the secondary degradation products from complex-type sugar chains. In this report, we describe comparatively the precise structures of asparagine-linked sugar chains of SAP-1 purified from normal human liver and GM1 gangliosidosis (type 1) liver, determined by microanalysis in combination with Bio-Gel P-4 column chromatography, methylation analysis, glycosidase digestion, and lectin affinity chromatography.

Materials and Methods

Lectins. Concanavalin A (Con A)–Sepharose was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. *Aleuria aurantia* lectin (AAL)–Sepharose (7 mg of AAL/mL of gel) was prepared as reported previously (Yazawa et al., 1984). Phytohemagglutinin E_4 (E-PHA)–agarose (7 mg of E-PHA/mL of gel) and phytohemagglutinin L_4 (L-PHA)–agarose (9 mg of L-PHA/mL of gel) were kindly supplied by Hohnen Oil Co., Ltd., Tokyo, Japan. *Datura stramonium* agglutinin (DSA)–Sepharose (3 mg of DSA/mL of gel) and *N*-acetylglucosamine oligomers were prepared according to the methods described in a previous paper (Yamashita et al., 1987).

Chemicals. NaB^3H_4 (348 mCi/mmol) was purchased from New England Nuclear, Boston, MA. NaB^2H_4 (98%) was obtained from Merck Co., Darmstadt, FRG. Methyl α-D-mannopyranoside, α-L-fucose, and chitin were purchased from Nakarai Tesque Inc., Kyoto, Japan. Bio-Gel P-4 (under 400 mesh) was obtained from Bio-Rad Laboratories, Richmond, CA.

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[1] Abbreviations: SAPs, sphingolipid activator proteins; SAP-1, -2, and -3, sphingolipid activator proteins 1, 2, and 3; SDS, sodium dodecyl sulfate; AAL, *Aleuria aurantia* lectin; E-PHA, phytohemagglutinin E_4; L-PHA, phytohemagglutinin L_4; DSA, *Datura stramonium* agglutinin; Con A, concanavalin A; subscript OT, NaB^3H_4-reduced oligosaccharides. All sugars mentioned in this paper have the D configuration except for fucose, which has the L configuration.

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Enzymes. Arthrobacter ureafaciens sialidase (Uchida et al., 1974) was purchased from Nakarai Tesque Inc., Kyoto, Japan. Bovine epididymal α-fucosidase and jack bean meal were purchased from Sigma Chemical Co., St. Louis, MO. Snail β-mannosidase was kindly provided by Seikagaku Kogyo Co., Tokyo, Japan. Jack bean β-*N*-acetylhexosaminidase and α-mannosidase were prepared by the method of Li and Li (1972). Diplococcal β-galactosidase, β-*N*-acetylhexosaminidase, and endo-β-*N*-acetylglucosaminidase D were purified from the culture fluid of *Diplococcus pneumoniae* according to the method of Glasgow et al. (1977).

Oligosaccharides. (Neu5Acα2→6)$_{0\sim2}$[Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$] (abbreviated as Neu5Ac$_{0\sim2}$·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$), [Neu5Acα2→6(3)]$_{1\sim3}${Galβ1→4GlcNAcβ1→2Manα1→6[Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Manα1→3]Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$} (abbreviated as Neu5Ac$_{1\sim3}$·Gal$_3$·GlcNAc$_3$·Man$_3$·GlcNAc·GlcNAc$_{OT}$), [Neu5Acα2→6(3)]$_4${Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2)Manα1→6[Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Manα1→3]Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$} (abbreviated as Neu5Ac$_4$·Gal$_4$·GlcNAc$_4$·Man$_3$·GlcNAc·GlcNAc$_{OT}$), and Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$ (abbreviated as Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·Fuc·GlcNAc$_{OT}$) were obtained from ceruloplasmin (Yamashita, et al., 1981a), α_1-acid glycoprotein (Yoshima et al, 1981), and urinary ribonuclease L (Hitoi et al., 1987), respectively, by hydrazinolysis followed by reduction with NaB^3H_4. Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (abbreviated as Man$_3$·GlcNAc·GlcNAc$_{OT}$), Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (abbreviated Man·GlcNAc·GlcNAc$_{OT}$), Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1-6)GlcNAc$_{OT}$ (abbreviated as Man$_3$·GlcNAc·Fuc·GlcNAc$_{OT}$), and Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$ (abbreviated as Man·GlcNAc·Fuc·GlcNAc$_{OT}$) were prepared from Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$ and Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·Fuc·GlcNAc$_{OT}$ by sequential digestion with β-galactosidase, β-*N*-acetylhexosaminidase, and α-mannosidase. Manα1→6Manβ1→4GlcNAcβ1→4(Fucα1→6)-GlcNAc$_{OT}$ (abbreviated as Man$_2$·GlcNAc·Fuc·GlcNAc$_{OT}$) was prepared from urinary glycopeptides of a fucosidosis patient (Yamashita et al., 1979).

Purification of Sphingolipid Activator Protein 1 (SAP-1). SAP-1 samples were prepared from 200-g liver samples from normal tissue and from a patient with GM1 gangliosidosis (type 1) according to the method of Li and Li (1976). After gel filtration on Sephadex G-75, the activator proteins were dialyzed and lyophilized. The residue, after dissolution in a small volume of water, was fractionated by HPLC on a Hitachi 655A-11 apparatus (Tokyo, Japan) equipped with ProRPCHR5/10 (Pharmacia LKB Biotech, Uppsala, Sweden). Fractions were eluted with a linear gradient of water containing 0.1% trifluoroacetic acid and acetonitrile–2-propanol (3:7) containing 0.1% trifluoroacetic acid. Peaks were detected by measuring absorbance at 220 nm. SAP-1 was eluted at about 35% acetonitrile–2-propanol (3:7), and the eluted protein that corresponded to SAP-1 had a solitary peak on repeated HPLC. Furthermore, the purity of the eluted protein was checked by SDS–polyacrylamide gel electrophoresis and Western blotting with anti-SAP-1 and anti-SAP-2 antibodies. The immunostaining pattern with anti-SAP-1 antibody showed a single band at the same location seen on the silver stained SDS–polyacrylamide gel, but no staining was found with the anti-SAP-2 antibody. By SDS–polyacrylamide gel electrophoresis, moleculr weights of the activator proteins purified from normal liver and GM1 gangliosidosis (type 1) liver were estimated to be around 9 500 and 12000, respectively (Inui & Wenger, 1984).

Isolation of the Asparagine-Linked Sugar Chains of SAP-1 of Human Normal Liver and GM1 Gangliosidosis (Type 1) Liver. One milligram of SAP-1 from normal liver and 300 μg of SAP-1 from GM1 gangliosidosis (type 1) liver were subjected to hydrazinolysis at 100 °C for 8 h as reported previously (Takasaki et al., 1982). After N-acetylation, one-fifth of the sugars released from normal liver SAP-1 and two-thirds of the oligosaccharides released from GM1 gangliosidosis liver SAP-1 were reduced with NaB^3H_4. The total yields of radioactive sugars obtained from SAP-1 of normal liver and GM1 gangliosidosis (type 1) liver were 4.2×10^5 and 3.7×10^5 cpm, and the numbers of sugar chains released from them were calculated as 1.0 and 1.1 on the basis of the specific activity of the NaB^3H_4 and the molecular weights of the activators, respectively. The remaining sugars from two SAP-1 samples were reduced with NaB^2H_4 to obtain samples for methylation analysis.

Glycosidase Digestion. Radioactive oligosaccharides [(2–20) × 10^3 cpm, 0.1–1 nmol] were incubated with one of the following reaction mixtures in sealed capillaries at 37 °C for 17 h: for sialidase digestion, 50 milliunits of enzyme in sodium acetate buffer, pH 5.0 (20 μL); for diplococcal β-galactosidase and/or β-*N*-acetylhexosaminidase digestion, 1 milliunit of each enzyme in 0.2 M citrate/phosphate buffer, pH 6.0 (10 μL); for jack bean β-*N*-acetylhexosaminidase digestion, 0.3 unit of enzyme in 0.2 M citrate/phosphate buffer, pH 5.5 (10 μL); for jack bean α-mannosidase digestion, 0.2 unit of enzyme in 0.05 M sodium acetate buffer, pH 4.5, containing 1 mM $ZnCl_2$ (20 μL); and for epididymal α-fucosidase digestion, 10 milliunits of enzyme in 0.2 M citrate buffer, pH 6.0 (20 μL). A small amount of toluene was added to the reaction mixture to prevent bacterial growth.

Fractionation or Analysis of Oligosaccharides by Affinity Chromatography on Immobilized Lectin Columns. The column containing 1 mL of AAL–Sepharose was equilibrated with phosphate-buffered saline (buffer A: 6.7 mM KH_2PO_4, 0.15 M NaCl, pH 7.4) containing 0.02% NaN_3. Tritium-labeled oligosaccharide [(1 to ~10) × 10^4 cpm, 0.5 to ~5 nmol] dissolved in 100 μL of buffer A was applied to the column. After the column was kept at 2 °C for 30 min, elution was started at 2 °C with 5 mL of buffer A followed by 5 mL of buffer A containing 5 mM L-fucose at 20 °C.

The column containing 1 mL of DSA–Sepharose was equilibrated with 10 mM Tris-HCl buffer, pH 7.4, containing 0.02% NaN_3 (buffer B). Tritium-labeled oligosaccharide (100 to ~300 cpm, 5 to ~15 pmol) dissolved in 100 μL of buffer B was applied to the column and allowed to stand at 2 °C for 30 min. Elution was performed at 2 °C with 10 mL of buffer B followed by 5 mL of buffer B containing 1% *N*-acetylglucosamine oligomers at 20 °C.

The columns containing 1 mL of Con A–Sepharose, E-PHA–agarose, and L-PHA–agarose were equilibrated with 10 mM Tris-HCl buffer, pH 7.4, containing 0.02% NaN_3, 0.15 M NaCl, and 1 mM each of $MnCl_2$, $MgCl_2$, and $CaCl_2$ (buffer C). Tritium-labeled oligosaccharide (100 to ~300 cpm, 5 to ~15 pmol) dissolved in 100 μL of buffer C was applied to the column, which was allowed to stand at 2 °C for 30 min. Elution from the Con A column was performed at 2 °C with 5 mL of buffer C followed by 5 mL of buffer C containing

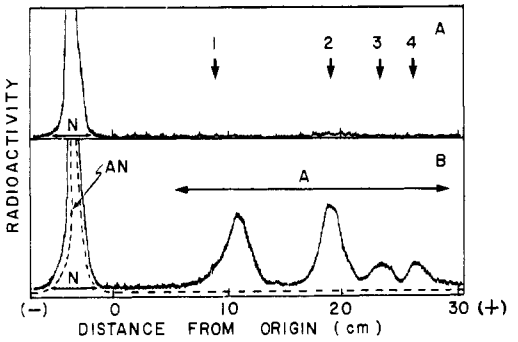


FIGURE 1: Paper electrophoresis of oligosaccharides released from human control liver SAP-1 and GM1 gangliosidosis (type 1) liver SAP-1 by hydrazinolysis followed by reduction with NaB^3H_4. Arrows indicate the migration positions of authentic oligosaccharides: 1, Neu5Ac·Gal_2·$GlcNAc_2$·Man_3·GlcNAc·$GlcNAc_{OT}$; 2, $Neu5Ac_2$·Gal_2·$GlcNAc_2$·Man_3·GlcNAc·$GlcNAc_{OT}$; 3, $Neu5Ac_3$·Gal_3·$GlcNAc_3$·Man_3·GlcNAc·$GlcNAc_{OT}$; 4, $Neu5Ac_4$·Gal_4·$GlcNAc_4$·Man_3·GlcNAc·$GlcNAc_{OT}$. Panel A, normal human liver SAP-1; panel B, GM1 gangliosidosis (type 1) liver SAP-1 (solid line) and the radioactive fraction A digested with sialidase (dotted line).

0.1 M methyl α-D-mannopyranoside, while oligosaccharides bound to an L-PHA column were eluted with 5 mL of the same buffer at 2 °C followed by the buffer at 20 °C, and oligosaccharides bound to an E-PHA column were eluted with 10 mL of the same buffer at 2 °C (Kobata & Yamashita, 1989).

Methylation Analysis. Methylation analysis of oligosaccharides was as described previously (Endo et al., 1979), except that the time of hydrolysis of permethylated oligosaccharides released from SAP-1 of GM1 gangliosidosis (type 1) liver was prolonged from 2 to 6 h by using 0.5 N H_2SO_4 containing 90% acetic acid and NaB^2H_4 was used as a reducing reagent. Analysis of partially O-methylated hexitols and *N*-acetylglucosaminitols was performed with a gas chromatograph–mass spectrometer, Model GC–MS QP-1000 (Shimadzu Co., Ltd., Kyoto, Japan), by using a fused silica capillary column coated with a cross-linked SPB-35 (0.53-mm i.d., 30-m length) or a packed column (2.5-mm i.d., 1-m length) with 2% OV-210 coated on Gas-Chrom Q (80–100 mesh). The column temperature was programmed from 170 to 230 °C at a rate of 2 °C/min, and the flow rate of carrier gas was 20 mL/min. The molar ratios of partially O-methylated alditol acetates were calculated from the total ion intensities in GC–MS.

Other Analytical Methods. Descending paper chromatography was performed with 1-butanol/ethanol/water (4:1:1) as solvent. High-voltage paper electrophoresis was performed by using pyridine/acetate buffer, pH 5.4 (pyridine/acetic acid/water, 3:1:387), at a potential of 73 V/cm for 90 min. Radiochromatoscanning was performed with a Packard radiochromatogram scanner, Model 7201.

Bio-Gel P-4 (under 400 mesh) column chromatography (2-cm i.d., 1.25-m length) was performed as reported previously (Yamashita et al., 1982). Radioactivity was determined with a Beckman liquid scintillation spectrometer, Model LS-9000.

RESULTS

Structural Studies of Sugar Chains of SAP-1 Purified from Normal Liver

Fractionation of Oligosaccharides Obtained from Normal Liver SAP-1. With paper electrophoresis at pH 5.4, all of the tritium-labeled oligosaccharides released from control liver SAP-1 did not move from the spotted positions as shown in Figure 1A, indicating that they are exclusively neutral sugar chains. After extraction of tritium-labeled oligosaccharides from the paper, they were subjected to Bio-Gel P-4 column chromatography. Since their elution patterns were very heterogeneous (data not shown), they were fractionated by AAL-Sepharose column chromatography; 47% of the total oligosaccharides passed through the column, and the remaining 53% of the oligosaccharides bound to the column and eluted with buffer containing 5 mM fucose. The fractions not bound and bound to the column were named as AAL^- and AAL^+, respectively. When oligosaccharides of both fractions were subjected to Bio-Gel P-4 column chromatography, oligosaccharides in the AAL^- fraction were separated into five radioactive peaks as shown in Figure 2A (named from a to e), and oligosaccharides in the AAL^+ fraction were separated into three radioactive peaks (named from f to h) as shown in Figure 2B.

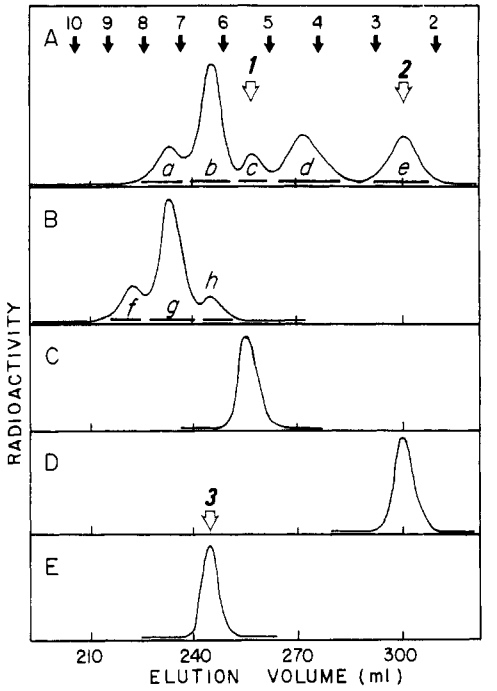


FIGURE 2: Bio-Gel P-4 column chromatography of the neutral oligosaccharide fraction in Figure 1A after separation by AAL-Sepharose column chromatography and partial degradation with exoglycosidase. Black arrows indicate the elution positions of glucose oligomers, and the numbers indicate those of glucose units. White arrows indicate the elution positions of authentic sugars: 1, Man·GlcNAc·$GlcNAc_{OT}$; 2, $GlcNAc_{OT}$; 3, Man·GlcNAc·Fuc·$GlcNAc_{OT}$. Panel A, AAL^- fraction; panel B, AAL^+ fraction; panel C, component a digested with α-mannosidase; panel D, component d digested with β-*N*-acetylhexosaminidase; panel E, component f digested with α-mannosidase. Components b and g gave the same results as in panels C and E, respectively.

Structures of the Neutral Components a–h. The elution position on the Bio-Gel P-4 column of component c was the same as that of Manβ1→4GlcNAcβ1→4$GlcNAc_{OT}$, and components a and b were converted to the same elution position as that of Man·GlcNAc·$GlcNAc_{OT}$ by α-mannosidase digestion, releasing 2 and 1 mol of α-mannosyl residues, respectively (Figure 2C). Those reduced trisaccharides were converted to *N*-acetylglucosaminitol by sequential digestion with β-mannosidase and β-*N*-acetylhexosaminidase (data not shown), indicating that the monosaccharide compositions and anomeric conformations of components a, b, and c are

a: $(Man\alpha)_2$·Manβ·GlcNAcβ·$GlcNAc_{OT}$

b: Manα·Manβ·GlcNAcβ·$GlcNAc_{OT}$

c: Manβ·GlcNAcβ·$GlcNAc_{OT}$

Table I: Methylation Analysis of Neutral Sugars Released from Control Liver SAP-1 (N in Figure 1A) and GM1 Gangliosidosis (Type 1) Liver SAP-1 (N + AN in Figure 1B)

partially O-methylated sugars	N, molar ratio[a]	N + AN
fucitol		
2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl	0.5	+[b]
galactitol		
2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl	0.0	+
mannitol		
2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl	1.0	+
3,4,6-tri-*O*-methyl-1,2,5-tri-*O*-acetyl	0.0	+
2,3,6-tri-*O*-methyl-1,4,5-tri-*O*-acetyl	0.0	+
2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl	0.6	+
3,6-di-*O*-methyl-1,2,3,5-tetra-*O*-acetyl	0.0	+
3,4-di-*O*-methyl-1,2,5,6-tetra-*O*-acetyl	0.0	+
2,4-di-*O*-methyl-1,3,5,6-tetra-*O*-acetyl	0.1	+
2-(*N*-methylacetamido)-2-deoxyglucitol		
1,3,4,5,6-penta-*O*-methyl	0.1	+
1,3,5,6-tetra-*O*-methyl-4-mono-*O*-acetyl	0.4	+
1,3,5-tri-*O*-methyl-4,6-di-*O*-acetyl	0.3	+
3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl	0.2	+
3,6-di-*O*-methyl-1,4,5-tri-*O*-acetyl	0.8	+

[a] Numbers in the table were calculated by taking the value of 2,3,4,6-tetra-*O*-methylmannitol as 1.0. [b] +: detected by mass spectroscopy monitoring *m/z* 102, 113, 117, 118, 129, 130, 131, 139, 143, 159, 161, 175, 189, 190, 202, 205, 233, 234.

The elution position of component e was the same as that of *N*-acetylglucosaminitol, and component d was converted to *N*-acetylglucosaminitol by β-*N*-acetylhexosaminidase digestion (Figure 2D), indicating that component d is GlcNAcβ·GlcNAc$_{OT}$.

By α-mannosidase digestion, components f and g were converted to the same elution position as that of Man·GlcNAc·Fuc·GlcNAc$_{OT}$, releasing 2 and 1 mol of α-mannosyl residues, respectively (Figure 2E). Component h showed the same elution position on the Bio-Gel P-4 column as that of Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$. Since the respective reduced tetrasaccharides were converted to *N*-acetylglucosaminitol by sequential digestion with β-mannosidase, β-*N*-acetylhexosaminidase, and α-fucosidase (data not shown), the monosaccharide compositions and anomeric conformations of components f, g, and h should be

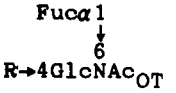
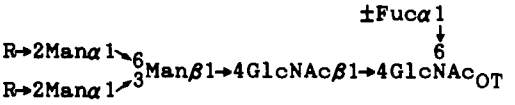
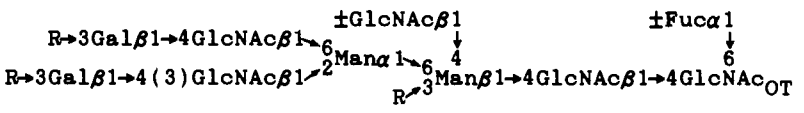
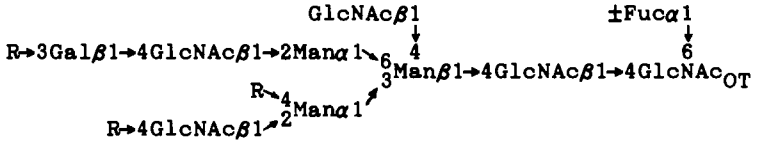
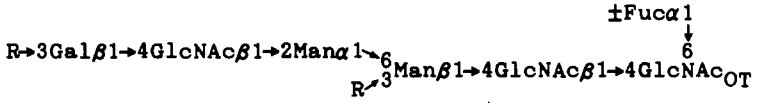
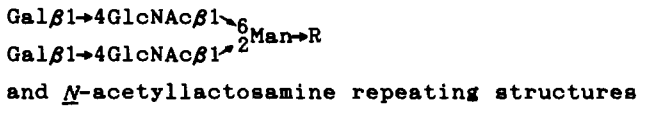
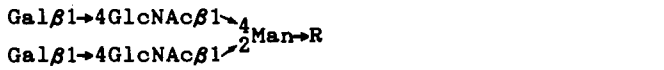
f: (Manα)$_2$·Manβ·GlcNAcβ·(Fucα)·GlcNAc$_{OT}$

g: Manα·Manβ·GlcNAcβ·(Fucα)·GlcNAc$_{OT}$

h: Manβ·GlcNAcβ·(Fucα)·GlcNAc$_{OT}$

In order to elucidate the glycosidic linkages of components a–h released from control liver SAP-1, methylation analysis of these neutral components was performed. The results are summarized in Table I. Detection of 2,3,4,6-tetra-*O*-methyl- and 2,3,4-tri-*O*-methylmannitol and small amounts of 2,4-di-*O*-methylmannitol indicates the occurrence of Manα1→6Manβ1→ and Manα1→6(Manα1→3)Manβ1→ groups in their components. Since components a and f bound to Con A–Sepharose, they should not contain the Manα1→6Manα1→6Manβ1→ group but instead the Manα1→6(Manα1→3)Manβ1→ group in the molecules on the basis of the carbohydrate-binding specificity of Con A–Sepharose (Table II), while components b and g should contain exclusively the Manα1→6Manβ1→ group, because they passed through the Con A–Sepharose column.

Table II: Carbohydrate-Binding Specificities of Immobilized AAL, Con A, L-PHA, E-PHA, and DSA Columns Used To Elucidate the Structures of Complex-Type Sugar Chains

lectin	binding specificity[a]	ref
A. aurantia lectin (AAL)		Yamashita et al., 1985
concanavalin A (Con A)		Ogata et al., 1975
phytohemagglutinin L$_4$ (L-PHA)[b]		Merkle & Cummings, 1987
phytohemagglutinin E$_4$ (E-PHA) strong binding[c] (E-PHA^R fraction at 20 and 2 °C)		Yamashita et al., 1983b
weak binding[d] (E-PHA^R fraction at 2 °C)		Kobata & Yamashita, 1989
D. stramonium agglutinin (DSA) strong binding (DSA$^+$ fraction)		Yamashita et al., 1987
weak binding (DSA^R fraction)		

[a] R represents either hydrogen or sugars. [b] Manuscript in preparation. [c] The nonasaccharide is retarded in the column at 20 °C and is more extensively retarded at 2 °C. [d] Although the hexasaccharide passes through an E-PHA column without interaction at 20 °C, it is retarded at 2 °C.

a (5.5%) Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (Manα1→3)

b (17.3%) Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$

c (4.1%) Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$

d (11.8%) GlcNAcβ1→4GlcNAc$_{OT}$

e (8.3%) GlcNAc$_{OT}$

f (9.2%) Fucα1→6 on Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (Manα1→3)

g (37.6%) Fucα1→6 on Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$

h (9.2%) Fucα1→6 on Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$

FIGURE 3: Presumed structures of components a–h in Figure 2.

Detection of 1,3,5,6-tetra-*O*-methyl- and 1,3,5-tri-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitol supports the fact that both fucosylated and nonfucosylated reducing terminal *N*-acetylglucosaminotols are included in components a–h. The presence of only 3,6-di-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitol as a di-*O*-methyl derivative of *N*-acetylglucosamine residues indicated that the reduced *N,N'*-diacetylchitobiose is exclusively substituted at the C-4 position. Further, detection of 3,4,6-tri-*O*-methyl- and 1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitol shows that components d and e are GlcNAcβ1→4GlcNAc$_{OT}$ and *N*-acetylglucosaminitol, respectively.

From the results so far described, the structures of the sugars released from normal liver SAP-1 by hydrazinolysis were proposed as summarized in Figure 3. The structures of sugar chains of normal liver SAP-1 are considered as secondary degradation products of complex-type sugar chains because a part of the proximal *N*-acetylglucosamine of the sample is fucosylated. But it still remains a possibility that components a–e may be derived from high-mannose-type sugar chains. In order to resolve the problem, the structures of sugar chains released from GM1 gangliosidosis (type 1) liver SAP-1 were studied.

Structural Studies of Sugar Chains of SAP-1 Purified from GM1 Gangliosidosis (Type 1) Liver

Fractionation of Oligosaccharides from GM1 Gangliosidosis (Type 1) Liver SAP-1. With paper electrophoresis at pH 5.4, 85% of the tritium-labeled oligosaccharides released from SAP-1 of GM1 gangliosidosis (type 1) liver were neutral (N) and the remaining 15% were acidic (A) (Figure 1B, solid line). The acidic oligosaccharides were converted to neutral components (AN) by sialidase digestion (Figure 1B, dotted line), indicating that their acidic nature can be ascribed to their sialic acid residues. The elution patterns of oligosaccharide fractions N and AN after Bio-Gel P-4 column chromatography were very heterogeneous and different from those of control liver as described in the preceding part (data not shown). Accordingly, they were further fractionated by AAL–Sepharose column chromatography. As with the SAP-1 of control liver, a part of fractions N and AN passed through the column and the remaining part bound to the column and was eluted with buffer containing 5 mM fucose; these were named as AAL$^-$ and AAL$^+$, respectively. Oligosaccharides in the AAL$^-$ fractions of N and AN were separated into seven and five components (Figure 4, panels A and C), and oligosaccharides in AAL$^+$ fractions of N and AN were separated into nine and four components (Figure 4, panels B and D), respectively. The radioactive peaks from the neutral fractions N and AN were named from I to XVI as shown in Figure 4.

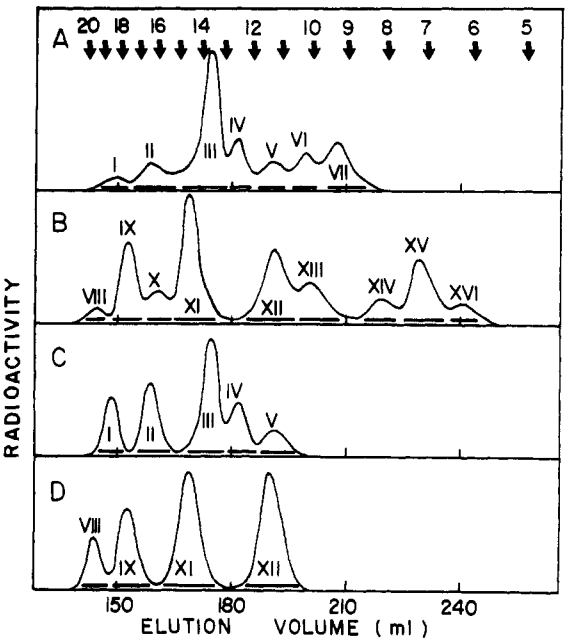


FIGURE 4: Bio-Gel P-4 column chromatography of fractions N and AN in Figure 1B separated by AAL–Sepharose column chromatography. Black arrows and numbers are the same as in Figure 2. Panel A, AAL$^-$ fraction of fraction N; panel B, AAL$^+$ fraction of fraction N; panel C, AAL$^-$ fraction of fraction AN; panel D, AAL$^+$ fraction of fraction AN.

Exoglycosidase Digestion of Components I–XVI. The monosaccharide compositions and anomeric conformations of components I–XVI were determined by sequential digestion with exoglycosidases. The elution profiles on a Bio-Gel P-4 column of components I–XVI after sequential digestion with diplococcal β-galactosidase and jack bean β-*N*-acetylhexosaminidase, which together hydrolyze specifically the Galβ1→4GlcNAc group (Glasgow et al., 1977), are shown with solid lines and dotted lines, respectively, in Figure 5, panels A–P; the elution position of each component before enzymatic digestion is indicated by a white triangle with the name of the component. By comparison of the mobilities of each radioactive component before and after the sequential β-galactosidase and β-*N*-acetylhexosaminidase digestion, the number of galactose residues and β-*N*-acetylglucosamine residues released from each component was elucidated as summarized in Table III. One Galβ1→4 residue and one *N*-acetylglucosamine residue in the outer chains of complex-type sugar chains behave as 1.0 to ~1.1 glucose units and 1.8 to ~2.0 glucose units, respectively, on the basis of glucose equivalents of authentic mono- to tetraantennary sugar chains

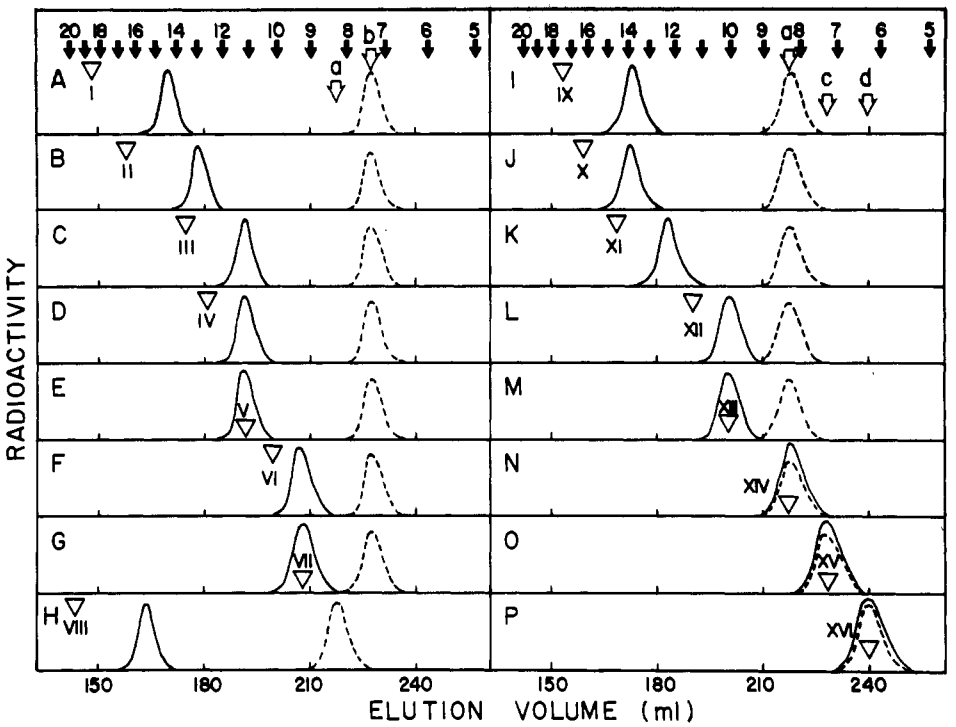


FIGURE 5: Sequential glycosidase digestion of components I–XVI in Figure 4. Black arrows and numbers are the same as in Figure 2. White arrows indicate the elution positions of authentic oligosaccharides: a, Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$; b, Man_3·GlcNAc·$GlcNAc_{OT}$; c, Man_2·GlcNAc·Fuc·$GlcNAc_{OT}$; d, Man·GlcNAc·Fuc·$GlcNAc_{OT}$. White triangles indicate the elution positions of components I–XVI before the β-galactosidase treatment. Panels A–P represent components I–XVI after sequential incubation with diplococcal β-galactosidase (solid line) and jack bean β-*N*-acetylhexosaminidase (dotted line).

on a Bio-Gel P-4 column, as previously reported (Yamashita et al., 1982).

By sequential digestion with diplococcal β-galactosidase and jack bean β-*N*-acetylhexosaminidase, components I–VII were converted to the same elution position as that of Man_3·GlcNAc·$GlcNAc_{OT}$ (Figure 5, panels A–G). The reduced pentasaccharide was converted to *N*-acetylglucosaminitol by sequential exoglycosidase digestion with α-mannosidase, β-mannosidase, and β-*N*-acetylhexosaminidase or by endo-β-*N*-acetylglucosaminidase D digestion (data not shown), indicating that the structure of the reduced pentasaccharide is Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4$GlcNAc_{OT}$.

Components VIII–XIV digested with β-galactosidase and β-*N*-acetylhexosaminidase were converted to the same elution position as that of Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$ (Figure 5, panels H–N). That the reduced hexasaccharide has the structure Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4-(Fucα1→6)$GlcNAc_{OT}$ was confirmed as described previously (Hitoi et al., 1987; Yamashita et al., 1983a).

By α-mannosidase digestion, component XV was converted to the same elution position as that of component XVI, releasing 1 mol of α-mannosyl residue, and the reduced tetrasaccharide was converted to *N*-acetylglucosamnitol by sequential digestion with β-mannosidase, β-*N*-acetylhexosaminidase, and α-fucosidase (data not shown). These results indicated that the monosaccharide compositions and anomeric conformations of components XV and XVI are

XV: Manα·Manβ·GlcNAcβ·(Fucα)·$GlcNAc_{OT}$

XVI: Manβ·GlcNAcβ·(Fucα)·$GlcNAc_{OT}$

Methylation Analysis of Components I–XVI. Methylation analysis of pooled components I–XVI obtained from GM1 gangliosidosis (type 1) SAP-1 revealed that the mannosyl residues occur as Man1→, →2Man1→, →4Man1→, →6Man1→, $\rightarrow^{4}_{2}$Man1→, $\rightarrow^{6}_{2}$Man1→, and $\rightarrow^{6}_{3}$Man1→ (Table I). These results indicated that any β-mannosyl residue of the trimannosyl core is not substituted with a bisecting *N*-acetylglucosamine residue and that two nonreducing terminal

Table III: Number of Galactose and *N*-Acetylglucosamine Residues Released from Components I–XVI[a] by Sequential Digestion with Diplococcal β-Galactosidase[b] and Jack Bean β-*N*-Acetylhexosaminidase[c] and Their Hydrolyzed Products

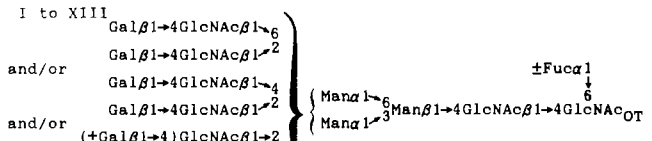
component (% molar ratio)	residues released: Galβ1→4[d]	residues released: GlcNAc-β1→[e]	hydrolyzed product
I (2.0)	4	4	Man_3·GlcNAc·$GlcNAc_{OT}$
II (6.6)	3	3	Man_3·GlcNAc·$GlcNAc_{OT}$
III (20.1)	2	2	Man_3·GlcNAc·$GlcNAc_{OT}$
IV (6.1)	1	2	Man_3·GlcNAc-$GlcNAc_{OT}$
V (4.2)	0	2	Man_3·GlcNAc·$GlcNAc_{OT}$
VI (5.9)	1	1	Man_3·GlcNAc·$GlcNAc_{OT}$
VII (7.7)	0	1	Man_3·GlcNAc·$GlcNAc_{OT}$
VIII (2.2)	4	4	Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$
IX (6.9)	3	3	Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$
X (2.0)	2	3	Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$
XI (13.1)	2	2	Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$
XII (10.6)	1	1	Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$
XIII (3.8)	0	1	Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$
XIV (2.1)	0	0	Man_3CGlcNAc·Fuc·$GlcNAc_{OT}$
XV (5.3)	0	0	Man_2·GlcNAc·Fuc·$GlcNAc_{OT}$
XVI (1.4)	0	0	Man·GlcNAc·Fuc·$GlcNAc_{OT}$

[a] See Figure 4. [b] Figure 5, solid lines. [c] Figure 5, dotted lines. [d] One Galβ1→4 residue in mono- to tetraantennary sugar chains behaves as 1.0 to ~1.1 glucose units as described in a previous paper (Yamashita et al., 1982). [e] Each *N*-acetylglucosamine residue in the side chains of complex-type sugar chains behaves as 1.8 to ~2.0 glucose units on the basis of glucose equivalents of authentic complex-type sugar chains on a Bio-Gel P-4 column as previously reported (Yamashita et al., 1982).

mannosyl residues of the trimannosyl core are substituted at C-2, C-4, C-6, C-2 and -4, and C-2 and -6 positions with *N*-acetyllactosamine and *N*-acetylglucosamine residues. Furthermore, pooled components I–XVI (50 cpm each) passed through an E-PHA–agarose column at 20 °C, although bisected oligosaccharides are retarded on the column under these conditions (see Table II). This result suggests that they are exclusively nonbisected oligosaccharides. The α-mannosyl residue of component XV should be linked at the C-6 position, because component XV is the partial degradation product of the trimannosyl core and is produced by the same degradation mechanism as component released from normal liver SAP-1 (see Figure 3).

Detection of 1,3,5,6-tetra-*O*-methyl- and 1,3,5-tri-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitols is in accordance with the fact that both fucosylated and nonfucosylated proximal *N*-acetylglucosaminitols are included among the oligosaccharides described already. The presence of only 3,6-di-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitol as a di-*O*-methyl derivative of *N*-acetylglucosamine indicated that the *N*-acetylglucosamine residues included in the sugar chains are substituted at the C-4 position. Detection of only 2,3,4,6-tetra-*O*-methylgalactitol as a partially O-methylated galactitol supports the idea that these oligosaccharides do not contain a Galβ1→4GlcNAcβ1→3Gal repeating structure but instead an *N*-acetyllactosamine structure.

Therefore, structures of components XIV–XVI were elucidated as shown in Table IV, and components I–XIII can be written as



Structures of Components I–XIII. Glycosidic linkages and the location of outer chains in components I–XIII were determined by using the behavior of oligosaccharides on immobilized DSA, E-PHA, L-PHA, and Con A columns with the data of methylation analysis and limited digestion with diplococcal β-*N*-acetylhexosaminidase. The behavior of components I–XIII on these four lectin columns is summarized in Table IV.

The carbohydrate-binding specificities of these four lectin columns are summarized in Table II. Oligosaccharides with two nonsubstituted or C-2-substituted α-mannosyl residues bind to a Con A–Sepharose column and can be eluted with buffer containing 0.1 M methyl α-D-mannopyranoside. Bi-, tri-, and tetraantennary oligosaccharides containing the Galβ1→4GlcNAcβ1→6[Galβ1→4(3)GlcNAcβ1→2]-Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ group are retarded on an L-PHA–agarose column.[2] Nonbisected mono-, bi-, and triantennary oligosaccharides containing the Galβ1→4GlcNAcβ1→2Manα1→6Manβ1→4GlcNAcβ1→-4GlcNAc$_{OT}$ group pass through an E-PHA–agarose column at 20 °C but are retarded at 2 °C (weak binding), while bisected bi- and triantennary oligosaccharides containing the Galβ1→4GlcNAcβ1→2Manα1→6Manβ1→4GlcNAcβ1→-4GlcNAc$_{OT}$ group are retarded on the column at 20 °C and are more extensively retarded at 2 °C (strong binding). Oligosaccharides containing the Galβ1→4GlcNAcβ1→6-(Glaβ1→4GlcNAcβ1→2)Man group or the *N*-acetyllactosamine repeating structure bind to a DSA–Sepharose column and can be eluted with buffer containing 1% *N*-acetylglucosamine oligomers (strong binding), whereas bi- and triantennary oligosaccharides with the Galβ1→-4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Man group are retarded on the column (weak binding).

Accordingly, the DSA$^+$ L-PHA^R fractions of tetra-, tri-, and biantennary components I, VIII, II, IX, X, III, and XI should contain a Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→-2)Manα1→6Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ group in the molecules; the DSA^R fractions of tri- and biantennary components II, IX, X, III, and XI should include a Galβ1→-4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Manα1→-3Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ group in the molecules; the E-PHA^R fractions of tri-, bi-, and monoantennary components II, IX, X, III, XI, IV, VI, and XII should have a Galβ1→4GlcNAcβ1→2Manα1→6β1→4GlcNAcβ1→-4GlcNAc$_{OT}$ group in the molecules; and the Con A$^+$ fractions of bi- and monoantennary components III, XI, IV, V, VI, XII, VII, and XIII should contain two C-2-substituted or nonsubstituted α-mannosyl residues in the molecules.

Furthermore, by digestion with diplococcal β-*N*-acetylhexosaminidase, which specifically does not cleave the GlcNAcβ1→6(GlcNAcβ1→2)Man group but does cleave the GlcNAcβ1→2 linkage of the GlcNAcβ1→2(GlcNAcβ1→-4)Man group and the GlcNAcβ1→2Man group (Yamashita et al., 1981c), degalactosylated tetraantennary components I and VIII were converted to the same elution position as that of GlcNAcβ1→6(GlcNAcβ1→2)Manα1→6(GlcNAcβ1→-4Manα1→3)Manβ1→4GlcNAcβ1→4(±Fucα1→6)-GlcNAc$_{OT}$, releasing one *N*-acetylglucosamine residue each. These results showed that two *N*-acetyllactosamine groups on the Manα1→3 side should be linked at the C-2 and C-4 positions to the α-mannosyl residue. The DSA$^+$ L-PHA^R fractions of triantennary components II and IX digested with the mixture of diplococcal β-galactosidase and β-*N*-acetylhexosaminidase were converted to the same elution position as that of GlcNAcβ1→6(GlcNAcβ1→2)Manα1→6-(Manα1→3)Manβ1→4GlcNAcβ1→4(±Fucα1→6)-GlcNAc$_{OT}$, indicating that the released *N*-acetylglucosamine residue is linked at the C-2 position of the α-mannosyl residue on the Manα1→3 side. These results indicated that the Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2) group in complex-type sugar chains and its partially degraded products, (±Galβ1→4)GlcNAcβ1→6 and (±Galβ1→4)GlcNAcβ1→2 groups, are located on the Manα1→6 arm, while the Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2) group and its partial degradation products, (±Galβ1→4)GlcNAcβ1→4 and (±Galβ1→4)GlcNAcβ1→2 groups, are located on the Manα1→3 arm.

On the basis of the results so far described, the possible structures of components I–XIII are summarized in Table IV.

Discussion

The present studies provide evidence that the sugar chains of SAP-1 purified from normal liver are the secondary degradation products from the hexasaccharide to a single *N*-acetylglucosamine as shown in Figure 3, which cannot be illustrated by the usual biosynthetic mechanism of asparagine-linked sugar chains (Hubbard & Ivatt, 1981). These results suggest that sugar chains of native SAP-1 were degraded sequentially by exoglycosidases in lysosomes and changed to oligosaccharides that are smaller in size. Therefore, it seems difficult to speculate on the role in the transport

[2] Manuscript in preparation.

Table IV: Behavior on Immobilized DSA, L-PHA, E-PHA, and Con A Columns of Components I–XVI and Their Possible Structures

fractions[a]	structures[b]
I and VIII DSA^+ $L\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_1$ (R_1: I, R_2: VIII)
II and IX DSA^R $E\text{-}PHA^R$[c]	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to3$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_1$ (R_1: II, R_2: IX)
DSA^+ $L\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to6Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_1$ (R_1: II, R_2: IX)
X DSA^+ $L\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to6Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_2$ $GlcNAc\beta1\to2(4)Man\alpha1\to3$
DSA^R $E\text{-}PHA^-$[d]	$GlcNAc\beta1\to2(6)Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4R_2$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3$
DSA^- $E\text{-}PHA^-$	$Gal\beta1\to4\{GlcNAc\beta1\to2Man\alpha1\to6$ / $GlcNAc\beta1\to4Man\alpha1\to3\}Man\beta1\to4R_2$ $Gal\beta1\to4GlcNAc\beta1\to2(4)Man\alpha1\to3$
DSA^- $E\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4\{GlcNAc\beta1\to4Man\alpha1\to3\}Man\beta1\to4R_2$ $GlcNAc\beta1\to2Man\alpha1\to3$
III and XI $Con\ A^+$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4$ (R_1: III, R_2: XI)
Con_RA^- DSA^R	$Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4$ (R_1: III, R_2: XI) $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3$
Con_+A^- DSA^+ $L\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6Man\alpha1\to3Man\beta1\to4$ (R_1: III, R_2: XI)
Con_-A^- DSA $E\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4$ (R_1: III, R_2: XI)
Con_-A^- DSA $E\text{-}PHA^-$	$Gal\beta1\to4GlcNAc\beta1\to6Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2(4)Man\alpha1\to3Man\beta1\to4$ (R_1: III, R_2: XI)
IV $E\text{-}PHA^R$ $Con\ A^+$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_1$
$E\text{-}PHA^R$ $Con\ A^-$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4R_1$
$E\text{-}PHA^-$ $Con\ A^+$	$GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_1$
IV $E\text{-}PHA^-$ $Con\ A^-$	$Gal\beta1\to4\{GlcNAc\beta1\to6Man\alpha1\to6$ / $GlcNAc\beta1\to2Man\alpha1\to3\}Man\beta1\to4R_1$ $Gal\beta1\to4\{GlcNAc\beta1\to4Man\alpha1\to6$ / $GlcNAc\beta1\to2Man\alpha1\to3\}Man\beta1\to4R_1$ $Gal\beta1\to4\{GlcNAc\beta1\to6Man\alpha1\to6$ / $GlcNAc\beta1\to2(4)Man\alpha1\to3\}Man\beta1\to4R_1$ $GlcNAc\beta1\to2Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4R_1$
VI and XII $Con\ A^+$ $E\text{-}PHA^R$	$Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to6$ $Man\alpha1\to3Man\beta1\to4$ (R_1: VI, R_2: XII)
$Con\ A^+$ $E\text{-}PHA^-$	$Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4$ (R_1: VI, R_2: XII)
$Con\ A^-$	$Gal\beta1\to4GlcNAc\beta1\to6Man\alpha1\to6$ $Man\alpha1\to3Man\beta1\to4$ (R_1: VI, R_2: XII) $Man\alpha1\to6$ $Gal\beta1\to4GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4$ (R_1: VI, R_2: XII)
V $Con\ A^+$	$GlcNAc\beta1\to2Man\alpha1\to6$ $GlcNAc\beta1\to2Man\alpha1\to3Man\beta1\to4R_1$
$Con\ A^-$	$GlcNAc\beta1\to6Man\alpha1\to6$ $GlcNAc\beta1\to2(4)Man\alpha1\to3Man\beta1\to4R_1$ $GlcNAc\beta1\to2Man\alpha1\to6$ $GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4R_1$
VII and XIII $Con\ A^+$	$GlcNAc\beta1\to2\{Man\alpha1\to6$ / $Man\alpha1\to3\}Man\beta1\to4$ (R_1: VII, R_2: XIII)
$Con\ A^-$	$GlcNAc\beta1\to6Man\alpha1\to6$ $Man\alpha1\to3Man\beta1\to4$ (R_1: VII, R_2: XIII) $Man\alpha1\to6$ $GlcNAc\beta1\to4Man\alpha1\to3Man\beta1\to4$ (R_1: VII, R_2: XIII)
XIV	$Man\alpha1\to6$ $Man\alpha1\to3Man\beta1\to4R_2$
XV	$Man\alpha1\to6Man\beta1\to4R_2$
XVI	$Man\beta1\to4R_2$

[a] Behavior on lectin columns: –, pass through; R, retarded; +, bounded and eluted with 5 mM fucose (AAL), 1% *N*-acetylglucosamine oligomer (DSA), or 0.1 M methyl α-D-mannopyranoside (Con A). [b] R_1 and R_2 indicate $GlcNAc\beta1\to4GlcNAc_{OT}$ and $GlcNAc\beta1\text{-}4(Fuc\alpha1\to6)GlcNAc_{OT}$, respectively. [c] $E\text{-}PHA^R$: the respective oligosaccharides passed through an E-PHA column at 20 °C, but they were retarded extensively at 2 °C (weak binding). [d] $E\text{-}PHA^-$: the elution profiles of the passed-through fractions on the E-PHA column were slightly broad.

mechanism of those sugar chains of native proteins like SAP-1 purified from normal organs. Degraded sugar chains like those seen in SAP-1 purified from normal liver are also observed in several lysosomal enzymes (Howard et al., 1982; Taniguchi et al., 1985; Takahashi et al., 1984). However, nondegraded complex-type sugar chains have been found in other lysosomal enzyme (Figura & Hasilik, 1986). The variation in accessibility to lysosomal acid hydrolases may be due to differences in the three-dimensional conformations of the respective enzyme proteins.

In order to elucidate whether the degraded sugar chains of normal liver SAP-1 are derived from complex-type sugar chains, sugar chains of SAP-1 purified from the liver of GM1 gangliosidosis (type 1) patients were comparatively investi-

gated. Consequently, they were shown to be mono-, bi-, tri-, and tetraantennary complex-type sugar chains containing sialyated and nonsialylated *N*-acetyllactosamine groups as shown in Table IV, although they are slightly degraded. These results indicate that some complex-type sugar chains may act as the recognition signal to transport SAP-1 from the Golgi apparatus to the lysosomes in human liver, although other possible alterations in biosynthesis and localization resulting from an altered secretory pathway of the diseased liver should be taken into account. Furthermore, as previously reported (Inui & Wenger, 1984), the amount of SAP-1 was very low in the cultured skin fibroblasts of I-cell disease patients, whereas it was normal in the liver of the patient. These phenomena also support the presence of an alternate transport system of glycoprotein mediated by something other than the mannose-6-phosphate receptor in human liver.

The structures of sugar chains of SAP-1 purified from GM1 gangliosidosis (type 1) liver resemble those of oligosaccharides accumulated in the liver and those excreted in the urine of GM1 gangliosidosis patients, although they are hydrolyzed at *N*,*N*′-diacetylchitobiose as reported previously (Wolfe et al., 1974; Yamashita et al., 1981b). These results may be due to the metabolic defect in acid β-galactosidase activity toward glycoproteins in GM1 gangliosidosis liver.

About 50% of the sugar chains of human liver SAP-1 contained a fucosylated trimannosyl core. This characteristic is common to the sugar chains of ribonucleases (Yamashita et al., 1986) and γ-glutamyltranspeptidase (Yamashita et al., 1989b), which are located in human liver hepatocytes, while all secretory glycoproteins in serum produced by normal liver contain nonfucosylated trimannosyl cores (Yamashita et al., 1989a). These lines of evidence indicate that GlcNAc:α1→6 fucosyltransferase of hepatocytes selectively acts on the sugar chains of nonsecretory glycoproteins in the cells.

As reported in this study, lectin affinity chromatography is a very effective tool for elucidating the glycosidic linkages and the location of outer chains in complex-type sugar chains in limited amounts, in combination with Bio-Gel P-4 column chromatography and digestion by glycosidases with narrow aglycon specificities. Especially, the location of Galβ1→4GlcNAcβ1→2Manα1→, Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2)Manα1→, and Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAcβ1→2)Manα1→ groups of complex-type sugar chains with as little as several picomoles of material can be easily determined in combination with E-PHA, DSA, and L-PHA column chromatography as shown in this study. However, structural analysis using E-PHA and L-PHA columns is limited to the hydrolysis products of *N*-glycanase and hydrazinolysis of glycopeptides, because the *N*,*N*′-diacetylchitobiose portions of the respective oligosaccharides is required to interact with these columns.

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Low-Resolution Structure of the Tetrameric Phenylalanyl-tRNA Synthetase from *Escherichia coli*. A Neutron Small-Angle Scattering Study of Hybrids Composed of Protonated and Deuterated Protomers

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Appendix: Considerations on Stuhrmann Plots of Particles with Two Constituents of Different Scattering Length Density

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ABSTRACT: *Escherichia coli* phenylalanyl-tRNA synthetase is a tetrameric protein composed of two types of protomers. In order to resolve the subunit organization, neutron small-angle scattering experiments have been performed in different contrasts with all types of isotope hybrids that could be obtained by reconstituting the $\alpha_2\beta_2$ enzyme from the protonated and deuterated forms of the α and β subunits. Experiments have been also made with the isolated α promoter. A model for the $\alpha_2\beta_2$ tetramer is deduced where the two α promoters are elongated ellipsoids ($45 \times 45 \times 160$ Å^3) lying side by side with an angle of about 40° between their long axes and where the two β subunits are also elongated ellipsoids ($31 \times 31 \times 130$ Å^3) with an angle of 30° between their axes. This model was obtained by assuming that the two pairs of subunits are in contact in an orthogonal manner and by taking advantage of the measured distance between the centers of mass of the α_2 and β_2 pairs ($d = 23 \pm 2$ Å).

Aminoacyl-tRNA synthetases catalyze the esterification of amino acids to tRNAs (Schimmel et al., 1979). For the same mode of reaction, this family of enzymes displays a variety of quaternary structures (monomeric, dimeric, and tetrameric). Among the 20 *Escherichia coli* aminoacyl-tRNA synthetases, only two (phenylalanyl- and glycyl-tRNA synthetases) form $\alpha_2\beta_2$ structures.

It was shown (Ducruix et al., 1983) that it is possible to reversibly dissociate the phenylalanyl-tRNA synthetase enzyme subunits in the presence of sodium thiocyanate. Dissociated α and β subunits can be isolated by gel filtration, neither of the subunits showing any significant enzymatic activity. Upon mixing of stoichiometric amounts of each subunit, a fully active $\alpha_2\beta_2$ enzyme is recovered.

The two genes pheS and pheT, coding for the two subunits of phenylalanyl-tRNA synthetase, were cloned in plasmid pBR322 (Plumbridge et al., 1980). The recombinant plasmid pB1 is responsible for the overproduction of the enzyme by a factor of more than 100 (Fayat et al., 1983a).

Because of the overproduction and the dissociation properties of the enzyme, it became possible to study the structure of the tetrameric enzyme at low resolution by combining the small-angle neutron scattering technique and specific deuteration of the promoters. Since neutron scattering offers the unique advantage that the scattering density of the solvent can be adjusted by varying the percentage of ^2H_2O in the solvent (Jacrot, 1976), we expected to measure the structural parameters of the enzyme by triangulation as shown for RNA polymerase (Stöckel et al., 1979, 1980a,b) or for methionyl-tRNA synthetase (Dessen et al., 1983a,b).

A study of the protonated form of the enzyme and its binding to $tRNA^{Phe}$ has already been published (Dessen et al., 1983a,b). It showed that the radius of gyration is independent of the presence of $MgCl_2$ and that there is no detectable conformational change upon $tRNA^{Phe}$ binding. It also allowed us to determine the enzyme molecular mass ratio, which was confirmed by the enzyme sequence, as deduced from genetic studies (Fayat et al., 1983b; Mechulam et al., 1985).

Materials and Methods

Bacterial Strains and Growth Conditions. Escherichia coli strain IBPC 1671 carrying plasmid DNA pB1 (Plumbridge et al., 1980) was grown at 30 °C in glucose minimal medium

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