OLIGOSACCHARIDES ACCUMULATING IN THE LIVER FROM A PATIENT WITH G_{M2}-GANGLIOSIDOSIS VARIANT O (SANDHOFF-JATZKEWITZ DISEASE)

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SUMMARY

A heptasaccharide which contained N-acetylglucosamine at the non-reducing termini and a trimannosyl core has been isolated from the liver of a case of G_{M2}-gangliosidosis, variant O. Its structure was determined by permethylation analyses and resembles the decasaccharide present in great excess in the liver of G_{M1}-gangliosidosis, Type I except that no β-galactosyl residues were present. The oligosaccharides likely derive from the defective catabolism of erythrocyte stromal glycoproteins during development.

Sandhoff-Jatzkewitz disease is an inherited glycosphingolipid storage disease in which the activity of both the A and B components of β-N-acetylhexosaminidase are absent. G_{M2}-ganglioside accumulates in the central nervous system and globoside in the viscera (1). There have been recent reports of abnormally large amounts of water-extractable materials containing glucosamine and mannose in the brain gray matter (2) and also of mannose and N-acetylglucosamine containing oligosaccharides in the urine (3). Wolfe et al (4) have recently described the isolation and characterization from the liver of G_{M1}-gangliosidosis type I patients of oligosaccharides which are likely derived from erythrocyte glycoproteins as a result of incomplete catabolism. Similar oligosaccharides are also present in the urine of G_{M1}-gangliosidosis type I patients (Kin and Wolfe, unpublished data). In this communication we describe the isolation and characterization of structurally related oligosaccharides also present in great excess in the liver from a patient with Sandhoff's disease.

MATERIALS AND METHODS. Extraction of the oligosaccharides from a freshly frozen sample of liver from a child who died of Sandhoff's disease was done by the method of Wolfe et al (4). The oligosaccharide mixture was first desalted on a Sephadex G-25 column and the salt-free oligosaccharide mixture re-chromatographed several times on the same column. Fractions were monitored by a Pharmacia refractive index monitor and by the phenol-sulphuric acid reaction. Periodate oxidation was carried out as described by Wolfe et al (4). The composition of the oligosaccharides was determined by g.l.c. of their reduced and acetylated compounds on a 3% ECNSS column (5). Permethylation of the oligosaccharide was performed essentially by the method of Hakomori (6) and the resulting permethylated compounds acetolyzed hydrolysed, borohydride reduced and acetylated (7) for g.l.c.-mass spectrometry identifications on an LKB-9000 instrument interfaced with a Varian MAT Model 100-SS computer.

The proton n.m.r. spectra were determined on a Varian HA-100 spectrometer, the compound being dissolved in ^2H_2O (10% w/v) in a coaxial capillary tube with tetramethylsilane as an external standard.

RESULTS. The elution pattern of the oligosaccharides from Sephadex G-25 showed two overlapping fractions (Fig. 1). The major peak, fraction 2 was isolated as a homogeneous fraction by repeated Sephadex G-25 chromatography in yields of 30 mg/10 g wet weight of liver.

Compositional analyses of the homogeneous fraction 2 showed that it contained mannose and N-acetylglucosamine. Galactose and N-acetylgalactosamine were completely absent. Borohydride treatment of the oligosaccharide followed by hydrolysis of the reduced product yielded mannose, N-acetylglucosamine and N-acetylglucosaminitol in the following ratio of 3:2.4:1 respectively. The non-reducing glucosamine moieties were completely destroyed by periodate oxidation and some of the mannose was also oxidized. The reducing N-acetylglucosamine molecule was resistant to oxidation (Table I).

TABLE I

Periodate oxidation studies on fraction 2 oligosaccharide

	Before treatment	After treatment
	moles/mole of N-acetylglucosamine (reducing)	
Mannose	3	2.3
N-acetylglucosamine (non-reducing)	2.4	0
N-acetylglucosamine (reducing)	1	1

Permethylation studies of the fraction 2 oligosaccharide before and after borohydride reduction are shown in Figures 2A and 2B and Table II. In both instances, the results were very similar except for the appearance of a peak with T-value of 3.00 in fraction 2 reduced which was identified by its mass spectral fragments as 4-O-acetyl-2(N-methylacetamido)-2-deoxy-1,3,5,6-tetra-O-methyl-D-glucitol (4,8). This indicates that the sugar at the reducing terminal was a 4-linked N-acetylglucosamine and was responsible for peak b present in Figure 2A but not Figure 2B. The presence of an amino-deoxy alditol derivative in the hydrolysate from fraction 2 reduced was confirmed by irreversibly absorbing all the reducing sugars on a Dowex 1-X8(OH^-) and selectively eluting the reduced amino-sugar with CO_2-free distilled water. The mass spectrum of peak a (Figure 2A) showed it was due to 1,5-di-O acetyl-2-(N-methylacetamido)-2-deoxy-3,4,6-tri-O-methyl-D-glucitol (9). Thus, N-acetyl-D-glucosamine moieties were present at both the non-reducing and reducing terminii of the oligosaccharide and were present in a ratio of 2.7:1. The remaining peaks in the gas chromatograms were all due to mannose residues and on the basis of their spectra attributed to 2-linked, 3,6-linked and 2,4-linked mannosyl residues (Table II).

TABLE II

Retention times (T-values) and area ratios of partially methylated sugars as their alditol and aminodeoxy alditol acetates obtained from fraction 2 oligosaccharide

	T-values[1]	Area ratios	
		Untreated oligosaccharide	$NaBH_4$ reduced oligosaccharide
3,4,6-trimethylmannitol	1.90	1.62	1.55
2-deoxy-2(N-methylacetamido)1,3,5,6-tetramethylglucitol	3.00	0	0.64
3,6-dimethylmannitol	3.85	0.45	0.59
2,4-dimethylmannitol	5.45	1.00	1.00
2-deoxy-2(N-methylacetamido)-3,4,6-trimethylglucitol (peak a)	- [2]	1.50	1.60
2-deoxy-2(N-methylacetamido)-3,6-dimethylglucitol (peak b)	- [2]	0.45	0

[1] Retention time relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol (see Björndal et al, ref. 14).

[2] T-value not measured because of long retention time and temperature program.

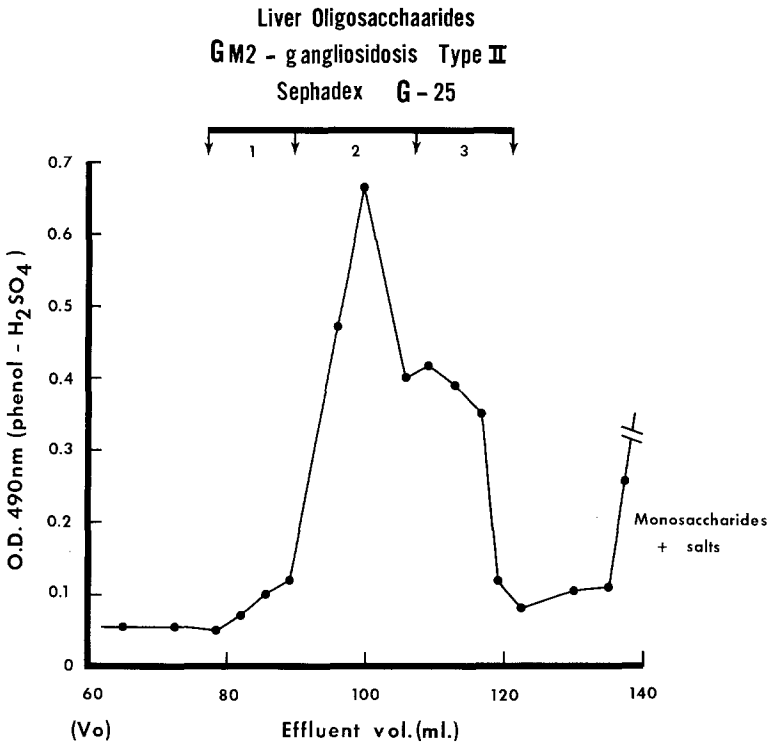

Figure 1. Sephadex G-25 column chromatography of water-soluble oligosaccharides from the liver of a case of G_{M2}-gangliosidosis, variant O.

The presence of a trimannosyl core structure in the oligosaccharide was strongly indicated from the proton n.m.r. spectrum which showed similar anomeric absorptions to those found in the G_{M1}-gangliosidosis type I oligosaccharide (4). Except for the absence of the β-galactoside signals and the presence of a well resolved doublet (J=7 Hz), assignable to the H-1 protons of a N-acetyl-β-D-glucosaminide moiety, the spectra obtained for both these oligosaccharides were similar in the anomeric region. Also prominent in the spectrum of the fraction 2 oligosaccharide was the presence of a strong N-acetyl signal at high field.

The smaller molecular weight fraction 3 (see Fig. 1) also contained only N-acetylglucosamine and mannose. N-acetylglucosamine at the non-reducing and reducing termini were present in a ratio of 2.1:1.0

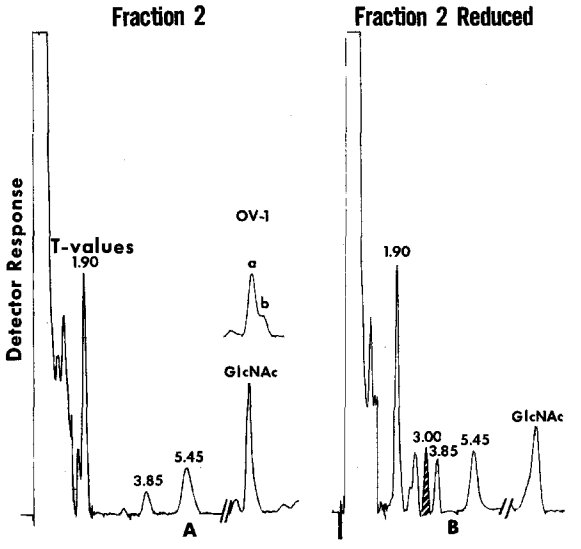

Figure 2. Gas-liquid chromatograms of the partially methylated alditol and aminodeoxy alditol acetates of fraction 2 untreated (A) and reduced with sodium borohydride (B). The retention times (T-values) relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol (T=1.00) are indicated. The insert in 2A shows separation of the GlcNAc into two components on an OV-1 column. The structures of each of the peaks was determined by mass spectrometry.

respectively. Permethylation studies of fraction 3 showed heterogeneity in the linkage of the mannose residues not seen in fraction 2. Although 2-linked and 2,4-linked mannosyl residues were present, no 3,6-linked mannosyl residue was detected. Instead there was a substantial amount of a 3-linked mannosyl residue. Due to limitations in the amount of this oligosaccharide available, the exact linkages of the mannose residues in this fraction are uncertain. However, it is clear that the non-reducing termini are N-acetylglucosamine.

DISCUSSION. Wolfe et al (4) postulated recently that the oligosaccharide accumulating in the liver in G_{M1}-gangliosis type I resulted from incomplete catabolism of the erythrocyte stromal glycoprotein described by Thomas and Winzler (10) due to the absence of β-galactosidase

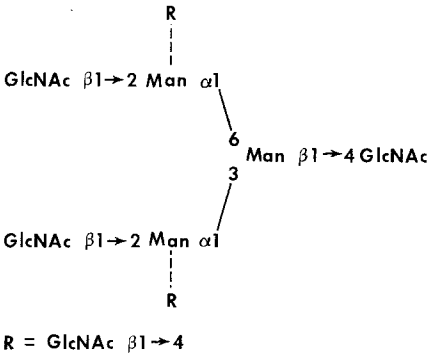

Figure 3. Structure of the major heptasaccharide (fraction 2) from the liver. The additional non-reducing GlcNAc is linked to either one of the 2-linked mannosyl residues.

activity. Since patients with G_{M2}-gangliosidosis, variant O are deficient in both hexosaminidase A and B but not in β-galactosidase (1), it could be expected that oligosaccharides similar to those in G_{M1} type I patients would accumulate in such patients except that galactoside linkages would be absent. This was indeed the case. The major oligosaccharide contained N-acetylglucosamine at the reducing and non-reducing termini and a trimannosyl core which according to n.m.r. and permethylation had the structure shown in Figure 3. The smaller fraction 3 exhibited heterogeneities with regard to the mannosyl linkages but N-acetylglucosamine was also identified as the sugar residue present at both the reducing and non-reducing termini of the oligosaccharide. It would appear that these oligosaccharides accumulate as a result of the deficiency of both hexosaminidase A and B components and thus catabolism of the erythrocytes glycoproteins is incomplete. Strecker and Montreuil (3) have isolated several oligosaccharides from the urine of a G_{M2}-gangliosidosis variant O patient and found that the constituent sugars were mannose and N-acetylglucosamine only, part of the latter being at the reducing terminal of the oligosaccharide. These urinary oligosaccharides may have similar structures to those

in the liver. However, no increase in sulfated glycosaminylglycans could be found in the urine (3,11) even though Thompson et al (12) found that N-acetylhexosaminidase from extracts of skin fibroblasts of a patient did not have any activity on a hexasaccharide obtained from chondroitin 4-sulphate.

Finally, it appears that the presence of one of the isoenzymes in N-acetylhexosaminidase, namely the B form, is sufficient to degrade the oligosaccharides described, since these could not be detected in the liver of a Tay-Sachs patient (deficiency of hexosaminidase A) (Kin and Wolfe, unpublished data). Accordingly, recent reports by Srivastava et al (13) show clearly that the B form of the hexosaminidase isoenzyme is not the precursor to the A form.

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