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Note

Structure of the minor oligosaccharides in the liver of a patient with G_{M2}-gangliosidosis variant O (Sandhoff–Jatzkewitz disease)

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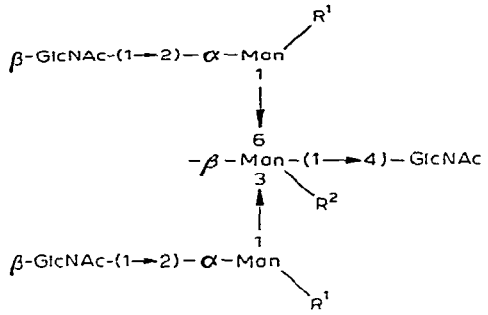
The absence of both the A and B forms of *N*-acetyl-β-hexosaminidase in patients with Sandhoff–Jatzkewitz disease leads to the accumulation of G_{M2}-ganglioside in the central nervous system and of globoside in the viscera[1]. In addition, oligosaccharides accumulate in the brain[2,3], and are also excreted in the urine[4–6]. In 1974, we described the isolation by gel chromatography of two oligosaccharide-containing fractions from the liver of a patient with Sandhoff–Jatzkewitz disease, the major fraction containing mainly the heptasaccharide[7] **1***, together with smaller proportions of the structurally related hexasaccharide[5] **2**. In addition, a minor fraction was found to contain an oligosaccharide having 2-acetamido-2-deoxyglucose residues at the reducing and 2-acetamido-2-deoxyglucosyl groups at the nonreducing termini, and large proportions of a 3-linked mannosyl residue[6]. Due to the limited amount of material available, we were then unable to obtain more details on the structure of this oligosaccharide. However, we recently had the opportunity to analyze the oligosaccharide by Fourier-transform, ^{1}H-nuclear magnetic resonance (F.t. ^{1}H-n.m.r.) spectroscopy, and now report these results and also those obtained from more-detailed permethylation analyses of the oligosaccharide.

RESULTS AND DISCUSSION

Chromatography of the oligosaccharides isolated from 10 g (wet weight) of liver on a column of Sephadex G-25 gave fractions 1 and 2 (see Fig. 2). These were further purified by repeated chromatography on the same column, and 30 mg of fraction 1 and 12 mg of fraction 2 were isolated. As described in earlier papers[5,7], fraction 1 contained the heptasaccharide **1**, and smaller amounts of the hexasaccharide **2**.

Fraction 2 was also found to contain mannose and 2-acetamido-2-deoxyglucose, exclusively, in the molar ratio of 1:0.9; but, in contrast to fraction 1, the borohydride-reduced fraction 2 had the following composition: 2-acetamido-2-deoxyglucose, 2-acetamido-2-deoxyglucitol, and mannose in the molar ratios of 1.1:1:2. Permethylation analyses of the oligosaccharide in fraction 2 showed only a small

*See Fig. 1 for the structures of all oligosaccharides mentioned in the text.



1 R^1 = β-GlcNAc (1→4)-linked to one of 2-linked mannose, R^2 = H
2 R^1 = H, R^2 = H
3 R^1 = H, R^2 = β-GlcNAc-(1→4)
4 α-Man-(1→3)-β-Man-(1→4)-GlcNAc
5 β-GlcNAc-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc
6 β-GlcNAc-(1→4)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc

Fig. 1. Structures of oligosaccharides isolated from liver of the Sandhoff–Jatzkewitz disease and mannosidosis.

proportion of doubly substituted mannose residues: also, as noted in our original communication[7], a substantial proportion of a 3-linked mannosyl residue and smaller proportions of other mono-substituted mannosyl residues were present. The ratios of these residues were as follows: 3-linked:2-linked:4-linked = 5:3:1.4. Both the 4-linked and the unsubstituted 2-acetamido-2-deoxyglucose residues were found in approximately equal proportions. The 4-linked 2-acetamido-2-deoxyglucose residue was clearly at the reducing end of the oligosaccharide, as methylation analysis of the borohydride-reduced oligosaccharide gave a compound having a T-value of 3.0, identified by mass spectrometry as 4-*O*-acetyl-2-deoxy-1,3,5,6-tetra-*O*-methyl-2-(*N*-methylacetamido)-glucitol. The methylation analysis indicated that fraction 2 was a linear oligosaccharide having 2-acetamido-2-deoxyglucose residues at both the nonreducing and the reducing termini of the molecule, and mannosyl residues in between. There appeared to be heterogeneity in the mannose linkages. The F.t. ^{1}H-n.m.r. spectrum of fraction 2 (see Fig. 3A) proved to be most informative. The major signals in the anomeric regions were: a well resolved doublet (J 8.2 Hz) centered at 5.05 p.p.m., and three broad singlets at 5.26, 5.63, and 5.72 p.p.m. The intensities of these signals suggested that the sugar residues were in equimolar proportions, so that the compound was probably a tetrasaccharide. The latter appears to be an analog of the well characterized trisaccharide **4**, excreted in the urine of patients with mannosidosis[8], as the signals at 5.26, 5.63, and 5.72 p.p.m. were also present in the F.t. ^{1}H-n.m.r. spectrum (see Fig. 3B) of this trisaccharide (recently isolated in our laboratory; unpublished results). These signals are respectively assigned to the β-mannosyl, α-mannosyl, and reducing 2-acetamido-2-deoxy-α-glucose residues (the β anomer appears as a small shoulder slightly upfield of the signal at 5.26 p.p.m.). The doublet at 5.05 p.p.m. present in the spectrum of fraction 2

is due to a terminal 2-acetamido-2-deoxyglucose residue linked β-(1→4), or β-(1→2), or both, to the terminal α-mannosyl group of trisaccharide **4**. Such linkages should not affect the anomeric signal of the α-mannosyl group, as already shown in our earlier studies on the oligosaccharides present in G_{M1}-gangliosidosis[9]. The β-linkage is assigned on the basis of the large coupling-constant observed.

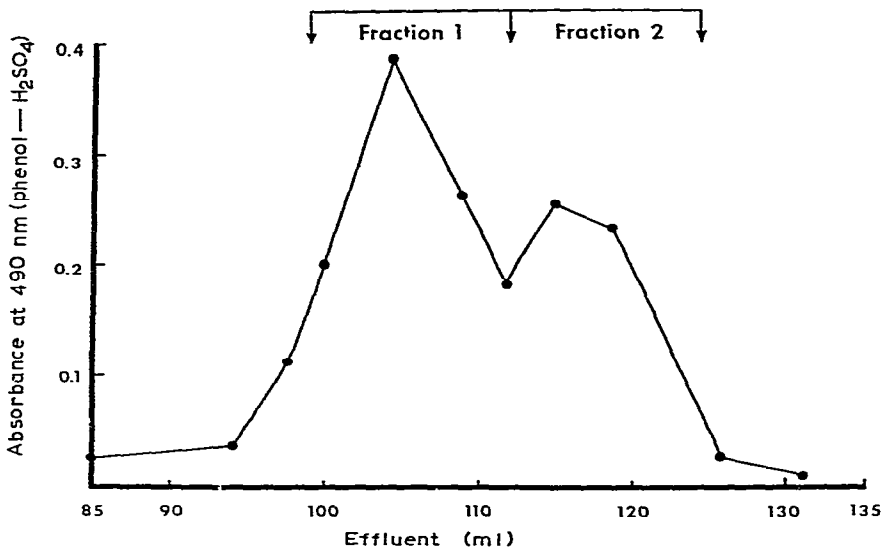


Fig. 2. Sephadex G-25 column-chromatography of oligosaccharides from the liver of Sandhoff–Jatzkewitz disease.

From the permethylation analyses and the ^{1}H-n.m.r. spectroscopic data, we may now conclude that fraction 2 is a mixture of tetrasaccharides **5** and **6**. Compound **5** is the major component, as evidenced by the ratio of the 2- to the 4-linked mannosyl residues found by the permethylation analyses.

The accumulation, in the liver, of oligosaccharides having 2-acetamido-2-deoxy-β-glucosyl groups at the nonreducing termini reflects the absence of both A and B forms of β-*N*-acetylhexosaminidase in patients with Sandhoff–Jatzkewitz disease. Furthermore, the wide variety of structures indicates that several types of glycoprotein[10] are not completely catabolized by these patients. Recently, Strecker *et al.*[6] described the excretion of seven oligosaccharides in the urine of a patient with this disease. These compounds were similar to those just described, except that the major urinary oligosaccharide was described as (*a*) a heptasaccharide (**3**) containing a novel, trisubstituted β-mannosyl residue, and (*b*) an isomer of the major heptasaccharide (**1**) accumulating in the liver[7]. The novel compound **3** was clearly a minor component in the liver, as the F.t. ^{1}H-n.m.r. spectrum of fraction 1 (see Fig. 2) showed that it contained mostly the heptasaccharide **1** and the hexasaccharide **2**, the characteristic signals of the anomeric protons of the trimannosyl core in these compounds having been well documented[6,7,9]. The anomeric signals assigned[6] to the mannosyl residues.

particularly the α anomers, in **3** were barely present in our spectrum. The differences noted between the liver and urinary oligosaccharides are probably due to different enzyme systems in the liver and kidney. It could well be that the unusual oligosaccharide **3** in the urine is derived from particular glycoproteins of the kidney.

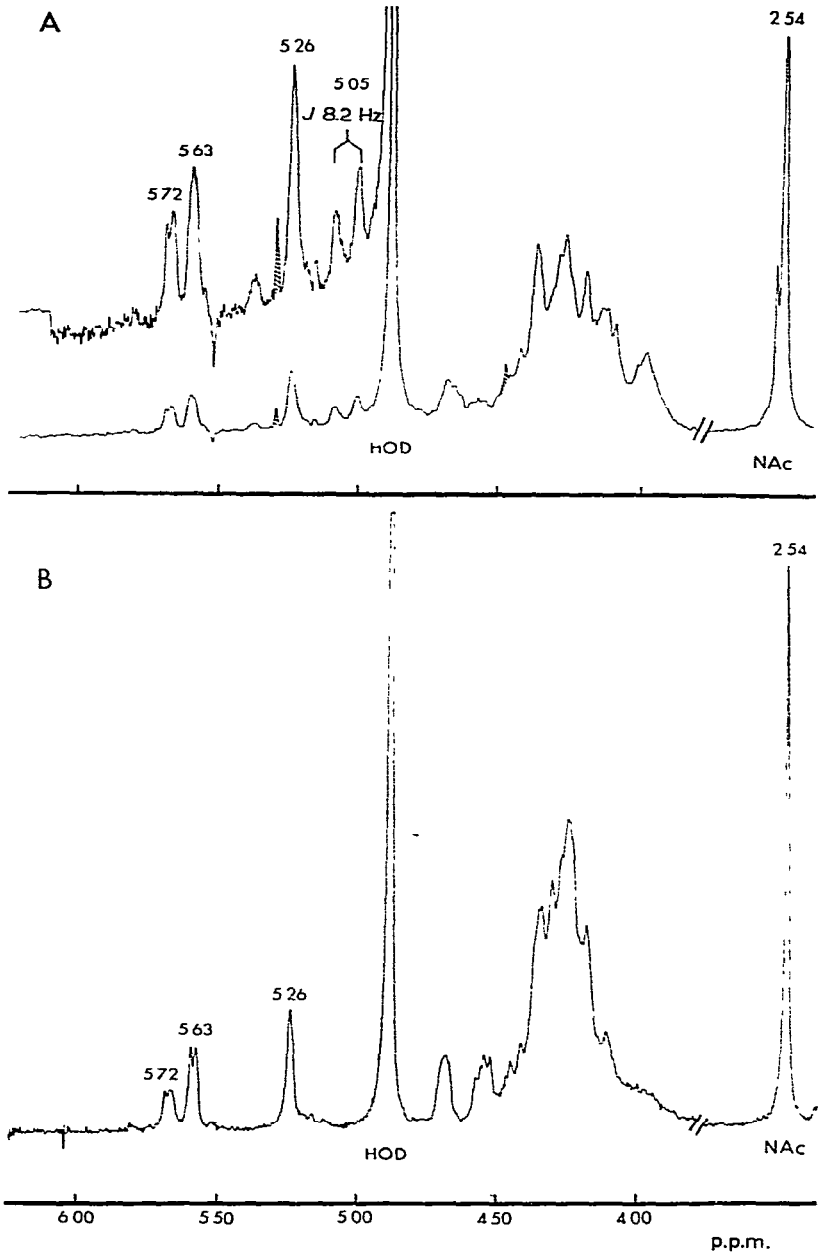


Fig. 3. A. F.t. ^{1}H-n.m.r. spectrum of fraction 2 oligosaccharides in D_2O solutions. (Upper tracing was recorded at higher sensitivity. Shaded areas represent spinning side-bands.) B. F.t. ^{1}H-n.m.r. spectrum of trisaccharide **4** isolated from the urine of a mannosidosis patient, in D_2O.

EXPERIMENTAL

Oligosaccharides were isolated from a freshly frozen sample of liver from a child who had died of Sandhoff–Jatzkewitz disease, and were fractionated on a column of Sephadex G-25, as previously described[7,9].

Compositions of the oligosaccharides were determined by gas–liquid chromatography (g.l.c.) of their reduced and then acetylated compounds on a column[11] of 3% of ECNSS-M. Permethylation of the oligosaccharides was achieved essentially by the method of Hakamori[12], and the resulting compounds were acetolyzed, the products hydrolyzed, the sugars reduced with borohydride, and the alditols acetylated[13] for g.l.c.-mass spectrometric identifications in an LKB-9000 instrument interfaced with a Varian MAT model 100-SS computer.

The F.t. ^{1}H-n.m.r. spectra were recorded with a Bruker HFX-90 instrument. The oligosaccharides were repeatedly exchanged in deuterium oxide. The 90-MHz spectra of the deuterium-exchanged compounds in D_2O solutions were then obtained, with the spectrometer operating in a Fourier-transform mode at a probe temperature of 60°. The signal lock was on the deuterium nucleus. Chemical shifts are in parts per million (p.p.m.) from an external, tetramethylsilane standard.

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