CHARACTERIZATION OF OLIGOSACCHARIDES AND GLYCOPEPTIDES EXCRETED IN THE URINE OF G_{M1}-GANGLIOSIDOSIS PATIENTS

N. M. K. Ng Ying Kin and Leonhard S. Wolfe

Donner Laboratory of Experimental Neurochemistry,
Montreal Neurological Institute, McGill University,
Montreal, Quebec H3A 2B4, Canada.

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SUMMARY

Three structurally related carbohydrate-rich fractions which are not present in normal urine have been isolated from the urine of G_{M1}-gangliosidosis patients, Types 1 and 2. Their carbohydrate structures have been determined by permethylation studies and p.m.r. spectroscopy and shown to be identical to compounds isolated previously from the liver in this disease. The main compound has the structure [(Gal β1→4 GlcNAc β1→2)$_2$ (Gal β1→4 GlcNAc β1→4)] (Man α1→6, Man α1→3) Man β1→4 GlcNAc. These compounds appear to derive from the incomplete degradation of certain erythrocyte glycoproteins and plasma immunoglobulins.

G_{M1}-gangliosidosis, an inherited disorder of ganglioside metabolism in children, is biochemically characterized by a virtual absence of activity of the enzyme G_{M1}-ganglioside β-galactosidase in the infantile form (Type 1) and abnormally low activity in the juvenile form (Type 2) (1). G_{M1} ganglioside (galactosyl β1→3 N-acetylgalactosaminyl β1→4[α2→3 N-acetylneuraminyl]lactosylceramide) thus accumulates in the central nervous system and to some extent in non-neuronal tissues, particularly the viscera (2-7). Furthermore, various earlier reports (8-12) have described the presence of galactose-containing compounds in visceral tissues and urine which were thought to resemble chemically undersulfated forms of the cartilage glycosaminoglycan, keratan sulfate. Recently, however, Wolfe et al. (13) showed by rigorous chemical methods that these highly water-soluble compounds were in fact structurally related oligosaccharides and glycopeptides, containing 8 or 10 sugar residues with a common trimannosyl-N-acetylglucosaminyl core. Galactose residues were at the non-reducing terminii and linked β1→4 to N-acetylglucosamine. These compounds were thought to be derived from the incomplete catabolism of

erythrocyte stromal glycoproteins (14,15). In this communication, we report the isolation and characterization of highly abnormal amounts of oligosaccharides and glycopeptides excreted in the urine of G_{M1}-gangliosidoses patients and the demonstration of their chemical similarity to the compounds which accumulate in the liver.

Materials and Methods

Oligosaccharides and glycopeptides were isolated from 24-hour urine samples of three G_{M1}-gangliosidosis patients by the following procedure. A 100 ml urine sample, after removal of sediments by centrifugation, was concentrated in vacuo at 25° to about 20 ml. The concentrate was dialysed three times in distilled water containing 0.02% of sodium azide for three days at 4°. The dialysate was subsequently concentrated and desalted on a 2x45 cm Biogel P-6 column. The salt-free oligosaccharide and glycopeptide fractions were rechromatographed on Biogel P-6 columns monitored by both a refractive index monitor (Pharmacia) and the phenol-sulfuric acid reaction.

The purified oligosaccharide and glycopeptide fractions were analyzed by physico-chemical methods described in detail in a previous paper (13). Briefly, the proton magnetic resonance (p.m.r.) spectra were obtained at both 100 and 220 MHz for 1 to 10% w/v solutions of D_2O-exchanged oligosaccharides in D_2O using the appropriate Varian spectrometers. The compositions of the oligosaccharides were determined by first hydrolyzing them in N HCl at 100° for 10 hr and analyzing the resulting sugars and amino-sugars as their reduced and acetylated derivatives by gas-liquid chromatography (g.l.c.) using a 4-foot column of 3% ECNSS-M on Gas-Chrom Q, 100-120 mesh (Applied Science Laboratories). Both the intact and borohydride treated oligosaccharides and glycopeptides were subjected to permethylation studies as described earlier (13,16). An LKB-9000 mass spectrometer was used for the identifications of the permethylated compounds.

Results

Figure 1 shows the elution profile from Biogel P-6 of the urinary oligosaccharides and glycopeptides isolated from Case 1 compared to the elution pattern obtained from the liver of the same patient. The overlapping fractions 3, 4 and 5 each contained galactose, mannose and N-acetylglucosamine as their major constituents and accounted for the majority of the oligosaccharides and glycopeptides isolated from the urine as was the case in the liver sample(13). Almost identical chromatographic patterns were obtained for Cases 2 and 3 and the oligosaccharides and glycopeptides isolated contained only galactose, mannose and N-acetylglucosamine as sugar components. The yield of the combined fractions from the three cases is shown in Table 1. The Type 2 case yielded lower amounts of the compounds than Type 1 but still in abnor-

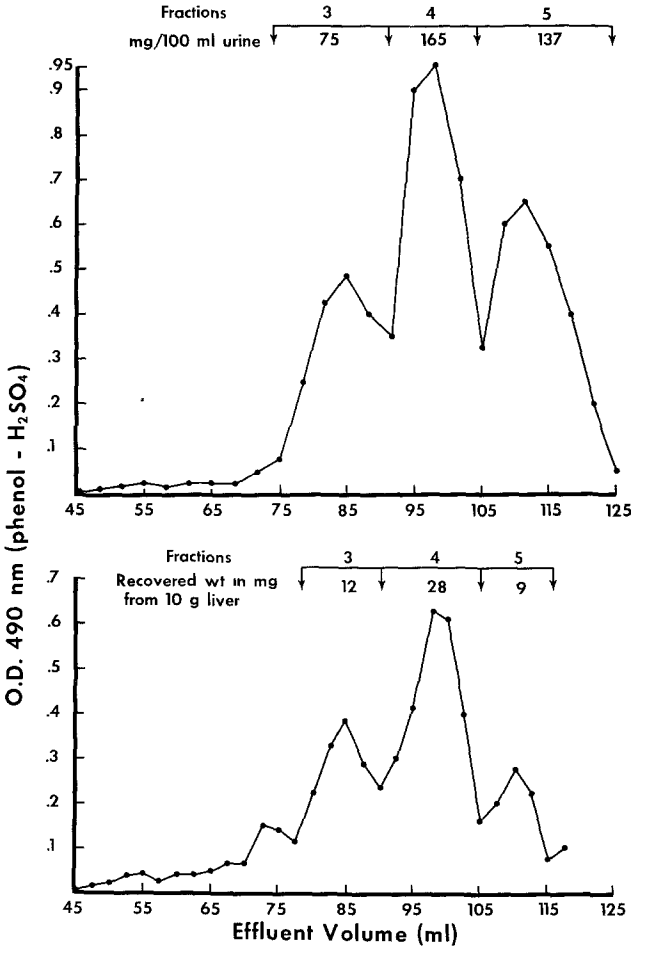

Figure 1. Elution profile on Biogel P-6 of the major salt-free oligosaccharides from liver and urine of a G_{M1}-gangliosidosis patient (Case 1, Table 1). Approximate molecular weight of fractions 3, 4 and 5 were 2200, 1800 and 1400 respectively (see 13).

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mal amounts since in urine from normal subjects these fractions were not detectable.

The 220 MHz p.m.r. spectrum of the major fraction in the urine of Case 1, namely the oligosaccharide fraction 4, was almost superimposable on the spectrum obtained for the identical fraction from the liver of the same patient (Fig. 2). Prominent absorptions in the anomeric region of the spec-

TABLE 1.

Yield of oligosaccharides from the urine of G_{M1}-gangliosidoses patients

Patients	mg/100 ml urine*
Case 1, Type 1	377
Case 2, Type 1	67
Case 3, Type 2	12
Normal urine (6)	Not detectable

*Based on the combined weights of the 3 major fractions purified by Biogel P-6 chromatography (see Figure 1).

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trum for the urine compound were observed at 5.71, 5.63, 5.41, 5.25, 5.09 and 4.96 (doublet, J = 7 Hz) ppm which can be assigned to the C-1 protons of reducing N-acetylglucosaminyl, three different mannosyl, non-reducing N-acetylglucosaminyl, and terminal non-reducing β-galactosyl residues respectively (see 13). The two high-field absorptions at 2.54 and 2.55 ppm, attributable to N-acetyl groups, confirmed the presence of the two different types of N-acetylglucosaminyl residues. The spectral similarities obtained for the liver and urine compounds strongly suggest that the major oligosaccharides present in the liver and the urine samples have identical structures. Their sugar composition showed mannose, galactose and N-acetylglucosamine in the ratio of approximately 3:3:4 respectively. Furthermore, permethylation analyses of the urine and liver oligosaccharides gave identical g.l.c. peaks of the resulting partially methylated alditol and amino-deoxyalditol acetates. From the T-values (16) and mass spectra of each of these peaks it was possible to conclude that the following glycosidic linkages were present in the urine oligosaccharide fraction 4: terminal non-reducing galactosyl, 2-linked, 2,4-linked and 3,6-linked mannosyl, 1,4-linked N-acetylglucosaminyl and reducing 4-linked N-acetylglucosaminyl residues. These data are compatible with the branched chain structure (Gal β1→4 GlcNAc β1→)$_3$ (Man α1→)$_2$ Man β1→4 GlcNAc

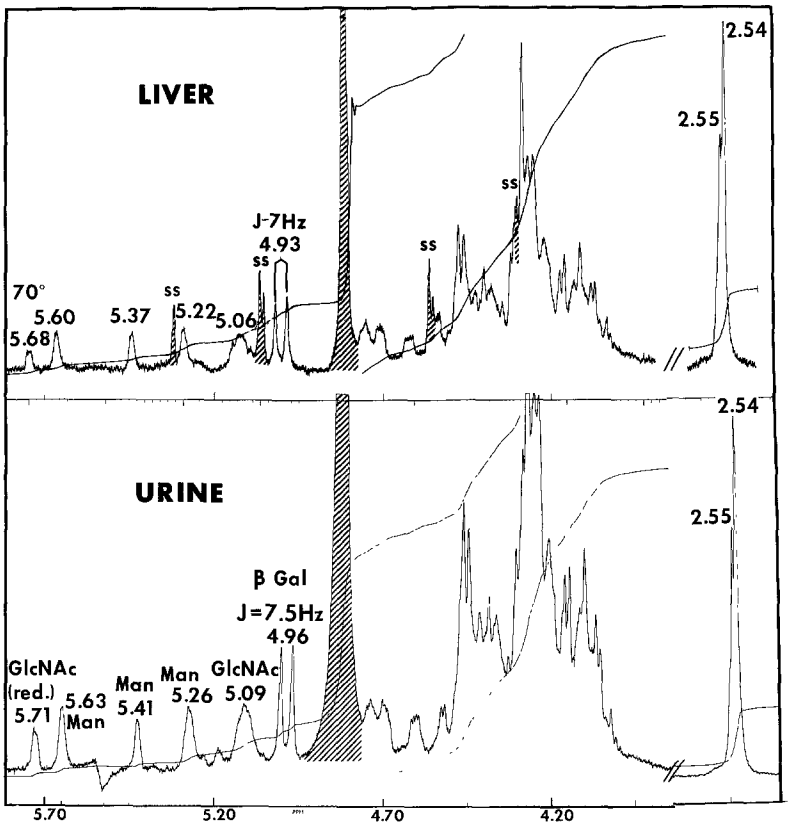

Figure 2. 220 MHz p.m.r. spectra of fraction 4 oligosaccharides (see Figure 1) from urine and frozen liver specimens from G_{M1}-gangliosidosis, Type 1. Shaded areas represent water and its spinning side bands (ss).

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as reported previously for the liver decasaccharide (13).

The 220 MHz p.m.r. spectra of fraction 3 and 5 obtained from Biogel P-6 chromatography of the urine sample of Case 1 (see Figure 1) showed that the same anomeric absorptions seen already in fraction 4 were also found in fractions 3 and 5, strongly suggesting that all these compounds were structurally related. However, the intensity of the signal at 5.71 ppm attributed to the H-1 reducing N-acetylglucosaminyl residue was markedly decreased in fraction 3 and increased in fraction 5 relative to the other anomeric protons. This is indicative of a glycopeptide linkage in fraction

3, in contrast to a terminal reducing N-acetylglucosamine residue in fraction 5. The different proportions of 4-linked reducing N-acetylglucosaminyl residues present in these two fractions was demonstrated by methylation analyses after sodium borohydride treatment. Fraction 3 contained trace amounts of a peak with T-value 3.00, whereas in fraction 5 this was a prominent peak. The mass spectrum of this compound showed it represented 4-O-acetyl-2-deoxy-2-(N-methylacetamido)-1,3,5,6-tetra-O-methyl glucitol which must have originated from a reduced 4-linked N-acetylglucosamine (see 13). Thus, the N-acetylglucosamine residue at the reducing terminus must be linked, most likely to asparagine or GlcNAc-Asn. The appearance of an additional signal at 2.57 ppm in the p.m.r. spectrum of fraction 3 assignable to the N-acetyl group substantiates this. It is concluded that fraction 3 contains the identical oligosaccharide structure to fraction 4 but linked to a small, uncharacterized amino-acid/peptide. Fraction 5, on the other hand, is an oligosaccharide with a terminal reducing N-acetylglucosamine residue similar to fraction 4 but its lower molecular weight, composition and the absence of 2,4-linked mannosyl residues showed that it is an octasaccharide derived from the fraction 4 decasaccharide by loss of one Gal β1→4 GlcNAc β1→ residue.

In Cases 2 and 3, the major oligosaccharide fractions isolated were shown to be similar to those obtained in Case 1, on the basis of their composition, p.m.r. spectra and methylation analysis.

Discussion

It is clear from the studies described here that the major oligosaccharide and glycopeptide fractions isolated from the urine of G_{M1}-gangliosidosis patients have structures identical to those previously reported by Wolfe *et al.* (13) from the liver in this glycolipid storage disease. The amounts excreted in the Type 1 form of the disease are quite large amounting to approximately 1 g/day. One possible source of these compounds is the MN-blood group sialoglycoproteins whose asialo-oligosaccharide structures resemble very closely the deca- and octasaccharides of fraction 4 and 5 from the urine and

liver (14,15). Recently, Baenzinger et al. (17,18) have proposed a structure for the B-type glycopeptides of IgE which is identical to the octasaccharide we have isolated from the tissue and urine in all its fine structure except for the presence of terminal sialic acid and fucose and a chitobiosyl residue linked to asparagine at the reducing terminal. Tarrentino et al. (19) also concluded that similar structures existed in human IgM. It is thus highly probable that the oligosaccharides stored in viscera and excreted in the urine result from an incomplete catabolism of these glycoproteins. The glycoproteins would first be degraded by proteolytic enzymes and then by an endoglucosaminidase (20) to expose N-acetylglucosamine at the reducing terminal and by a fucosidase and sialidase to expose galactose residues at the non-reducing terminii. These oligosaccharides which would be undegradable in G_{M1}-gangliosidosis subjects consequently accumulate in lysosomes principally in the liver and spleen, overflow into the blood and are excreted unchanged in the urine. In quantitative terms, the amounts of oligosaccharides and glycopeptides accumulating and excreted in Type 1 G_{M1}-gangliosidosis is considerably greater than the combined ganglioside storage in the brain and extraneural tissues. This no doubt reflects the faster rate of metabolism of blood glycoproteins to membrane gangliosides. Cerebral cortex of the Case 1 patient was found to contain only 0.3 mg/g wet weight of the oligosaccharides, 25-30 times less than found in the liver (unpublished results). The brain oligosaccharides appear not to be derived from neuronal membrane glycoproteins but from the blood circulation. Finally, it is worth noting that despite the presence of wide varieties of glycoproteins in the mammalian tissues, the compounds we have isolated so far from G_{M1} and also G_{M2} (Variant 0) gangliosidoses patients (13,21) appear to be derived from an incomplete catabolism of a select group of structurally related glycoproteins, notably IgE, IgM and human erythrocyte stromal glycoproteins. The β-galactosidase deficient in G_{M1}-gangliosidosis patients hydrolyses only certain specific natural substrates. Recent publications (22,23) on the specificity of purified human β-galactosid-

ases towards natural substrates support this view.

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