Characterization and Analysis of Branched-chain N-Acetylglucosaminyl Oligosaccharides Accumulating in Sandhoff Disease Tissue

EVIDENCE THAT BIANTENNARY BISECTED OLIGOSACCHARIDE SIDE CHAINS OF GLYCOPROTEINS ARE ABUNDANT SUBSTRATES FOR LYSOSOMES*

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Branched chain N-acetylglucosaminyl oligosaccharides accumulating in visceral and neural tissues of two patients with Sandhoff disease were isolated and quantified using high performance liquid chromatography. Detailed structural analysis of the three most abundant fractions, oligosaccharides 4, 5, and 6, was carried out using 360 MHz proton magnetic resonance spectroscopy. The biantennary bisected heptasaccharide, oligosaccharide 6, was ubiquitously distributed and a major component of the stored oligosaccharides in all tissues analyzed including, liver, spleen, kidney, lung, pancreas, and brain. This analysis indicates that glycoproteins containing biantennary bisected oligosaccharide side chains are abundant substrates for lysosomes in human tissues. Moreover, oligosaccharide 6 was the predominant storage product in brain comprising 70% of the total accumulating water-soluble glycoconjugates. Oligosaccharide 5, a triantennary heptasaccharide, had a similar distribution in visceral tissues and it was the major storage product in pancreas but was at very low levels in brain. These results suggest that the biosynthetic enzymes, GlcNAc transferase III (Narasimham, S. (1982) J. Biol. Chem. 257, 10235-10242; and IV (Gleeson, P. A., and Schachter, H. (1983) J. Biol. Chem. 258, 6162-6173), which are responsible for synthesis of these structures, have a generalized distribution with varying levels of expression in human viscera, moreover, transferase IV may have limited expression in neural tissue. The proposed structures for the branched-chain compounds are as follows:

\[
\text{OLIGOSACCHARIDE 4} \quad \text{GlcNAc}(1-2)\text{Man}(1-6) \quad \text{Man}(1-4)\text{GlcNAc} \\
\text{GlcNAc}(1-2)\text{Man}(1-3) \\
\]

Lysosomal storage disorders resulting from deficiencies of glycohydrolytic enzymes are characterized, in part, by urinary excretion and tissue accumulation of a wide variety of high molecular weight oligosaccharides (1). Presumably, these storage products represent the remnants of incomplete degradation of the oligosaccharide side chains of the cellular and serum glycoproteins that are subject to lysosomal catabolism. Isolation and detailed structure analysis of the compounds accumulating in several of these diseases, including GM1 gangliosidosis (2, 3), α-fucosidosis (4), and α-mannosidosis (5, 6), has recently been carried out. These studies have provided valuable information about the possible mechanistic pathways of cellular glycoprotein degradation and they have also revealed the partial structures of a vast number of glycoconjugates. In some cases, mutation differences between clinical variants or phenotypic subtypes is reflected by marked differences in the urinary metabolites (4). Although the excreted glycoconjugates in several glycoprotein diseases have been analyzed in detail, thus far an extensive survey and characterization of oligosaccharides accumulating in visceral or neural tissues for any storage disease has not been reported.

Sandhoff disease (GM1 gangliosidosis, Type II) is an inherited storage disorder affecting humans; both the lipid GM2 ganglioside as well as urinary and visceral oligosaccharides accumulate. The stored glycoconjugates contain β-linked N-
acetylglucosamine residues at their nonreducing terminus due to the generalized absence of the lysosomal β-hexosaminidase (1). Several linear pentasaccharides as well as higher molecular weight branched-chain oligosaccharides have been isolated from urine of affected individuals and their structures assigned (7-9). The latter compounds, whose structures are shown in Fig. 1, include (a) a biantennary hexaosaccharide, oligosaccharide 4, (b) a triantennary heptasaccharide, oligosaccharide 5, and (c) a triantennary heptasaccharide, oligosaccharide 6, termed biantennary bisected.

The glucosaminyl oligosaccharides that accumulate in Sandhoff tissues, particularly the branched-chain compounds, are of interest since the degree of branching or the number of antennae on lactosaminic-type oligosaccharides is mediated during biosynthesis by the addition of N-acetylglucosamine residues onto the growing oligosaccharide chain (10). This process is carried out by at least four distinct N-acetylglucosamine transferase enzyme systems, each employing UDP-GlcNAc as substrate and incorporating N-acetylglucosamine at specific sites on the oligosaccharide molecule at different stages of biosynthesis (11-14). We have investigated, here, the visceral branched-chain storage products in Sandhoff disease in order to obtain some estimate of the diversity and relative abundance of oligosaccharide branching of glycosconjugates which undergo lysosomal degradation in humans. These studies are based on the assumption that, as a result of the near total hexosaminidase deficiency, lysosomal catabolism of cellular glycosconjugates in Sandhoff disease terminates when an N-acetylglucosamine residue is encountered by the catalytically impaired enzyme. Although partial degradation of oligosaccharides takes place with removal of sialic acid, fucose, and other carbohydrate residues, the number of antennae or branches in the original glycosconjugate can be discerned since N-acetylglucosamine residues are not cleaved and the number or type of branch points are preserved. These results may also provide insight, albeit indirect, into the relative levels and extent of action of the N-acetylglucosaminyl transferase enzyme systems in different human tissues.

MATERIALS AND METHODS

Reagents and Tissues—Acetonitrile, water, and methanol, were HPLC grade and were obtained from Baker Chemical Co. Sodium borohydride, orcinol, activated charcoal, and diatomaceous silica (Celite) were from Sigma. Silicic acid thin layer plates were Silica Gel 60 on 20 × 20 cm glass-backed plates obtained from E. Merck, Darmstadt, Germany.

Tissues from normal individuals and Sandhoff patients were taken immediately after autopsy and were stored frozen at −20 °C prior to use. Sandhoff patients were identified by clinical phenotype (infantile or late onset form), enzyme assays for hexosaminidase and ultrastructural studies of autopsy specimens. Two patients, L. O. and V. C. were from Cordoba, Argentina and a third patient, T. C., was from Toronto, Canada.

Preparative Isolation of N-Acetylgalactosaminyl Oligosaccharide with Thin Layer Chromatography—Oligosaccharides were isolated on a preparative level by homogenization of 4 g of frozen tissue in 4 ml of methanol/water, 9:1 (v/v), in a ground glass tissue homogenizer, followed by centrifugation (2000 × g, 15 min). After removal of the supernatant, the pellet was extracted two additional times in a similar manner followed by a final extraction with methanol/water, 1:1 (v/v). The supernatants were pooled and the solvent removed under vacuum. The resulting residue was suspended in 6 column volumes of water. The sample, the column was washed with 10 ml of water and the eluate collected. The progress of the separation was followed by thin layer chromatography on a column of Bio-Gel P-2, 1.0 × 90 cm (Bio-Rad), eluting with water, and collecting 2-ml fractions. The progress of the column was monitored with orcinol spray, the appropriate fractions pooled, and solvent removed by lyophilization.

Preparative Isolation of N-Acetylgalactosaminyl Oligosaccharide-alditols with High Performance Liquid Chromatography—The oligosaccharides in Sandhoff tissues were also purified as oligosaccharide-alditols on a preparative scale with high performance liquid chromatography. Extraction of the oligosaccharides from frozen tissues and partial purification with charcoal/Celite chromatography was carried out as described above. The oligosaccharide-alditol was eluted with 200 μl of 100 mM borate buffer, pH 9.3, and the oligosaccharides converted to oligosaccharide-alditols by addition of 2.0 mg of NaBH₄. After reaction for 1 h at room temperature the excess reductant was destroyed by addition of 10 μl of glacial acetic acid. The resulting salts in the reaction mixture were removed by gel filtration chromatography using a Bio-Gel P-2 column (200-400 mesh, H₃ form, Bio-Rad), 1.0 × 0.5 cm, overlayered with AG 1-X8 (200-400 mesh, acetate form), 1.0 × 1.5 cm, and a final layer of Amberlite IRA-743, 1.0 × 2.0 cm (Sigma). After three applications of the sample, the column was washed with 10 ml of water and the solvent removed under vacuum. The residue containing the oligosaccharide-alditols was suspended in 300 μl of acetone, 10 mM phosphate, pH 3.0, 65:35 (v/v) and the mixture fractionated on a commercial HPLC column, Micro Pak AX-5 (4.0 × 30 cm, Varian, Associates Inc.), equilibrated in the same solvent. Elution of the column was carried out using a Varian liquid chromatograph, model 5000, with a linear gradient of acetone, 10 mM phosphate, pH 3.0, with increasing concentrations of 10 mM phosphate buffer in the gradient, a 0.3% increase/min for 50 min up to 50% 10 mM phosphate buffer. The flow rate was maintained at 1.0 ml/min and the location of the oligosaccharide-alditols was determined by monitoring the absorbance (210 nm) of the column eluate. A total of six major fractions were collected and the later eluting fractions, oligosaccharides 4, 5, and 6, were subjected to gel filtration chromatography (Bio-Gel P-2) after HPLC separation. Structural analysis of each fraction was carried out using 1H magnetic resonance spectroscopy.

Structural Analysis of Oligosaccharides—Preparative Isolation of the oligosaccharide fractions isolated by TLC and HPLC were made using a 360 MHz Oxford superconducting magnet coupled with a Nicolet 1180 E computer, operating in the Fourier transform mode using quadrature phase detection. Spectra were obtained at a sweep width of ± 2000 Hz using a near 90° pulse. Spectra were recorded with the samples maintained at ambient temperature or, in some experiments, at 65 °C in order to reveal signals situated under the HOH signal. Reported spectra were computer expanded to 1600 or 500 Hz and coupling constants were taken from the directly observed HO'H signal. Acetone was included as an internal standard (δ = 2.243 ppm) and chemical shifts are reported relative to external [5-(trimethylsilyl)tetraedetium]sodium propionate.

Prior to recording spectra, paramagnetic contaminants were removed from the oligosaccharide fractions by chromatography on an ion exchange column containing Chelex resin (Bio-Rad), 200–400 mesh, OH form, 0.1 × 0.1 cm, followed by five successive lyophilizations from HOH, low magnetic field grade (W. Beck Sharp and Doehme, Canada).

Survey Analysis of N-Acetylgalactosaminyl Oligosaccharides in Visceral Tissues—The accumulating oligosaccharides in visceral tissues were isolated and normal tissues were fractionated as previously described for isomerization to acetylgalactosamine and then isolated on a preparative scale as the alditol acetates as radioactively labeled 3H-oligosaccharide-alditols using HPLC. Samples of frozen tissue (10–20 mg) were extracted in 0.5 ml of water at 4°C using a ground glass tissue homogenizer. After the protein content of the suspension was determined (15), methanol (0.5 ml) was added and particulate matter was removed by centrifugation (5 min, 5000 × g, 4°C). After removal of the supernatant, the pellet was extracted in a similar manner, twice with 1.0 ml of methanol/water,
1:1 (v/v), and once with 1.0 ml of water. The supernatants were pooled and the solvent removed under vacuum. The resulting residue was suspended in water (2.0 mg of tissue protein/ml) and cleared with centrifugation (5 min, 5000 x g, 4 °C).

The extracted oligosaccharides were converted to 3H-oligosaccharide-alditols by reaction with NaB[3H]4 using a modification of the method employed for labeling oligosaccharides in urine and amniotic fluid taken from individuals with other glycoprotein storage disease as described elsewhere (16). In order to quantify the individual fractions an internal oligosaccharide standard (2.48 nmol) composed of two hexaasaccharide isomers, Galb1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcγ1-GlcNAc, and Galb1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcα1-GlcNAc, was added to aliquots (~200 µl) of each tissue extract and the mixture lyophilized. The residue was suspended in 30 µl of 100 mM borate buffer, pH 9.3, followed by 30 µl of 50 mM NaOH containing 0.25 mCi of NaB[3H]4, >100 mCi/mmol (ICN, Irvine, CA). After heating at 37 °C for 1 h, the reaction was terminated with acetic acid and the mixture desalted on a layered-bed ion exchange column (Amberlyst A2) with a contaminant (*20%) as a biantennary hexasaccharide with 2 GlcNAc residues at C-1 and C-2 protons of each carbohydrate residue were in good agreement with those reported for the aldose form of this compound isolated from Sandhoff urine (7) and glycopeptides of similar structure (17, 18). Since the crude liver extract containing the oligosaccharide mixture was initially treated with NaBH4 prior to HPLC chromatography, the oligosaccharides were isolated and characterized as oligosaccharide-alditols. Thus the HPLC-purified compounds, including oligosaccharide 4-alditol lacked anomeric proton signals at 5.240 ppm which arise from the C-1 protons of both GlcNAc residues, Fig. 1, upper insert. With this compound, the C-1 α and β anomers were observed for the terminal, reducing GlcNAc residue (data not shown), giving an identical spectra to that previously reported for this material isolated from urine of Sandhoff patients (7).

The proton spectra obtained from oligosaccharide 5-alditol was consistent with a triantennary heptasaccharide with structural features similar to oligosaccharide 4 except for the presence of an additional N-acetylgalcosamine residue (C-1, 4.540 ppm) in β1-4 linkage to the Man c residue. Support for this structural assignment is obtained by inspection of the C-2 proton region (4.244 ppm) arising from Man β and c residues. In this case, proton signals for these residues are not resolved.

RESULTS AND DISCUSSION

Proton Magnetic Resonance Analysis of Oligosaccharide-alditols 4, 5, and 6—Oligosaccharides 4, 5, and 6, isolated from liver of Sandhoff patient L. O., were confirmed as branched-chain glycoconjugates using proton magnetic resonance spectroscopy, Fig. 1 and Table I. Oligosaccharide 4 was identified as a biantennary hexaasaccharide with 2 GlcNAc residues at the nonreducing terminus. Signal assignments for the C-1 and C-2 protons of each carbohydrate residue were in good agreement with those reported for the aldose form of this compound isolated from Sandhoff urine (7) and glycopeptides of similar structure (17, 18). Since the crude liver extract containing the oligosaccharide mixture was initially treated with NaBH4 prior to HPLC chromatography, the oligosaccharides were isolated and characterized as oligosaccharide-alditols. Thus the HPLC-purified compounds, including oligosaccharide 4-alditol lacked anomeric proton signals at 5.240 ppm which arise from the C-1 protons of both GlcNAc residues, Fig. 1, upper insert. With this compound, the C-1 α and β anomers were observed for the terminal, reducing GlcNAc residue (data not shown), giving an identical spectra to that previously reported for this material isolated from urine of Sandhoff patients (7).

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Fig. 1. The 360 MHz 1H magnetic resonance spectra of branched-chain oligosaccharide-alditols isolated from Sandhoff liver. Shown are signals in the anomeric proton region. Signals for ring protons, acetamidomethyl protons, and H02H have been omitted for clarity. Spectra were taken at 23 °C and at 65 °C (to identify signals under the H02H envelope) using acetone as internal standard. A, oligosaccharide 4-alditol spectra were taken on a 2 nM solution using 256 acquisitions. Upper insert: anomeric proton signals of residues e and f for oligosaccharide 4 as the aldose form (oligosaccharide 4 without NaBH4 reduction). B, oligosaccharide 5-alditol spectra taken on a 0.8 mM solution using 256 acquisitions. C, oligosaccharide 6-alditol spectra taken on a 0.8 mM solution using 256 acquisitions.
from one another and appear as a single complex resonance. If Man d were substituted with an additional GlcNAc residue instead of Man c, then two distinct signals in the C-2 proton region would be detected, as has been observed with other triantennary compounds in which the third branch of the molecule imminates from the α1-6 mannose residue (18). Further evidence for a triantennary structure of this type is the presence of the C-3 proton signals of the Man c residue in a downfield shift of the C-3, 4.089 ppm, which appears as a doublet of doublets. GlcNAc substitution of Man c results in a downfield shift of the C-3, proton resonance out of the ring proton envelope. In contrast, a similar downfield shift has not been observed when GlcNAc substitution is on the Man d residue (17). However, the presence of small amounts of GlcNAc linked to the Man d residue cannot be excluded based on the spectral data.

Oligosaccharide 6-alditol gave proton magnetic resonance spectra consistent with a biantennary bisected heptasaccharide structure. Similar spectra have previously been reported with a heptasaccharide isolated from Sandhoff urine (7) and biantennary bisected glycopeptides prepared biosynthetically using purified glycopeptides derived from IgG as substrate precursors (13). The doublet is 4.502 ppm is assigned to the C-3 proton signals of the Man c residue, 4.089 ppm, which appears as a doublet of doublets. GlcNAc substitution of Man c results in a downfield shift of the C-3, proton resonance out of the ring proton envelope. In contrast, a similar downfield shift has not been observed when GlcNAc substitution is on the Man d residue (17). However, the presence of small amounts of GlcNAc linked to the Man d residue cannot be excluded based on the spectral data.

The proton spectra were recorded at a spectral window ± 2000 Hz using quadrature phase detection. Acetone was included as an internal standard and chemical shifts (δ) are reported relative to external 3-[trimethylsilyl]tetradecuterio]sodium propionate taken from computer expanded spectra at 1600 Hz sweep width. Except where noted, spectra were acquired at ambient temperature. Oligosaccharides were isolated from Sandhoff liver and brain as described under "Materials and Methods.”

<table>
<thead>
<tr>
<th>Proton and residue</th>
<th>Oligosaccharide</th>
<th>4, liver</th>
<th>5, alditol</th>
<th>6, alditol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Aldose</td>
<td>Alditol</td>
<td>Liver</td>
<td>Brain</td>
</tr>
<tr>
<td>C-1 Man</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b, β1-4</td>
<td>4.740</td>
<td>4.829*</td>
<td>4.818*</td>
<td>4.829*</td>
</tr>
<tr>
<td>c, α1-3</td>
<td>5.143</td>
<td>5.131</td>
<td>5.147</td>
<td>5.077</td>
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<tr>
<td>d, α1-6</td>
<td>4.933</td>
<td>4.939*</td>
<td>4.937*</td>
<td>5.039</td>
</tr>
<tr>
<td>C-2 Man</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b, β1-4</td>
<td>4.275</td>
<td>4.277</td>
<td>4.244</td>
<td>4.216</td>
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<tr>
<td>c, α1-3</td>
<td>4.214</td>
<td>4.216</td>
<td>4.244</td>
<td>4.250</td>
</tr>
<tr>
<td>d, α1-6</td>
<td>4.136</td>
<td>4.157</td>
<td>4.153</td>
<td>4.216</td>
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<tr>
<td>C-3 Man</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>c, α1-3</td>
<td>4.089</td>
<td></td>
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<td></td>
</tr>
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</table>

* Taken from spectra recorded at 65 °C.

Oligosaccharides 5 and 6 were not resolved from one another with preparative thin layer chromatography and these fractions migrated as a single diffuse band on the TLC plate. For this reason proton magnetic resonance spectra could not be acquired on the aldose forms of these compounds.

Distribution of Stored N-Acetylgalactosaminyl Oligosaccharides in Sandhoff Tissues—Six major oligosaccharide fractions were identified in some of the visceral tissues taken from the two patients with Sandhoff disease when analyzed with high performance liquid chromatography. Similar compounds were not observed in tissues from normal individuals at this level of detection. Linear straight chain oligosaccharides eluted early in the chromatogram while the branched chain glycoconjugates, oligosaccharides 4, 5, and 6 eluted later, at higher concentrations of the buffered aqueous phase in the eluting gradient, Fig. 2. The chromatographic patterns of the storage materials were similar in the two cases investigated (L. O. and V. C.) although they were not identical. Also, the levels of the accumulating materials differed slightly, Table II. These variations are probably not due to differences in the altered hexosaminidase enzyme since both patients probably have an identical mutation at the hexosaminidase locus. Both individuals, although not directly related, were natives of the La Roja region surrounding Cordoba, Argentina, and are part of, what may be, a unique ethnic deme with an unusually high incidence of Sandhoff disease (20). Due to inbreeding and
geographic isolation, the people of this area are likely to share a common gene pool. Environmental, dietary, as well as metabolic influences on glycoconjugate metabolism may be important factors affecting the composition of the accumulating metabolites and this may account for the subtle differences observed.

One of the most striking features of the stored metabolites revealed with this analysis was the high levels of the bisected biantennary heptasaccharide, oligosaccharide 6, in all tissues, relative to the other branched-chain oligosaccharides. Although this material was previously identified as an abundant component of the excreted glycoconjugates in Sandhoff urine (7), it was found as a minor fraction of the oligosaccharides in liver of a single Sandhoff patient (8). The presence of this compound was not detected in tissues in earlier studies (9). Our analysis indicates that the bisected structure is present in liver of both affected individuals and at similar levels in other visceral tissues. Moreover, the bisected compound is the primary stored oligosaccharide in brain, comprising about 80% of the branched-chain oligosaccharide fraction and about 70% of the total oligosaccharides accumulating in this tissue. The bisected material in brain from a third patient of Canadian origin had a nearly identical concentration indicating that storage of this material is not unique to Argentine patients.

The bisected-type oligosaccharide side chains of glycoproteins arise during glycoprotein biosynthesis by the action of UDP-GlcNAc:glycopeptide β4-N-acetylglucosaminyl transferase III (transferase III) (13) which adds GlcNAc residues in β1-4 linkage to the inner most mannose residue of the biantennary oligosaccharide molecule. The enzyme has been suggested to play an important role in controlling carbohydrate composition and oligosaccharide structure by mediating, indirectly, both addition and removal of other carbohydrate residues such as α-fucose (21) and α-mannose (13), respectively. Transferase III activity has been found at high levels in hen oviduct membrane and correspondingly, Avian glycoproteins originating from this tissue contain bisected-oligosaccharide chains (19, 22). Since lysosomal catabolism involves, in part, autophagic degradation of cellular components at least some of the glycoprotein by-products accumulating in Sandhoff disease are likely to be remnants of glycoconjugates biosynthesized and originating from within the cells in which they accumulate. Our results demonstrate that bisected oligosaccharides are ubiquitous storage products in all visceral and neural tissues analyzed, and this suggests that transferase III may have a similar distribution. In addition, predominant storage of bisected oligosaccharide may reflect proportionately, the tissue or cellular levels of the enzyme. For example the transferase III may be at relatively high levels in brain and these oligosaccharides may be prevalent among brain glycoproteins. Bisected oligosaccharides have been identified on several human glycoproteins including: IgA1 (23) and IgG (24), glycoporin A (25, 26), and fetal and adult forms of lactosaminoglycan from band 3 of the human erythrocyte membrane (23, 27), although transferase III has not been characterized in human tissues and its presence in brain has not been demonstrated. It should be noted however, that a correlation between the presence or levels of biosynthetic enzymes and the amount of their resulting reaction by-products accumulating within lysosomes has not been documented in any storage disorder. Some of the accumulating metabolites may be extracellular in origin and may enter the cell by pinocytic mechanisms.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Patient</th>
<th>4, biantennary</th>
<th>5, triantennary</th>
<th>6, biantennary bisected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>L. O.</td>
<td>19 ± 1</td>
<td>46 ± 1</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>V. C.</td>
<td>10 ± 1</td>
<td>17 ± 0</td>
<td>10 ± 1</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>L. O.</td>
<td>9 ± 2</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>V. C.</td>
<td>10 ± 1</td>
<td>5 ± 1</td>
<td>7 ± 1</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>L. O.</td>
<td>1.7 ± 0.4</td>
<td>ND*</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>V. C.</td>
<td>3.8 ± 0.3</td>
<td>ND*</td>
<td>11 ± 1</td>
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<tr>
<td>T. C.</td>
<td>1.0 ± 0.4</td>
<td>ND*</td>
<td>8 ± 0.1</td>
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<td>Kidney</td>
<td>L. O.</td>
<td>4.9 ± 0.7</td>
<td>1.7 ± 0.1</td>
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<td>V. C.</td>
<td>1.1 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>3.6 ± 0.7</td>
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<td>Spleen</td>
<td>L. O.</td>
<td>3.6 ± 0.6</td>
<td>0.5 ± 0.1</td>
<td>2.7 ± 0.9</td>
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<tr>
<td>V. C.</td>
<td>1.0 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>1.7 ± 0.3</td>
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<tr>
<td>Lung</td>
<td>L. O.</td>
<td>3.2 ± 0.4</td>
<td>0.7 ± 0.2</td>
<td>2.2 ± 0.2</td>
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<td>V. C.</td>
<td>1.3 ± 0.5</td>
<td>0.4 ± 0.1</td>
<td>1.8 ± 0.5</td>
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</tbody>
</table>

* Not detected at this sensitivity, however, when the sample was increased 10-fold trace amounts were identified.

Since lysosomal catabolism involves, in part, autophagic degradation of cellular components at least some of the glycoprotein by-products accumulating in Sandhoff disease are likely to be remnants of glycoconjugates biosynthesized and originating from within the cells in which they accumulate. Our results demonstrate that bisected oligosaccharides are ubiquitous storage products in all visceral and neural tissues analyzed, and this suggests that transferase III may have a similar distribution. In addition, predominant storage of bisected oligosaccharide may reflect proportionately, the tissue or cellular levels of the enzyme. For example the transferase III may be at relatively high levels in brain and these oligosaccharides may be prevalent among brain glycoproteins. Bisected oligosaccharides have been identified on several human glycoproteins including: IgA1 (23) and IgG (24), glycoporin A (25, 26), and fetal and adult forms of lactosaminoglycan from band 3 of the human erythrocyte membrane (23, 27), although transferase III has not been characterized in human tissues and its presence in brain has not been demonstrated. It should be noted however, that a correlation between the presence or levels of biosynthetic enzymes and the amount of their resulting reaction by-products accumulating within lysosomes has not been documented in any storage disorder. Some of the accumulating metabolites may be extracellular in origin and may enter the cell by pinocytic mechanisms.

Triantennary oligosaccharides were found in highest concentration and the predominate storage product in pancreas which is unique among all viscera in this regard. Compounds of this type are formed during glycoprotein biosynthesis by the addition of GlcNAc residues to the oligosaccharide chain by the enzyme UDP-GlcNAc:GnGn(GlcNAc to Manα1-3) β4-N-acetylglucosaminyl transferase IV (transferase IV). Thus far, the enzyme has been partially characterized only in hen oviduct membrane preparations (14). This enzyme may be present at high levels (relative to transferase III) in human pancreas and, unlike other viscera, triantennary structures are abundant substrates for lysosomes in this tissue. In contrast, triantennary compounds were found at very low levels (~7% of total stored oligosaccharides) in brain of Sandhoff disease patients and this may reflect the limited transferase IV activity toward brain glycoproteins relative to transferase III.

At present, the temporal expression of oligosaccharide biosynthetic enzymes in developing tissues is not firmly established and developmental differences between visceral and neural tissues could account for the varying levels of the triantennary compounds. Sandhoff disease is usually fatal within the first 2 years of life and if complete expression of GlcNAc transferase IV in brain occurs after this period then this may account for the disproportionately lower levels of triantennary oligosaccharides accumulating in brain tissue of these patients.

Additional oligosaccharides eluting after oligosaccharide 6 were detected at low levels, and these were estimated to be about 4–5% of the total branched-chain oligosaccharide fraction. Unfortunately, due to the limited amounts of tissues and their dilute concentration, further characterization of the later eluting components was not possible. These storage products may be glycoconjugates of more complex structure such as tetra-antennary oligosaccharides and high molecular weight oligosaccharides with the repeating lactosaminic unit, Galβ1-4 GlcNAc. Although these compounds have not been observed previously in urine or liver of other Sandhoff patients (7, 8), structures of this type have been found on numerous human glycoproteins (17, 18, 27, 28) and they have been isolated and characterized from urine of patients with other storage disorders such as Gm1 gangliosidosis (2, 3) and sialidosis (29).
Their presence would also be anticipated among the stored metabolites in Sandhoff disease.

In summary, although the significance or implications of the stored and excreted glycoconjugates in this or other storage disorders can only be considered speculative, the results obtained here further elucidate the biochemical pathogenesis of this disease and they suggest that further investigation of the processes of oligosaccharide metabolism, in particular, possible regulatory systems in human tissues, is warranted.

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