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Isolation and structural characterization of sialic acid-containing storage material from mucolipidosis I (sialidosis) fibroblasts

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Sialic acid-containing storage material was isolated from cultured human mucolipidosis I (sialidosis) fibroblasts by gel permeation chromatography on Bio-Gel P-6 followed by medium-pressure anion-exchange chromatography on Mono Q. The structure determination of the isolated sialyloligosaccharides was carried out by 500-MHz ^{1}H-NMR spectroscopy in conjunction with sugar analysis and analytical HPLC. The storage material showed completely sialylated mono-, di- and triantennary *N*-glycosidic *N*-acetyllactosamine oligosaccharides having the Manβ1 → 4GlcNAc sequence at the reducing end in common. Heterogeneity occurred with respect to the linkages between terminal sialic acid and the penultimate galactose residues (α2 → 3/α2 → 6). It turned out that all the identified carbohydrate chains are consistent with the neuraminidase deficiency.

Introduction

Mucolipidosis I is a rare inherited metabolic disease which leads to severe clinical symptoms, such as psychomotor retardation and neurodegeneration [1]. The defect in the catabolism of glycoproteins is caused by an α-neuraminidase deficiency (sialidase) leading to an accumulation of sialic acid-containing material in tissues, leucocytes and fibroblasts [2] and to an excessive excretion of glycopeptides [3] and sialyloligosaccharides in urine. The structures of the urinary sialyloligosaccharides are rather well documented [4–9]. Much less is known about the nature of the accumulated sialic acid-containing storage material in cultured fibroblasts, although these cells are frequently used for the diagnosis of genetic diseases [10]. In fibroblasts from mucolipidosis I patients a 3–7-fold increase of the amount of bound sialic acid, in comparison to normal cell lines, has been observed [11–14]. Therefore, fibroblasts seem to be a representative cell type for studying the effect of the neuraminidase deficiency on lysosomal catabolism. In the case of human α-mannosidosis [15] and of β-mannosidosis in goats [16], the accumulation of oligosaccharides in fibroblasts has been demonstrated by thin-layer chromatography. To gain insight into the structural features of the storage material from mucolipidosis I fibroblasts, sialic acid-containing glycoconjugates have been isolated and analyzed by 500-MHz ^{1}H-NMR

Abbreviations: HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; Man, mannose; Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid.

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spectroscopy combined with sugar analysis and analytical HPLC. Here, we report on the identification of the primary structures of accumulated sialyloligosaccharides.

Materials and Methods

Fibroblasts

Skin fibroblasts of a dysmorphic mucolipidosis I patient (E.V.) with infantile onset were cultured in Ham's F10 medium supplemented with 10% fetal calf serum. In the fibroblasts, α-neuraminidase activity was greatly diminished, but all other enzymes tested, including β-galactosidase, showed normal activities [17].

Cells from eight 75 cm^2 Falcon flasks were harvested by trypsinization after 3 weeks of confluency, washed three times with phosphate-buffered saline and resuspended in 4 ml distilled water. The cells were disrupted by sonicating twice for 20 s at 0 °C. After centrifugation for 10 min at $10000 \times g$, the supernatant was collected and the pellet was extracted once more with 3.5 ml distilled water. The combined supernatants were used for further investigations.

Colorimetric methods

The sialic acid content was measured by the periodic acid–thiobarbituric acid method of Warren [18], before and after hydrolysis with 0.05 M H_2SO_4 (1 h, 80 °C). For the determination of the amount of free and total sialic acid, 100 and 50 μl aliquots of the supernatant were used, respectively. Free NeuAc was used as a standard.

The protein concentration in the supernatant was determined according to Lowry et al. [19], using 10 and 20 μl aliquots of the supernatant. Bovine serum albumin was applied as a standard.

Gel permeation chromatography

Part of the supernatant (7.25 ml) was fractionated on a Bio-Gel P-6 (200–400 mesh, Bio-Rad) column (120×2.4 cm) at 4 °C with a 0.1 M ammonia-acetic acid buffer (pH 5.4) as eluent (22 ml/h, 4.8 ml fractions). The elution profile was obtained by determination of the hexose content in the various fractions with the phenol-sulfuric acid assay [20]. In addition the ultraviolet absorption at 280 nm was measured.

Anion-exchange chromatography

Medium-pressure anion-exchange chromatography was carried out on a Mono Q HR 5/5 column utilizing a Fast Protein Liquid Chromatography apparatus, equipped with a Liquid Chromatography Controller LCC-500 and two P-500 pumps (Pharmacia) [21]. For the separation of sialyloligosaccharides a linear gradient of 0–100 mM NaCl in 10 ml water (Lichrosolv, Merck) at a flow rate of 2.0 ml/min and a pressure of 2.4 MPa was applied.

The eluate was monitored at 214 nm using a Pharmacia UV-1/214 detector, operating at 1.0 AU sensitivity, connected with a dual chart recorder. The injection volumes were 0.5 ml. Specific fractions were desalted on a Bio-Gel P-2 (100–200 mesh, Bio-Rad) column (18×1 cm) with distilled water as eluent and subsequently lyophilized.

Sugar analysis

Sugar analysis was carried out by gas-liquid chromatography on a capillary CPsil5 WCOT fused silica column (25 m × 0.32 mm i.d., Chrompack) using a Varian Aerograph 3700 gas chromatograph. The trimethylsilylated methyl glycosides were prepared by methanolysis (1.0 M methanolic HCl, 24 h, 85 °C), *N*-reacetylation and trimethylsilylation [22].

500-MHz ^{1}H-NMR spectroscopy

Sialyloligosaccharides were repeatedly exchanged in ^2H_2O (99.96 atom% ^{2}H, Aldrich) with intermediate lyophilization. ^{1}H-NMR spectra were recorded on a Bruker WM-500 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands) operating at 500 MHz in the Fourier transform mode at a probe temperature of 27 °C. Resolution-enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation [23]. Chemical shifts (δ) are expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured by reference to internal acetone (δ 2.225 ppm in ^2H_2O at 27 °C) with an accuracy of 0.002 ppm [24].

HPLC

Analytical HPLC was carried out on a Kratos

liquid chromatograph consisting of two Spectroflow 400 Solvent Delivery Systems, a Spectroflow 450 Solvent Programmer and a Rheodyne injection valve module. For the separation a 10 μm Lichrosorb-NH_2 column (250 × 4.6 mm, Chrompack) was used.

The elution of the monosialylated compounds was performed isocratically with a mixture of acetonitrile/30 mM KH_2PO_4, pH 4.7 (66 : 34, v/v) [25]. The di- and trisialylated compounds were eluted isocratically with a mixture of acetonitrile/30 mM KH_2PO_4-K_2HPO_4 buffer, pH 7.0 (62.5 : 37.5, v/v). In both cases the flow rate was 2.0 ml/min. The eluate was monitored by a Spectroflow 783 Programmable Absorbance Detector at 205 nm and a sensitivity of 0.01 AU. Peak areas were calculated by a Spectra Physics SP 4290 Integrator. The HPLC column was calibrated using well-defined sialyloligosaccharides isolated from urine of the same sialidosis patient (unpublished data).

Results

Determination of the protein content in the water-phase (7.5 ml) after disruption of the mucolipidosis I fibroblasts yielded 2.25 mg protein/ml. The amounts of free and total sialic acid were 7 and 72 nmol/mg protein, respectively. The quantity of bound sialic acid, 65 nmol/mg protein, is strongly enhanced as compared to control values (5–10 nmol/mg protein) [10–13].

The elution pattern of the supernatant on Bio-Gel P-6 is presented in Fig. 1. As is evident from the figure, the supernatant contained mainly high-molecular-mass material. The phenol-sulfuric acid assay turned out to be too insensitive to trace the presence of smaller hexose-containing carbohydrate chains. Because medium-size carbohydrate chains had to be expected, the fractions were pooled as indicated and subjected to sugar analysis.

Fraction I contained mainly glucose and trace

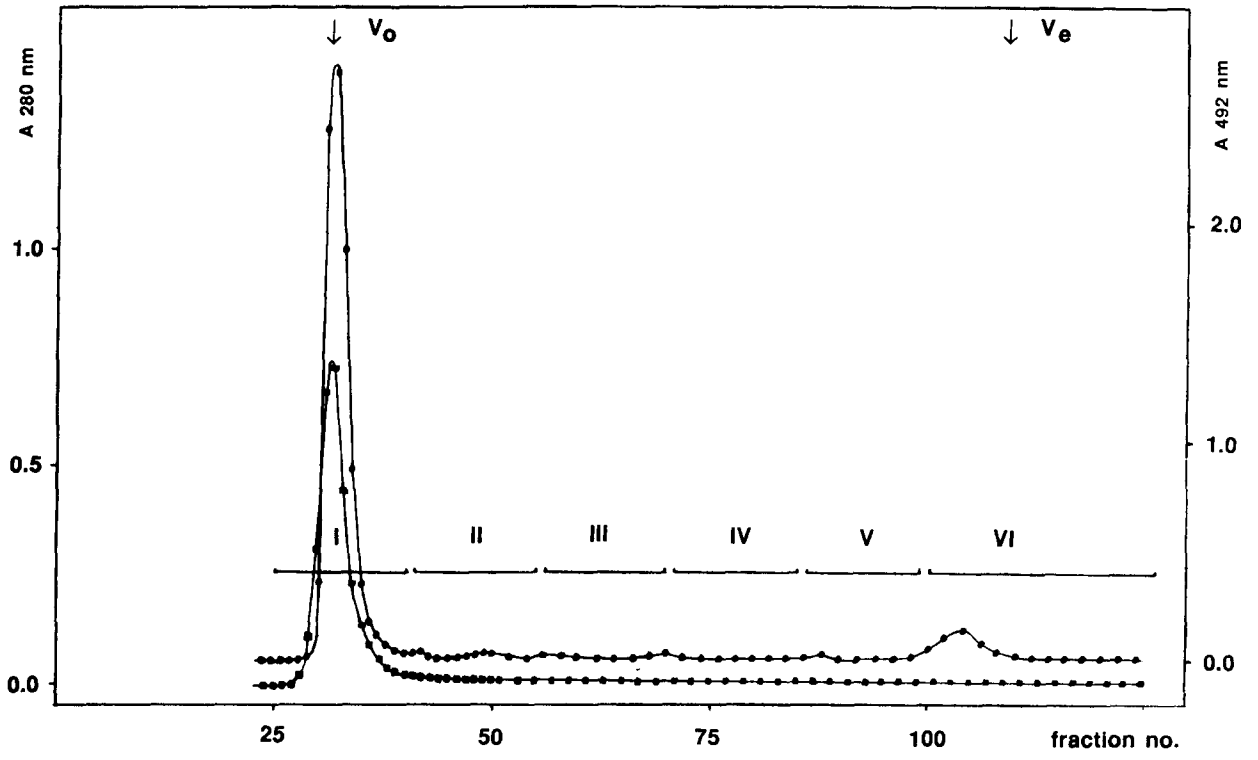


Fig. 1. Bio-Gel P-6 elution pattern of the supernatant of mucolipidosis I fibroblasts, using a 0.1 M ammonia-acetic acid buffer, pH 5.4, as eluent. The ultraviolet 280 nm profile is indicated by (■———■) and the results of the phenol-sulfuric acid determinations (492 nm), using 0.2 ml of the fractions, by (●———●). Fractions I–VI were pooled as indicated.

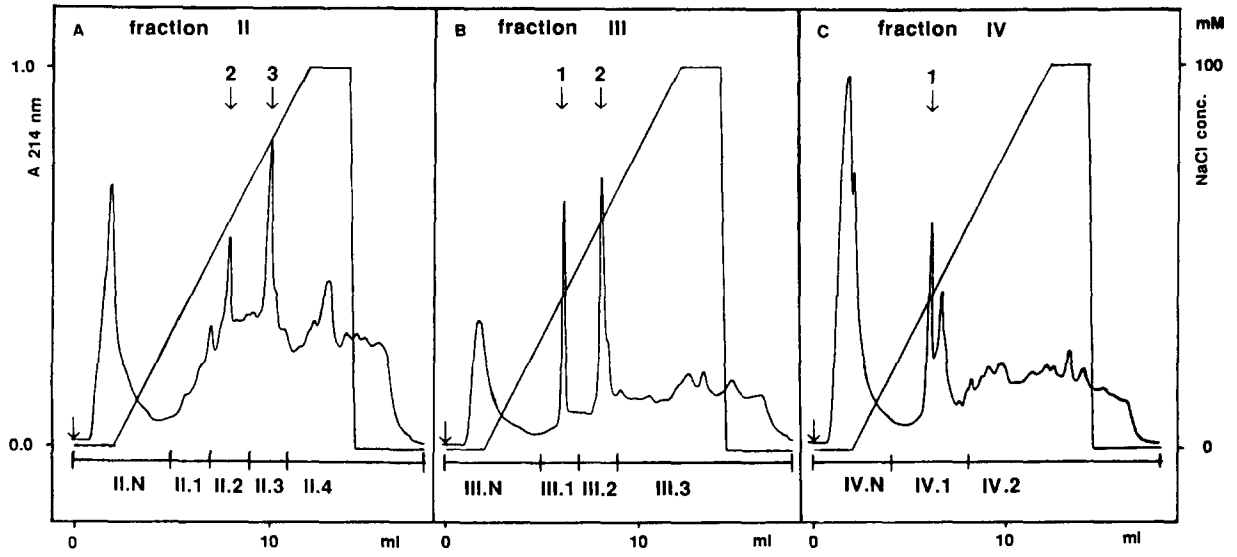


Fig. 2. Mono Q elution profiles of fractions II, III and IV at 214 nm, using a linear gradient of 0–100 mM NaCl in 10 ml water. The subfractions were pooled as indicated. The numerals above the arrows correspond to the number of sialic acid residues bound in reference compounds isolated from sialidosis urine [21].

amounts of mannose, galactose, GlcNAc and NeuAc, suggesting the occurrence of glycoproteins and glycogen fragments. Fractions II, III and IV showed the presence of mannose, galactose, GlcNAc and NeuAc. Fraction V contained no detectable amounts of sugar and fraction VI only glucose.

The sialic acid-containing fractions II, III and IV were further fractionated by anion-exchange chromatography on Mono Q (Fig. 2). Comparison of the retention times of the various peaks monitored at 214 nm with those of reference compounds isolated from sialidosis urine strongly suggested the presence of tri- and disialylated compounds in fraction II, di- and monosialylated compounds in fraction III and monosialylated compounds in fraction IV [21]. Pooled subfractions as indicated in Fig. 2A–C were subjected to sugar analysis. In Table I a summary of the sugar analysis data of the relevant Mono Q subfractions, together with the estimated amount of material, is given. The various results point to the presence of *N*-glycosidic type of carbohydrate chains with different types of branching and numbers of sialic acid residues. The additional fractions not included in Table I did not contain detectable amounts of sugar, except for the neutral fractions (N) which showed the presence of small amounts of glucose.

TABLE I

MOLAR CARBOHYDRATE COMPOSITION OF THE RELEVANT MONO Q SUBFRACTIONS OF THE SUPERNATANT OF MUCOLIPIDOSIS I FIBROBLASTS

Monosaccharide	II.2	II.3	III.1	III.2	IV.1
Man [a]	3.0	3.0	2.0	3.0	2.0
Gal	2.4	2.8	1.0	2.2	0.8
GlcNAc	3.6	3.9	1.5	3.1	1.4
NeuAc	1.6	2.9	0.6	2.0	0.8
Amount [b]	10	22	42	30	20

[a] Mannose taken as 3 or 2 as indicated.
[b] Estimated amount (nmol) of accumulated oligosaccharide.

The fractions II.2, II.3, III.1, III.2 and IV.1 were further investigated by 500-MHz ^{1}H-NMR spectroscopy. The structural-reporter-group ^{1}H-NMR data of these fractions, together with those of reference compounds [5,24], have been compiled in Table II.

In all fractions, heterogeneities in the type of NeuAc linkages ($\alpha 2 \rightarrow 3/\alpha 2 \rightarrow 6$) were observed

TABLE II

^{1}H-NMR CHEMICAL SHIFTS OF STRUCTURAL-REPORTER GROUPS OF CONSTITUENT MONOSACCHARIDES FOR THE SIALYLOLIGOSACCHARIDES ISOLATED FROM MUCOLIPIDOSIS I FIBROBLASTS, TOGETHER WITH THOSE FOR THE REFERENCE COMPOUNDS **21**, **29**, **35**, **38**, **39** AND **41** FROM REF. 24

Chemical shifts (δ) are expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate in ^2H_2O at 27 °C acquired at 500 MHz, but were actually measured by reference to internal acetone (δ 2.225). For numbering of the monosaccharides and complete structures, see text. In the table heading, the structures are represented by short-hand symbolic notation; for an explanation, see Scheme I.

Reporter group	Residue		Chemical shift in											
			21	**35**		III.1	**29**	**39**	**38**		III.2	**41**		II.3
H-1	GlcNAc	2α	5.208	5.206	5.206	5.206	5.216	5.213	5.213	5.211	5.211	5.215	5.211	5.211
		2β	4.72	4.72	n.d. [a]	n.d.	4.72	4.72	4.72	n.d.	n.d.	4.72	n.d.	n.d.
	Man	3α	4.788	4.79	n.d.	n.d.	4.786	4.780	4.775	n.d.	n.d.	4.77	n.d.	n.d.
		3β	4.785	4.78	n.d.	n.d.	4.777	4.767	4.763	n.d.	n.d.	4.76	n.d.	n.d.
		4α 4β	5.143	5.122	5.142	5.125	5.137	5.136	5.122 5.120	5.138	5.138	5.134	5.134	5.134
		4'α	–	–	–	–	4.952	4.924	4.923	4.955	4.931	4.943	4.939	4.918
		4'β	–	–	–	–		4.929	4.928					
	GlcNAc	5	4.602	4.579	4.599	n.d.	4.608	4.606	4.578	4.615	4.615	4.594	4.601	4.601
		5'	–	–	–	–	4.608	4.578	4.578	4.615	4.580	4.605	4.601	4.584
	Gal	6	4.446	4.544	4.443	4.54	4.445	4.444	4.544	4.443	4.443	4.443	4.445	4.445
		6'α	–	–	–	–	4.445	4.547	4.546	4.443	4.545	4.443	4.445	4.545
		6'β	–	–	–	–	4.449		4.547			4.447		
	GlcNAc	7	–	–	–	–	–	–	–	–	–	4.551	4.545	4.545
	Gal	8	–	–	–	–	–	–	–	–	–	4.545	4.545	4.545

H-2	Man	3α	4.244	4.245	4.240	4.240	4.266	4.263	4.256	4.254	4.254	4.228	4.223	4.223
		3β	4.233	4.234			4.255	4.251	4.244			4.217		
		4α	4.198	4.197	4.197	4.197	4.199	4.197	4.192	4.201	4.201	4.221	4.223	4.223
		4β	4.196											
		4′	–	–	–	–	4.121	4.119	4.117	4.119	4.119	4.119	4.121	4.121
H-3a	NeuAc	2 → 6 [b]	1.716	–	1.717	–	1.721	1.719	–	1.712	1.712	1.720	1.713	1.713
	NeuAc′	2 → 6	–	–	–	–	1.719	–	–	1.712	–	1.717	1.713	–
	NeuAc	2 → 3	–	1.799	–	1.794	–	–	1.797	–	–	–	–	–
	NeuAc′	2 → 3	–	–	–	–	–	1.800	1.799	–	1.797	–	–	1.799
	NeuAc*	2 → 3	–	–	–	–	–	–	–	–	–	1.802	1.799	1.799
H-3e	NeuAc	2 → 6 [b]	2.670	–	2.670	–	2.669	2.668	–	2.664	2.664	2.670	2.666	2.666
	NeuAc′	2 → 6	–	–	–	–	2.672	–	–	2.664	–	2.672	2.666	–
	NeuAc	2 → 3	–	2.758	–	2.758	–	–	2.758	–	–	–	–	–
	NeuAc′	2 → 3	–	–	–	–	–	2.756	2.758	–	2.750	–	–	2.754
	NeuAc*	2 → 3	–	–	–	–	–	–	–	–	–	2.757	2.754	2.754
NAc	GlcNAc	2α	2.043	2.043	2.042	2.042	2.063	2.061	2.061	2.059	2.059	2.062	2.060	2.060
		2β	2.041	2.041			2.060	2.057	2.058			2.059		
		5	2.069	2.049	2.071	2.045	2.071	2.069	2.049	2.067	2.067	2.067	2.068	2.068
		5′α	–	–	–	–	2.069	2.045	2.046	2.067	2.042	2.067	2.068	2.047
		5′β	–	–	–	–	2.066	2.044	2.043			2.065		
		7	–	–	–	–	–	–	–	–	–	2.074	2.075	2.075
	NeuAc		2.030	2.029	2.030	2.030	2.031 [c]	2.030 [c]	2.032 [c]	2.030 [c]	2.030 [c]	2.030 [d]	2.031 [d]	2.031 [d]

[a] n.d. = not determined.

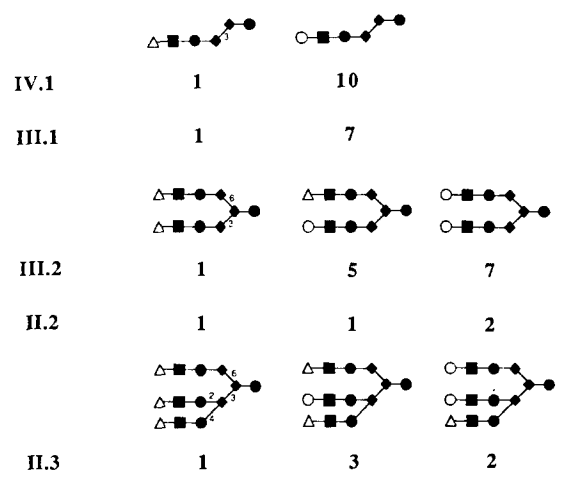
[b] NeuAc denotes the sialic acid linked to Gal-**6**, NeuAc′ the one linked to Gal-**6′** and NeuAc* the one linked to Gal-**8**.

[c] Protons stemming from two NAc groups.

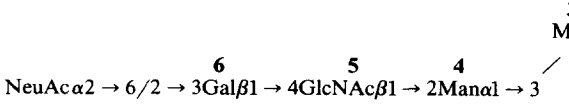
[d] Protons stemming from three NAc groups.

by ^{1}H-NMR spectroscopy. Although the presence of various structures differing merely in their NeuAc linkages was clear, analytical HPLC was used to confirm the presence of the isomers and to calculate their ratios more accurately. These ratios were obtained from the peak areas, which showed the same retention times as appropriate reference structures isolated from sialidosis urine and are presented in Scheme I. It should be noted that the amount of material in the investigated fractions was too small to permit structural analysis by ^{1}H-NMR spectroscopy after preparative HPLC separation. Therefore the data obtained from the ^{1}H-NMR spectra and the analytical HPLC profiles were combined to assess the structures presented below.

The ^{1}H-NMR spectrum of subfraction III.1 shows the presence of the following monosialylated monoantennary structures:



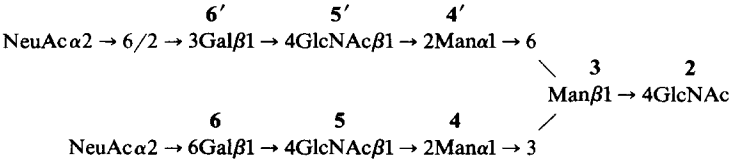
Scheme I. Structures and estimated relative amounts of sialyloligosaccharides, present in various fractions isolated from mucolipidosis I fibroblasts. △, NeuAcα2 → 3; ○, NeuAcα2 → 6; ■, Gal; ●, GlcNAc; ◆, Man.



The ^{1}H-NMR data can be explained completely in terms of a mixture of reference compounds **21** and **35** [5,24]. The heterogeneity with respect to the attachment of NeuAc (α2 → 3 or α2 → 6) is reflected by the specific chemical shift values of the NeuAc H-3e and H-3a signals (α2 → 6: H-3e, δ 2.670/H-3a, δ 1.717; α2 → 3: H-3e, δ 2.758/H-3a, δ 1.794), together with those of the H-1 signals of Man-**4** (α2 → 6: δ 5.142; α2 → 3: δ 5.125), the H-1 signals of Gal-**6** (α2 → 6: δ 4.443; α2 → 3: δ 4.54) and the NAc singlets of GlcNAc-**5** (α2 → 6: δ 2.071; α2 → 3: δ 2.045). Analytical HPLC confirmed the presence of both compounds, showing an α2 → 6/2 → 3 ratio of 7 : 1.

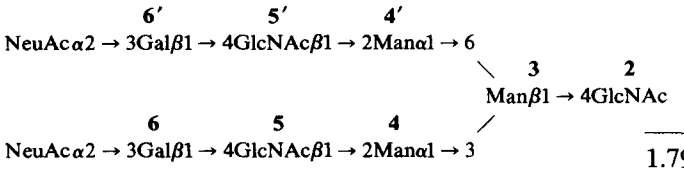
^{1}H-NMR spectroscopy of subfraction IV.1 (data not included in Table II) in combination with analytical HPLC reveals the occurrence of the same monosialylated monoantennary structures, whereby only the α2 → 6/2 → 3 ratio differs from that of fraction III.1, being 10 : 1.

The ^{1}H-NMR spectrum of subfraction III.2 provides evidence for the presence of the following disialylated diantennary structures:



The ^{1}H-NMR data match completely those of a mixture of reference compounds **29** and **39** [5,24]. The linkage heterogeneity of NeuAc in the Manα1 → 6 branch is reflected by the specific chemical shift values of the H-1 signals of Man-**4′** (α2 → 6: δ 4.955; α2 → 3: δ 4.931) and the NAc signals of GlcNAc-**5′** (α2 → 6: δ 2.067; α2 → 3: δ 2.042) in combination with the NeuAc H-3e and H-3a signals (α2 → 6: H-3e, δ 2.664/H-3a, δ 1.712; α2 → 3: H-3e, δ 2.750/H-3a, δ 1.797). Also the chem-

ical shifts of the H-1 signals of GlcNAc-**5′** and Gal-**6′** show both NeuAcα2 → 3 and α2 → 6 attachment. Analytical HPLC of III.2 confirmed the presence of both compounds in a ratio of 7 : 5. Furthermore, a small peak was observed with the same retention time as the reference diantennary compound with two NeuAc residues in α2 → 3 linkage (see Scheme I):



The ^{1}H-NMR spectrum of subfraction II.2 demonstrates the presence of disialylated diantennary structures, having heterogeneity with respect to the NeuAc linkages in both antennae. The chemical shift values of the H-1 signals of Man-**4** and Man-**4′** (α2 → 6: H-1 Man-**4**, δ 5.142/H-1 Man-**4′**, δ 4.95; α2 → 3: H-1 Man-**4**, δ 5.125/H-1 Man-**4′**, δ 4.92) in combination with the NeuAc H-3e and H-3a signals (α2 → 6: H-3e, δ 2.670/H-3a, δ 1.717; α2 → 3: H-3e, δ 2.754/H-3a, δ 1.799) indicate a heterogeneity of the NeuAc linkage type in both branches. This is confirmed by the H-1 signals and the NAc singlets of the GlcNAc-**5**/**5′** residues (α2 → 6: H-1, δ 4.60/NAc, δ 2.071; α2 → 3: H-1, δ 4.58/NAc-**5**, δ

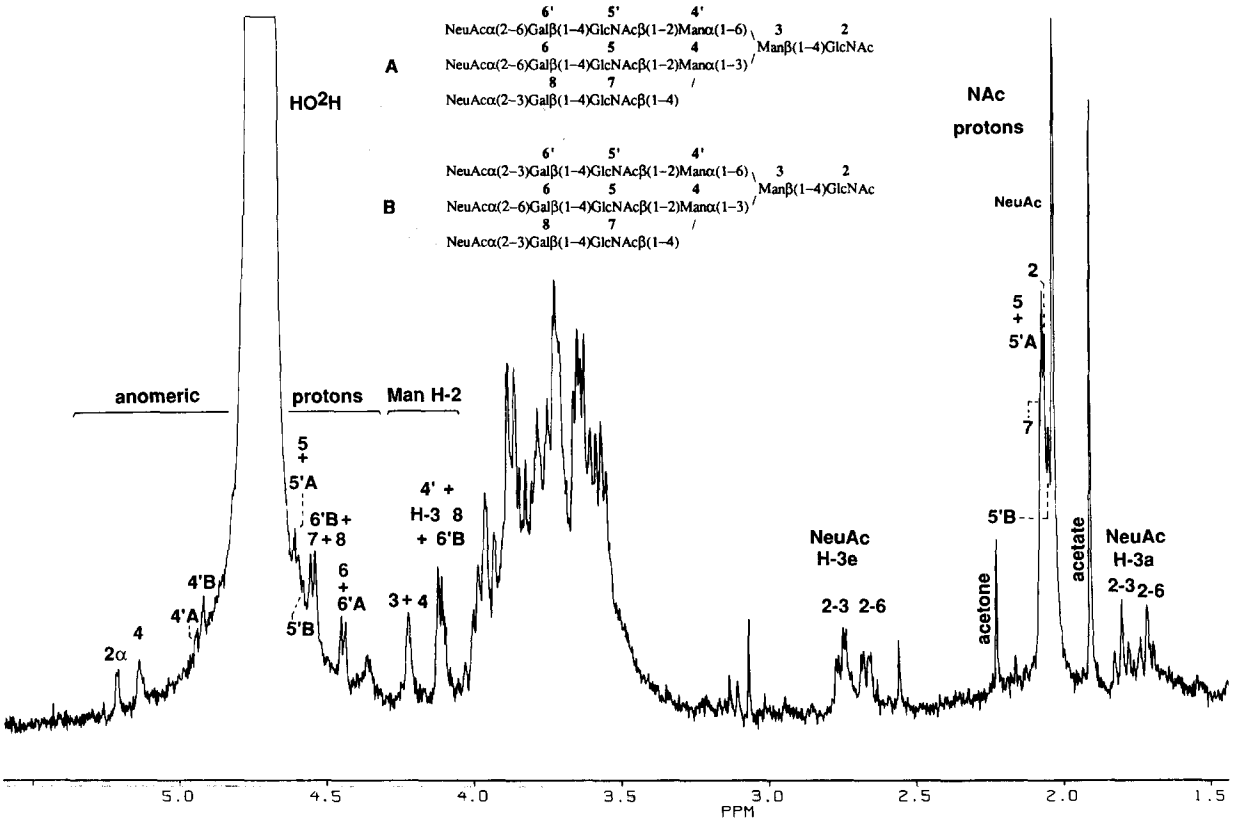
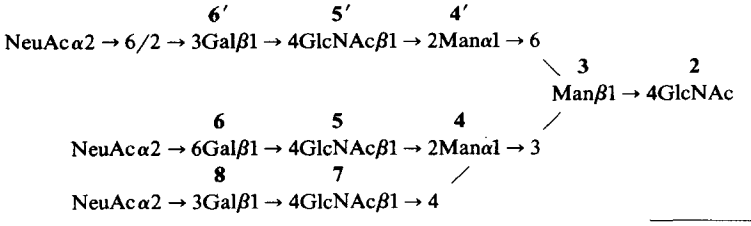


Fig. 3. 500-MHz ^{1}H-NMR spectrum (3600 scans) of fraction II.3 in ^2H_2O at 27 °C, obtained from mucolipidosis I fibroblasts after anion-exchange chromatography on Mono Q.

2.051/NAc-**5′**, δ 2.045) and the H-1 signals of the Gal-**6/6′** residues (α2 → 6: δ 4.445; α2 → 3: δ 4.546). The ^{1}H-NMR data (not included in Table II) are similar to those of a mixture of reference compounds **29**, **39** and **38** [5,24]. The analytical HPLC profile showed the presence of the same three structures as mentioned above for III.2, but now in ratio of 2 : 1 : 1 (Scheme I).

The ^{1}H-NMR spectrum of subfraction II.3 is given in Fig. 3 and shows the presence of the following trisialylated triantennary structures:



The chemical shifts of the NeuAc H-3a and H-3e signals show both α2 → 6 and α2 → 3 attachments (α2 → 6: H-3e, δ 2.666/H-3a, δ 1.713; α2 → 3: H-3e, δ 2.754/H-3a, δ 1.799). The NeuAc residue bound to Gal-**6′** is heterogeneous with respect to the linkage type, being α2 → 3 and α2 → 6. This is reflected in the chemical shift values of the H-1 signals of Man-**4′** (α2 → 6: δ 4.939; α2 → 3: δ 4.918) and the NAc signals of GlcNAc-**5′** (α2 → 6: δ 2.068; α2 → 3: δ 2.047). The Gal-**6′** and GlcNAc-**5′** H-1 signals confirm the NeuAc heterogeneity (α2 → 6: δ 4.445/δ 4.601; α2 → 3: δ 4.545/δ 4.584, respectively). The Gal-**6** is mainly α2 → 6-sialylated, as is deduced from the H-1 signal of Man-**4** (δ 5.134), in combination with the H-1 signals of Gal-**6** and GlcNAc-**5** (δ 4.445 and 4.601, respectively). Gal-**8** is exclusively α2 → 3-sialylated as can be concluded from the only and characteristic NAc signal of GlcNAc-**7** (δ 2.075) [24]. The chemical shift values of structure A from Fig. 3 are in accordance with those of reference compound **41** [24]. Structure B differs from structure A by one NeuAc, being bound α2 → 3 to Gal-**6′** instead of α2 → 6. The HPLC profile of II.3 revealed that the trisialylated triantennary structures occur in a ratio of 2 : 3. In addition, a peak with the same retention time as the triantennary reference structure with three α2 → 3-linked NeuAc residues was observed (see Scheme I).

Discussion

The increase of bound sialic acid in neuraminidase-deficient fibroblasts, as reported by several groups [10–13], is caused by the storage of completely sialylated oligosaccharides, derived from *N*-glycoproteins. The isolated structures are of the mono-, di- and triantennary *N*-acetyllactosamine type of oligosaccharides having GlcNAc-**2** at the reducing terminus. The observed heterogeneity with respect to the NeuAc linkages is consistent with the neuraminidase deficiency for both α2 → 3 and α2 → 6 linkages. The same types of structures have been found in sialidosis urine, but also partially sialylated structures have been isolated from this source [4,5,9]. The latter structures were not found in fibroblasts, which could be due to the limited amount of material available. On the other hand, it cannot be excluded that the urinary, partially sialylated structures have been formed during storage or working-up procedures. Scarce amounts of material prompted us to perform ^{1}H-NMR spectroscopy before HPLC separations were carried out. However, the combination of NMR spectroscopy with analytical HPLC turns out to be a sensitive and accurate method for the structural analysis of small amounts of heterogeneous mixtures of sialyloligosaccharides. It has to be noted that the insensitivity of the carbohydrate-detection method resulted in an inaccurate pooling of the Bio-Gel P-6 fractions. This accounts for the occurrence of monoantennary structures in fractions III and IV and of diantennary structures in fractions II and III.

The finding of GlcNAc-**2** at the reducing end indicates a very efficient removal of the peptide part and of the GlcNAc-**1** residue in the lysosomes of the fibroblasts. Previously an endoglucosaminidase splitting the *N*,*N*′-diacetylchitobiose

unit was believed to be responsible for this phenomenon [26], but recently indications have been found for another pathway. After the removal of the peptide moiety the asparagine residue is split off from the carbohydrate chain and then the GlcNAc-1 residue is cleft from the oligosaccharide [27,28].

The accumulated sialyloligosaccharides are most likely derived from intracellular metabolism of endogenous glycoproteins. The nature of the isolated structures is in agreement with naturally occurring human sialylated carbohydrate chains of glycoproteins. Moreover, a recent report shows the storage of sialic acid-containing material in mucolipidosis II fibroblasts. The latter material was formed after incubation with a radioactive precursor and was found in the trichloroacetic acid-soluble fraction [29]. Strikingly, part of the trisialylated triantennary compounds resembles the carbohydrate chains of fetuin [30–32], which was added to the culture medium. Therefore it cannot completely be excluded that part of the storage material is derived from fetuin or other glycoproteins present in the medium, which are digested by the lysosomes after endocytosis and of which the carbohydrate chains, due to the neuraminidase deficiency, cannot be broken down.

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