Eur. J. Biochem. *132*, 375 – 381 (1983)
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Primary Structure of Three Mannosyl-glycoasparagines and Nine Sialyl-oligosaccharides Isolated from the Urine of Two Patients with Gaucher's Disease (Infantile Form)

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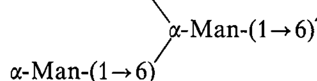
(Received November 23, 1982/January 19, 1983) – EJB 6246

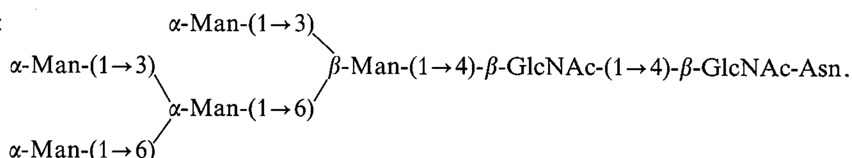
The urine of two patients with the infantile form of Gaucher's disease were analyzed for their carbohydrate content. Nine sialyl-oligosaccharides were isolated and characterized. Compounds with identical structures occur in the urine of patient with sialidosis. Furthermore, an abnormal excretion of mannosyl-glycoasparagines was observed. Three of these compounds were isolated and identified:

GP-I: α-Man-(1→6)-β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-Asn;

GP-III: α-Man-(1→3)[α-Man-(1→6)]α-Man-(1→6)-β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-Asn;



GP-IV: α-Man-(1→3)[α-Man-(1→6)]α-Man-(1→6)[α-Man-(1→3)]β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-Asn.



This carbohydrate material, which is found also in the spleen of the patients, probably originates from obstruction of lysosomal functioning by the accumulation of an excessive amount of glucocerebroside.

Gaucher's disease is an autosomal, recessively heritable inborn error of metabolism characterized by a deficiency in glucocerebrosidase [1], resulting in the accumulation of glucocerebroside in the spleen [2]. Three forms of the disease (infantile, juvenile and adult) can be distinguished on the basis of clinical parameters and age of the patients [3]. The infantile form is characterized by hepatosplenomegaly and neurological abnormalities during the first years of life.

Little is known about the biochemical or genetic basis of the variation observed among Gaucher's disease patients. There is no correlation between the clinical course of the disease and the levels of residual glucocerebrosidase activity, so it is quite possible that the clinical expression of Gaucher's disease depends upon factors different from the glucocerebrosidase deficiency. Several authors have reported an increase of lysosomal hydrolase activities, such as β-glucuronidase, acid phosphatase, both hexosaminidases A and B and β-galactosidase in spleen, liver or plasma of the patients [4, 5]. The origin of this phenomenon is not known, but Moffitt et al. [5] suggested that it may be due to an interaction of accumulated metabolites inside the lysosomes with cellular hydrolases. Some of these secondary changes in lysosomal enzyme activities might account for changes in the glycolipid or glycoprotein content of the tissue. Recently, De Gasperi et al. [6] described an increased amount of glycopeptide material in spleen of patients with Gaucher's disease.

These reports prompted us to investigate the urine of two patients with Gaucher's disease (infantile form), with regard to the content and the nature of carbohydrates related to glycoproteins. A preliminary report of the ^{1}H-NMR investigations of the urinary glycoasparagines has been published previously [7].

MATERIALS AND METHODS

Isolation of Carbohydrate Material

Urine of two siblings with the infantile form of Gaucher's disease was fractionated as described previously [8]: 10 l of urine were first demineralized in batches using Dowex 50 × 8 (25 – 50 mesh, H^+ form) and Dowex 1 × 8 (25 – 50 mesh, $HCOO^-$ form). The filtrate was concentrated to 1 l under reduced pressure and then applied to a column (5 × 40 cm) of

Abbreviations. Man, D-mannose; GlcNAc, *N*-acetyl-D-glucosamine; Gal, D-galactose; NeuAc, *N*-acetyl-D-neuraminic acid; Asn, L-asparagine; Asp, L-aspartic acid; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; GC-MS, gas-liquid chromatography coupled with mass spectrometry; NMR, nuclear magnetic resonance.

charcoal/celite. After washing with 2 l of distilled water, the adsorbed material was eluted with 5 l of 50% ethanol. The volume of the ethanol solution was reduced to about 100 ml by evaporation. Subsequently, the solution was applied to columns (2 × 30 cm) of Dowex 50 × 2 (200–400 mesh, H^+ form) and Dowex 1 × 2 (200–400 mesh, CH_3COO^- form) connected in series. After washing with 500 ml of distilled water, the columns were eluted separatly by a discontinuous gradient of 1–500 mM pyridine/acetic acid buffer (pH 5.5). Each fraction was then concentrated to 1 ml and analyzed by paper chromatography.

Chromatographic Procedures

Further separation and purification of oligosaccharides and glycopeptides was achieved by descending paper chromatography for 1–12 days on Whatman no. 3 paper, with ethyl acetate/pyridine/acetic acid/water (5:5:1:3, v/v) as solvent. Carbohydrate material was stained with the aniline oxalate reagent [9] or with a 1% ninhydrin solution in acetic acid/acetone (5:95, v/v).

Homogeneity of purified glycans was verified by thin-layer chromatography on Silicagel-60, precoated plates (Kieselgel 60, Merck, Darmstadt) with ethanol/*n*-butanol/pyridine/acetic acid/water (100:10:10:3:30, v/v) as solvent [10]. Sugar material was stained with a 0.2% orcinol solution (w/v) in sulfuric acid/water (20:80, v/v), followed by heating to 105 °C for 10 min.

Analytical Methods

The molar carbohydrate composition of the isolated compounds was determined according to Zanetta et al. [11], after methanolysis with 0.5 M HCl in methanol for 24 h at 80°C. Molar ratios of glucosamine and aspartic acid were determined using a Beckman amino-acid analyser, after hydrolysis of the glycoasparagines in 4 M HCl for 4 h.

Permethylation was carried out according to Hakomori [12], as modified by Björndal et al. [13]. After extraction with chloroform, the permethylated compounds were purified by passing through a Silicagel column (0.5 × 5 cm) (Kieselgel, 70–325 mesh, Merck, Darmstadt). The column was washed with 5 ml chloroform and eluted with 20 ml methanol/chloroform (5:95, v/v) [14]. The partially *O*-methylated monosaccharides obtained by methanolysis of the permethylated oligosaccharide-alditols (0.5 M HCl in methanol for 24 h at 80 °C) were analyzed by gas-liquid chromatography/mass spectrometry after acetylation in pyridine/acetic anhydride (1:1, v/v, 500 µl, 100 °C, 30 min) using a Girdell model 30 gas chromatograph (Suresnes, France) equipped with a capillary glass column (0.3 mm × 60 m) coated with Carbowax 20 M (column temperature: 130–225 °C with a temperature gradient of 2 °C/min; flow rate of carrier gas N_2: 20 ml/min) and subsequently identified by mass spectrometry (Riber-Mag 10-10 mass spectrometer, Rueil-Malmaison, France).

Prior to NMR spectroscopy, the glycoasparagines were repeatedly exchanged in D_2O (99.96 atom% D, Aldrich, Milwaukee), with intermediate lyophilization. For NMR spectral analyses approximately 2 mM solutions of the compounds in 0.4 ml D_2O were used. The 500-MHz ^{1}H-NMR spectra of the glycoasparagines were recorded on a Bruker WM-500 spectrometer, operating in the Fourier transform mode at a probe temperature of 300 K [7]. Chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (indirectly to acetone in D_2O: $\delta = 2.225$ ppm).

Table 1. *Molar carbohydrate composition of sialyl-oligosaccharides isolated from the urine of two patients with Gaucher's disease (infantile form)*
The molar ratios are based upon 2 or 3 mol of mannose/mol of oligosaccharide

Sialyl-oligosaccharide	Amount in normal urine	Amount in Gaucher's disease	Molar ratios Gal	Man	GlcNAc	NeuAc
	mg/ml					
1	0.1–0.3	5	1.05	2.0	1.94	0.87
2	0.2–0.3	16	0.95	2.0	2.04	0.94
3	<0.1	14	0.87	3.0	2.05	0.88
4	0.1–0.5	8	1.84	3.0	3.16	1.04
5	<0.1	16	1.74	3.0	3.05	1.07
6	<0.1	4	1.88	2.0	2.87	2.05
7	<0.1	5	1.94	3.0	3.07	2.08
8	0.1–1.0	8	1.87	3.0	2.94	1.72
9	–	22	2.01	3.0	2.94	1.92

Table 2. *Molar ratios of partially methylated methylglycosides derived from the methanolysates of the permethylated, reduced sialyl-oligosaccharides from the urine of patients with Gaucher's disease (infantile form)*
The molar ratios were determined on the basis of one residue of 1,3,5,6-tetra-*O*-Me-4-mono-*O*-Ac-GlcN(Me)Ac-itol; Me = methyl, Ac = acetyl

Partially methylated methylglycoside	Molar ratio in sialyl-oligosaccharide 1	2	3	4	5	6	7	8	9
2,3,4,6-Tetra-*O*-Me-Man	–	–	0.92	–	–	–	–	–	–
2,3,4,6-Tetra-*O*-Me-Gal	–	–	–	0.88	0.91	–	–	–	–
2,4,6-Tri-*O*-Me-3-mono-*O*-Ac-Man	1.04	1.09	–	–	–	0.88	–	–	–
3,4,6-Tri-*O*-Me-2-mono-*O*-Ac-Man	0.96	1.11	0.88	1.88	1.95	–	1.84	1.90	1.94
2,3,4-Tri-*O*-Me-6-mono-*O*-Ac-Gal	–	1.11	0.94	–	1.04	1.04	–	0.87	1.82
2,4,6-Tri-*O*-Me-3-mono-*O*-Ac-Gal	1.03	–	–	0.91	–	0.92	1.92	0.94	–
2,4-Di-*O*-Me-3,6-di-*O*-Ac-Man	–	–	0.88	0.94	1.11	–	1.12	1.03	1.00
3,6-Di-*O*-Me-2,4-di-*O*-Ac-Man	–	–	–	–	–	0.80	–	–	–
3,6-Di-*O*-Me-4-mono-*O*-Ac-GlcN(Me)Ac	0.84	0.92	0.90	1.94	1.90	1.84	1.80	1.70	1.96
1,3,5,6-Tetra-*O*-Me-4-mono-*O*-Ac-GlcN(Me)Ac-itol	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4,7,8,9-Tetra-*O*-Me-Neu(Me)Ac	0.70	0.76	0.80	0.75	0.80	1.55	1.60	1.54	1.80

Fig. 1. *Paper chromatogram of urinary sialyl-oligosaccharides from a patient with Gaucher's disease (infantile form).* The sialyl-oligosaccharides were eluted from a Dowex 1 × 2 column (200 – 400 mesh, CH_3COO^- form) with a discontinuous gradient of pyridine/acetic acid buffer (pH 5.5) from 1 mM to 500 mM (1 : 1 mM, 2 : 2 mM, 3 : 5 mM, 4 : 10 mM, 6 : 50 mM, 7 : 100 mM, 8 : 200 mM, 9 : 500 mM). Solvent: ethyl acetate/pyridine/acetic acid/water (5 : 5 : 1 : 3). Time of migration: over night

Resolution enhancement of the spectra was achieved by Lorentzian to Gaussian transformation according to Ernst [15].

RESULTS

Characterization of Sialyl-oligosaccharides

The sialyl-oligosaccharides eluted from the Dowex 1 × 2 column were qualitatively analyzed by paper chromatography. The chromatographic pattern (Fig. 1) was identical to that obtained for sialidosis urine [16]. The levels of these oligosaccharides are significantly increased as compared to normal urine (see Table 1); however, they are considerably lower than those in the urine of sialidosis patients [16].

The nine major sialyl-oligosaccharides were isolated by preparative paper chromatography and characterized by methylation analysis. The carbohydrate compositions (Table 1) and the molar ratios of the partially methylated methyl-glycosides derived from the permethylated reduced oligosaccharides (Table 2) indicate that the oligosaccharide structures are most probably identical to those described to occur in urine of sialidosis patients. The structures of the nine major sialyl-oligosaccharides present in Gaucher's disease urine, are listed in Fig. 2.

Characterization of Glycoasparagines

Paper chromatography of the fractions eluted from the Dowex 50 × 2 column (Fig. 3) showed the occurrence of

```
1 α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
                                                      \
                                                       β-Man-(1→4)-GlcNAc
2 α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
                                                      \
                                                       β-Man-(1→4)-GlcNAc
3 α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
                                                      \
                                                       β-Man-(1→4)-GlcNAc
                                                      /
                                           α-Man-(1→6)

4 α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
                                                      \
                                                       β-Man-(1→4)-GlcNAc
                                                      /
            β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)

5 α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)
                                                      \
                                                       β-Man-(1→4)-GlcNAc
                                                      /
            β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)

6 α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→4)
                                          \
                                           β-Man-(1→3)
                                          /           \
  α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)             β-Man-(1→4)-GlcNAc

7 α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-β-Man-(1→3)
                                                      \
                                                       α-Man-(1→4)-GlcNAc
                                                      /
  α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-β-Man-(1→6)

8 α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-β-Man-(1→3)
                                                      \
                                                       α-Man-(1→4)-GlcNAc
                                                      /
  α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→2)-β-Man-(1→6)

9 α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-β-Man-(1→3)
                                                      \
                                                       α-Man-(1→4)-GlcNAc
                                                      /
  α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-β-Man-(1→6)
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Fig. 2. *Structures of the nine major sialyl-oligosaccharides isolated from the urine of a patient with Gaucher's disease (infantile form)*

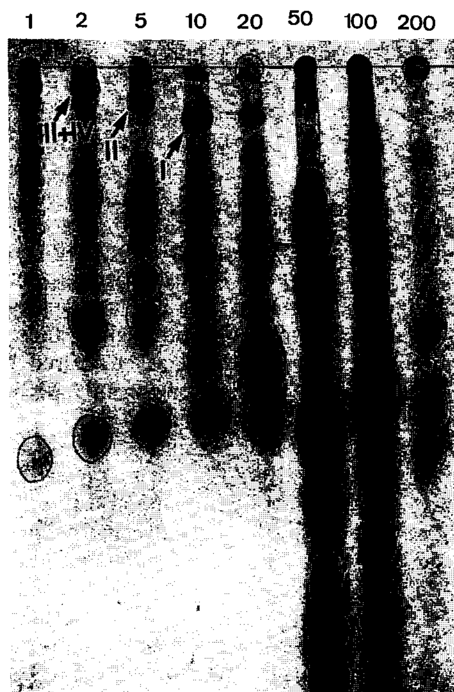


Fig. 3. *Paper chromatogram of urinary glycoasparagines from a patient with Gaucher's disease (infantile form).* The glycoasparagines were eluted from a Dowex 50 × 2 column (200–400 mesh, H^+ form) with a discontinuous gradient of pyridine/acetic acid buffer (pH 5.5) from 1 mM to 200 mM, as indicated at the top. Time of migration: over night

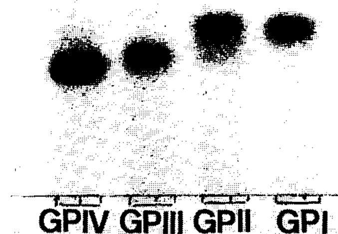
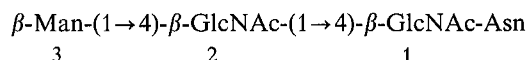


Fig. 4. *Thin-layer chromatogram of purified glycoasparagines isolated from the urine of a patient with Gaucher's disease (infantile form).* Solvent: ethanol/*n*-butanol/pyridine/acetic acid/water (100 : 10 : 10 : 3 : 30, v/v)

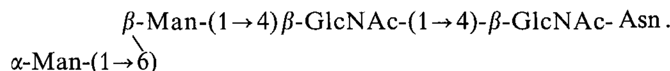
several glycoasparagines, which have not been observed in normal urine. Glycoasparagine GP-I, eluted with 10 mM pyridine/acetate, was isolated by paper chromatography and its purity was verified by TLC. Glycoasparagine GP-II, eluted with 5 mM pyridine acetate, was found to be a mixture of several components, so far unresolvable by paper chromatography or TLC. Glycoasparagines GP-III and GP-IV, eluted with 2 mM pyridine acetate, were isolated by paper chromatography performed during 6 days and also their purity was confirmed by TLC (see Fig. 4). The fraction eluted with 1 mM pyridine/acetate contained a mixture of more complex glycoasparagines which has not yet been resolved by paper chromatography.

The carbohydrate compositions and the molar ratios of the partially methylated methylglycosides present in the methanolysates of the permethylated glycopeptides GP-I, -III and -IV are given in Table 3.

GP-I. The methylation studies in combination with the molar carbohydrate composition (Table 3) demonstrate that GP-I contains a terminal mannose residue, whereas the other constituent monosaccharides are monosubstituted. Therefore, GP-I is a linear glycoasparagine. The 500-MHz ^{1}H-NMR spectrum of GP-I [7] reveals the sequence of the monosaccharides and the configuration of their glycosidic linkages. The presence of the structural element



can readily be inferred from comparison of the chemical shifts of the structural reporter groups of these residues (for GlcNAc-1, δ H-1 = 5.07 ppm and δ NAc = 2.014 ppm; for GlcNAc-2, δ H-1 = 4.618 ppm and δ NAc = 2.076 ppm; for Man-3, δ H-1 = 4.767 ppm, $J_{1,2}$ = 0.6 Hz) with those of numerous glycopeptides containing *N*-glycosidic carbohydrate chains, published so far [17–22]. Both from methylation analysis (Table 3) as well as from the ^{1}H-NMR spectral data (for Man-4′, δ H-1 = 4.915 ppm, $J_{1,2}$ = 1.8 Hz and δ H-2 = 3.968 ppm, while for Man-3 δ H-2 = 4.080 ppm) it is evident that the terminal mannose residue is α-(1→6)-linked to mannose-3 (cf. [17, 22, 23]). This affords the following structure for GP-I:



GP-III. The results of sugar and methylation analysis of GP-III (Table 3) show that two out of the four constituting mannose residues occupy terminal positions. Further, the presence of a di-3,6-substituted mannose and that of a mono-6-substituted mannose residue is indicated (see also Fig. 5). Assuming GP-III to possess the usual *N,N′*-diacetylchitobiose unit linked to asparagine, in principle two structures can be proposed on the basis of sugar and methylation analyses, namely:

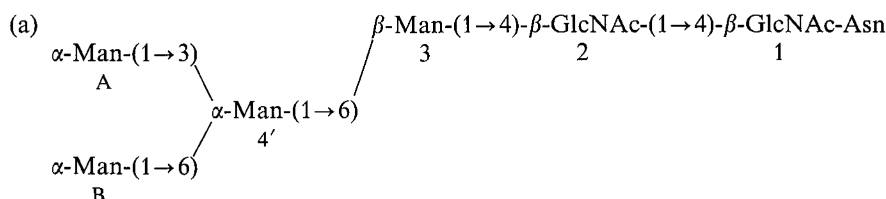
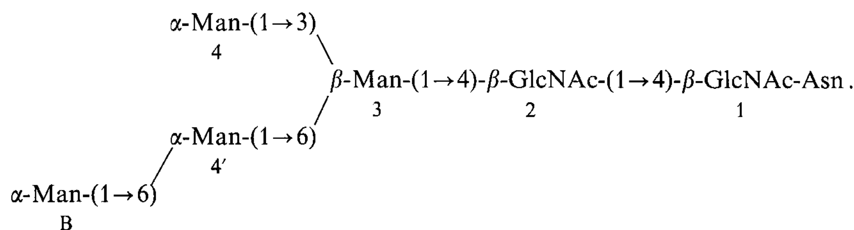


Table 3. *Molar ratios of constituent monosaccharides, and of partially methylated methylglycosides, after methylation analysis, of the major glycoasparagines from the urine of two patients with Gaucher's disease (infantile form)*
The carbohydrate and amino acid compositions are based upon 1 mol of aspartic acid/mol of glycoasparagine, after hydrolysis. The methylation analysis data are based upon 1, 2 or 3 mol of 2,3,4,6-tetra-*O*-methyl-mannoside/mol of glycoasparagine

Glyco-asparagine	Amount	Carbohydrate and amino acid composition			Methylation analysis data				
		Man	GlcNAc	Asp	2,3,4,6-tetra-*O*-Me-Man	2,3,4-tri-*O*-Me-6-mono-*O*-Ac-Man	3,4,6-tri-*O*-Me-2-mono-*O*-Ac-Man	2,4-di-*O*-Me-3,6-di-*O*-Ac-Man	3,6-di-*O*-Me-4-mono-*O*-Ac-GlcN(Me)Ac
	mg/l								
GP-I	1.2	2.00	1.94	1.00	1.00	1.25	0.12[a]	0.11[a]	1.45
GP-III	1.7	3.80	1.88	1.00	2.00	–	–	1.05	1.48
GP-IV	2.5	4.75	1.92	1.00	3.00	0.15[a]	0.17[a]	1.90	1.50

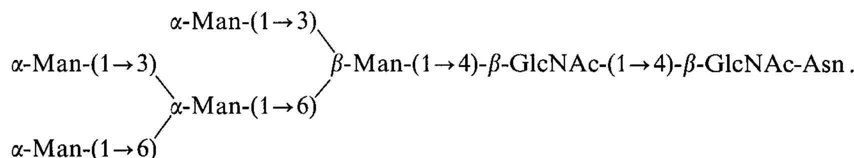
[a] Attributed to contaminants

and



Comparison of the 500-MHz ^{1}H-NMR spectrum of GP-III with that of GP-I (see [7]) confirms the common presence of the $\overset{3}{\text{Man}}\rightarrow\overset{2}{\text{GlcNAc}}\rightarrow\overset{1}{\text{GlcNAc}}$ unit. Furthermore, the characteristically shaped H-2 resonance of the β-linked mannose-3 is found again at $\delta = 4.076$ ppm; as for GP-I, this means that mannose-3 bears no substituent at C-3 [17, 22, 23]. In this way, structure (a) can be selected to be the correct one for GP-III. The branching mannose-4′ residue is characterized by δ H-1 = 4.870 ppm and δ H-2 = 4.140 ppm. The terminal α-(1→3) and α-(1→6)-linked mannose residues, designated A and B, respectively, show the chemical shift values which are typical for terminal mannose residues linked in this way (for Man-A, δ H-1 = 5.076 ppm and δ H-2 = 4.064 ppm; for Man-B, δ H-1 = 4.909 ppm and δ H-2 = 3.988 ppm).

GP-IV. Sugar analysis of GP-IV (Table 3) shows that this compound contains an additional Man residue as compared to GP-III. From the methylation analysis data (Table 3, Fig. 5) it is evident that this additional mannose occupies a terminal position. Moreover, the mono-6-substituted Man residue in GP-III is no longer found in GP-IV. It is replaced by a second di-3,6-substituted mannose. Therefore, the following structure can be proposed for GP-IV:



This structure is confirmed by the 500-MHz ^{1}H-NMR spectral data of GP-IV [7]. In particular, the change in chemical shift of H-2 of mannose-3 from $\delta = 4.076$ ppm to 4.251 ppm, in conjunction with the presence of an additional set of H-1 and H-2 signals for a terminal α-(1→3)-linked mannose residue (for Man-4, δ H-1 = 5.099 ppm; δ H-2 = 4.077 ppm; for Man-A δ H-1 = 5.093 ppm; δ H-2 = 4.066 ppm), are decisive in this respect for the attachment of mannose-4 in α-(1→3)-linkage to mannose-3 in the structure of GP-III.

Glycoasparagines GP-I and GP-IV are contaminated with one or more minor components as is evident from the results of methylation analysis (see Fig. 5 and Table 3). The structure of one of the contaminants of GP-IV, which represent about 10% of the mixture, was established by methylation analysis and NMR spectroscopy [7] to be the same as that of the major component of GP-I.

The structures of the major glycoasparagines that were found to occur in the urine of patients with the infantile form of Gaucher's disease, are compiled in Fig. 6.

DISCUSSION AND CONCLUSION

The structures of major sialyl-oligosaccharides and glycoasparagines accumulated in the urine of the two patients with Gaucher's disease are given in Fig. 2 and 6. From a quantitative point of view, their excretion is not comparable with that observed in other lysosomal storage diseases such as sialidosis, GM_1-gangliosidosis or mannosidosis [16, 24, 25]. The quantities of glycoasparagines are very low and cannot be detected by direct TLC of urine. Nevertheless, their excretion is significant, because these glycopeptides have never previously been described in either normal urine or urine from other types

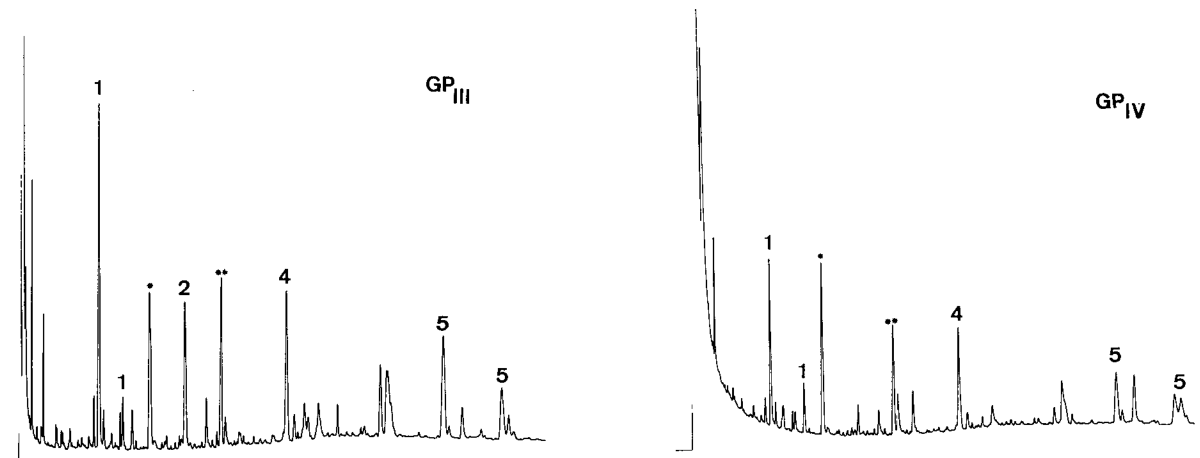


Fig. 5. *GLC analysis of partially methylated monosaccharide derivatives present in the methylanolysates of the permethylated glycoasparagines (III and IV).* (1) 2,3,4,6-Tetra-*O*-Me-Man; (2) 2,3,4-tri-*O*-Me-Man; (3) 3,4,6-tri-*O*-Me-Man; (4) 2,4-di-*O*-Me-Man; (5) 3,6-di-*O*-Me-Glc(Me)Ac; ** fatty acids. The retention time is plotted horizontally against the detector response

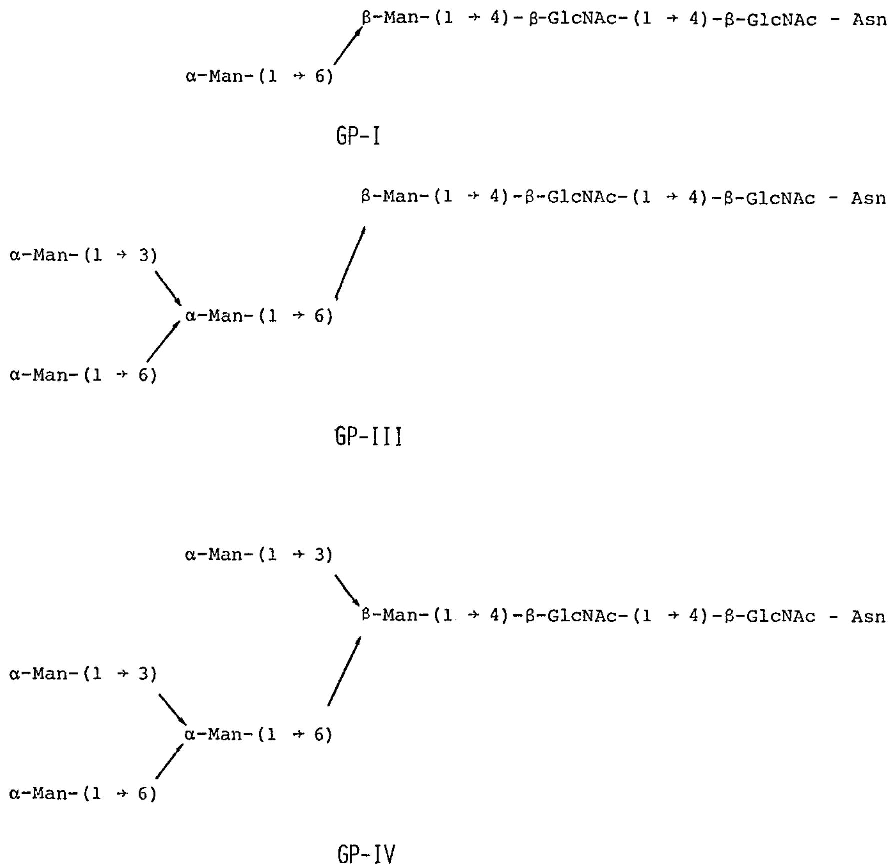


Fig. 6. *Structures of the major glycoasparagines present in the urine of a patient with Gaucher's disease (infantile form)*

of lysosomal diseases. The same glycopeptides have been found to accumulate in the spleen of a third patient (Michalski and Strecker, unpublished results). These findings are in line with the observation of de Gasperi et al. [6].

The structure of the oligosaccharides and glycopeptides strongly suggest that they are related to incomplete catabolism of *N*-acetyllactosaminic and high-mannose types of carbohydrate chains of glycoproteins. As the α-neuraminidase activity was found to be normal, and the α-mannosidase activity only slightly increased in the serum and fibroblasts of the two siblings, the occurrence of this accumulated material is not due to a primary enzymatic defect of lysosomal hydrolases, but must be considered to originate from obstruction of lysosomal functioning, probably due to the accumulation of glucocerebroside, which may reach 1% in weight in the spleen, or an interaction of this lipidic material with lysosomal hydrolases inside the lysosome.

The investigation was supported by the *Centre National de la Recherche Scientifique (Laboratoire Associé 217: Biologie physicochimique et moléculaire des glucides libres et conjugués)*, the *Institut National de la Santé et de la Recherche Médicale* (contract 78.1.0523), The Netherlands Foundation for Chemical Research (SON) with financial aid from The Netherlands

Organization for the Advancement of Pure Research (ZWO), and The Netherlands Foundation for Cancer Research (KWF, grant UUKC-OC 79-13).

REFERENCES

1. Brady, R. O., Kanfer, J. N. & Shapiro, D. (1965) *Biochem. Biophys. Res. Commun. 18*, 221–225.
2. Patrick, A. D. (1965) *Biochem. J. 97*, 17c.
3. Brady, R. O. (1978) in *The Metabolic Basis of Inherited Disease* (Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S., eds) pp. 73–76, McGraw Hill Book Company, New York.
4. Ockerman, P. A. & Kohlin, P. (1969) *Clin. Chem. 15*, 61–64.
5. Moffitt, K. D., Chambers, J. P., Diven, W. F., Glew, R. H., Wenger, D. A. & Farrell, D. F. (1978) *Arch. Biochem. Biophys. 190*, 247–260.
6. De Gasperi, R., Rapelli, S., Li, Y.-T. & Berra, B. (1981) *Abstracts of the Third E.S.G.L.D. Workshop*, Woudschoten, Zeist.
7. Van Halbeek, H., Dorland, L., Veldink, G. A., Vliegenthart, J. F. G., Michalski, J.-C., Montreuil, J., Strecker, G. & Hull, W. E. (1980) *FEBS Lett. 121*, 65–70.
8. Strecker, G. & Lemaire-Poitau, A. (1977) *J. Chromatogr. 143*, 553–569.
9. Partridge, S. M. (1950) *Biochem. Soc. Symp. 3*, 52–61.
10. Bayard, B., Kerckaert, J. P., Roux, D. & Strecker, G. (1979) *Protides Biol. Fluids Proc. Colloq. 27*, 153–156.
11. Zanetta, J. P., Breckenridge, W. C. & Vincendon, G. (1972) *J. Chromatogr. 69*, 291–304.
12. Hakomori, S. I. (1964) *J. Biochem. (Tokyo) 55*, 205–208.
13. Björndal, H., Hellerquist, C. G., Lindberg, B. & Svensson, S. (1970) *Angew. Chem. Int. Ed. Engl. 9*, 610–619.
14. Yamashita, K., Tachibana, Y. & Kobata, A. (1976) *Arch. Biochem. Biophys. 174*, 582–591.
15. Ernst, R. R. (1966) in *Advances in Magnetic Resonance* (Waugh, J. S., ed.) vol. 2, pp. 1–135, Academic Press, London, New York.
16. Strecker, G., Peers, M.-C., Michalski, J.-C., Hondi-Assah, T., Fournet, B., Spik, G., Montreuil, J., Farriaux, J. P., Maroteaux, P. & Durand, P. (1977) *Eur. J. Biochem. 75*, 391–403.
17. Vliegenthart, J. F. G., Van Halbeek, H. & Dorland, L. (1981) *Pure Appl. Chem. 53*, 45–77.
18. Fournet, B., Montreuil, J., Strecker, G., Dorland, L., Haverkamp, J., Vliegenthart, J. F. G., Binette, J. P. & Schmid, K. (1978) *Biochemistry, 17*, 5206–5214.
19. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Schmid, K., Montreuil, J., Fournet, B. & Hull, W. E. (1980) *FEBS Lett. 114*, 11–16.
20. Van Halbeek, H., Dorland, L., Vliegenthart, J. F. G., Montreuil, J., Fournet, B. & Schmid, K. (1981) *J. Biol. Chem. 256*, 5588–5590.
21. Dorland, L., Van Halbeek, H., Vliegenthart, J. F. G., Lis, H. & Sharon, N. (1981) *J. Biol. Chem. 256*, 7708–7711.
22. Strecker, G., Fournet, B., Montreuil, J., Dorland, L., Haverkamp, J., Vliegenthart, J. F. G. & Dubesset, D. (1978) *Biochimie (Paris) 60*, 725–734.
23. Herlant-Peers, M.-C., Montreuil, J., Strecker, G., Dorland, L., Van Halbeek, H., Veldink, G. A. & Vliegenthart, J. F. G. (1981) *Eur. J. Biochem. 117*, 291–300.
24. Yamashita, K., Ohkura, K., Okada, S., Yabuuchi, K. & Kobata, A. (1981) *J. Biol. Chem. 256*, 4789–4798.
25. Strecker, G., Fournet, B., Bouquelet, S., Montreuil, J., Dhondt, J. L. & Farriaux, J. P. (1976) *Biochimie (Paris) 58*, 579–586.

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