*J. Inher. Metab. Dis.* 14 (1991) 730–740


# A Comparative Study of Sialyloligosaccharides Isolated from Sialidosis and Galactosialidosis Urine

J. VAN PELT[1]*, J. P. KAMERLING[1], H. D. BAKKER[2] and J. F. G. VLIEGENTHART[1]

[1] *Department of Bio-Organic Chemistry, University of Utrecht, Transitorium III, Padualaan 8, NL-3584 CH Utrecht, The Netherlands;* [2] *Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands*

**Summary:** Sialic acid-containing carbohydrates were isolated from sialidosis urine by a combination of gel-filtration on Bio-Gel P-6 and medium-pressure anion-exchange chromatography on Mono Q. The Mono Q fractions were subjected to 500-MHz $^1$H-NMR spectroscopy, sugar analysis and analytical HPLC on Lichrosorb-$NH_2$. These methods indicated the presence of various *N*-acetyllactosamine type sialyloligosaccharides differing from each other in branching pattern and sialic acid linkage types. Among the structures were fully and partially sialylated mono-, di-, tri- and tetra-antennary compounds. A comparison with the results from galactosialidosis urine indicated that essentially the same carbohydrates were present in both urines, but that the relative amounts of the various sialyloligosaccharides differ to some extent. Sialidosis urinary oligosaccharides contained relatively more α2-6 linked sialic acid than oligosaccharides from galactosialidosis urine. It could be concluded that the additional β-galactosidase deficiency in galactosialidosis did not influence the nature of the excreted material and that the sialidase deficiency determined completely the defective catabolism of glycoproteins in both sialidosis and galactosialidosis.

The lysosomal storage diseases sialidosis (McKusick 25655) and galactosialidosis (McKusick 25654) are characterized by a sialidase (EC 3.2.1.18) and a combined sialidase/β-galactosidase (EC 3.2.1.23) deficiency, respectively (Lowden and O'Brien, 1979; Cantz, 1982). The latter combined enzyme deficiency is caused by an absence of a 32-kD protective protein, which contributes activity to sialidase and protects β-galactosidase against proteolytic degradation (Hoogeveen *et al.*, 1981; D'Azzo *et al.*, 1982; Van Diggelen *et al.*, 1982; Verheijen *et al.*, 1985). The sialidase deficiency in both diseases is reflected in the excretion of sialic acid-containing *N*-acetyllactosamine

*Correspondence: Dr J. van Pelt, De Wever Hospital, Klin. Chem. Lab., Henri Dunantstr. 5, NL-6419 PC Heerlen, The Netherlands

*MS received 12.2.91 Accepted 11.3.91*

type carbohydrates of which the structure of several mono- and disialylated compounds have been elucidated (Strecker *et al.*, 1977; Dorland *et al.*, 1978; Koseki and Tsurumi, 1978, 1979; Kuriyama *et al.*, 1981, 1985). However, the importance of the β-galactosidase deficiency in galactosialidosis with respect to glycoprotein catabolism is still not clear. In a previous study, the isolation and characterization of 21 different sialyloligosaccharides, including fully sialylated tri- and tetra-antennary structures, from galactosialidosis urine was described (Van Pelt *et al.*, 1989). To investigate the presence and relative amounts of sialyloligosaccharides in sialidosis urine, this urine was subjected to the same chromatographic procedure as applied for galactosialidosis urine.

## METHODS

*Urine:* Sialidosis urine was obtained from a dysmorphic sialidosis patient (E.V.) with onset at infantile age. The urine was chilled after collection and stored at −20°C with a few drops of chloroform as preservative.

*Chromatographic procedures:* Chromatographic procedures were performed as described in detail for galactosialidosis urine (Van Pelt *et al.*, 1989). A Bio-Gel P-6 column (120 × 2.5 cm, 200–400 mesh, Bio-Rad) was used for gel permeation chromatography. The column was eluted with 0.1 mol/L ammonia–acetic acid buffer, pH 5.5, at 4°C and at a flow rate of 21 ml/h. The elution profiles were obtained by hexose measurements at 492 nm with the phenol/sulphuric acid method (Dubois *et al.*, 1956) and by sialic-acid determinations at 570 nm with the orcinol/conc. $HCl/FeCl_3$ method (Schauer and Corfield, 1982). Medium-pressure anion-exchange chromatography was performed on a Mono Q column (HR 5/5, Pharmacia), eluted with a gradient of 0–100 mmol/L NaCl in 10 ml water. The eluate (flow rate 2.0 ml/min) was monitored at 214 nm (Van Pelt *et al.*, 1987). Analytical high-performance liquid chromatography was carried out by injecting a small part (1/50–1/250) of the sample dissolved in the eluent on a Lichrosorb-10-$NH_2$ column (250 × 4.6 mm, Chrompack). The monosialylated compounds were eluted isocratically with a mixture of acetonitrile–30 mmol/L $KH_2PO_4$, pH 4.7 (65:35, v/v). The higher-sialylated compounds were eluted isocratically with a mixture of acetonitrile–30 mmol/L $KH_2PO_4$ – $K_2HPO_4$, pH 7.0 (62.5:37.5, v/v). The eluate (flow rate 2.0 ml/min) was monitored at 205 nm.

*Sugar analysis:* Sugar analysis was carried out by gas–liquid chromatography on a capillary CP-Sil 5 WCOT fused silica column (25 m × 0.32 mm, Chrompack) using a Varian Aerograph 3700 gas chromatograph. The trimethylsilylated methyl glycosides were prepared by methanolysis (1.0 mol/L methanolic HCl, 24 h, 85°C), *N*-reacetylation and trimethylsilylation (Kamerling and Vliegenthart, 1982).

*500-MHz $^1$H-NMR spectroscopy:* Sialyloligosaccharides were repeatedly exchanged in $^2H_2O$ (99.96 atom% $^2$H, Aldrich) with intermediate lyophilization. Resolution-enhanced 500-MHz $^1$H-NMR spectra were recorded at $p^2H \sim 7$ on a Bruker

WM-500 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands) operating at a probe temperature of 27°C. Chemical shifts ($\delta$) are expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate, but were actually measured by reference to internal acetone ($\delta$ 2.225 ppm in $^2H_2O$ at 27°C) (Vliegenthart *et al.*, 1983).

## RESULTS

Sialidosis urine (40 ml) was lyophilized and, after resuspension in 7.5 ml of water, subjected to Bio-Gel P-6 chromatography (Figure 1). In the pooled fractions 1–5 the total amounts of sialic acid and hexose were 21 and 36 mg, respectively. The sugar analysis data of 1–5, given in Table 1, indicated the presence of sialic acid-containing carbohydrates derived from *N*- and *O*-glycosidic types of chains. The molar ratios

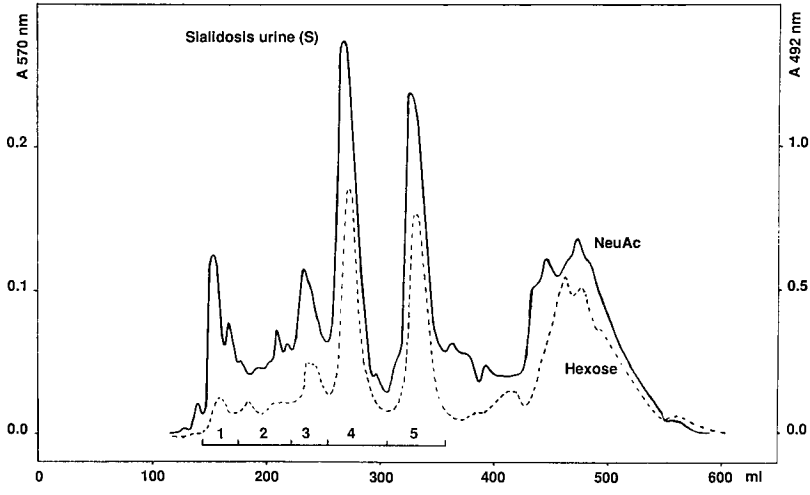



**Figure 1** Bio-Gel P-6 chromatography of sialidosis urine. The hexose profile (492 nm; -----) and the sialic acid profile (570 nm; ———) were obtained by using 50-$\mu$l and 100-$\mu$l aliquots of the 5.0-ml fractions, respectively. Fractions 1–5 were pooled as indicated

**Table 1 Molar carbohydrate composition of the Bio-Gel P-6 fractions 1–5 relative to Man = 3.00**

| | *Fraction* | | | | |
|---|---|---|---|---|---|
| *Monosaccharide* | 1 | 2 | 3 | 4 | 5 |
| Fuc | 1.2 | 0.7 | 0.2 | — | 0.2 |
| Man | 3 | 3 | 3 | 3 | 3 |
| Gal | 10.0 | 5.6 | 3.3 | 2.2 | 1.8 |
| GalNAc | 3.5 | 1.2 | 0.5 | — | 0.5 |
| GlcNAc | 7.8 | 6.3 | 4.5 | 3.2 | 3.0 |
| NeuAc | 9.8 | 4.7 | 3.4 | 2.0 | 2.3 |

of the constituent monosaccharides in the various Bio-Gel fractions of sialidosis urine corresponded to the findings in galactosialidosis urine.

500-MHz $^1$H-NMR spectroscopy of fractions 1–5 showed the occurrence of various *N*-acetyllactosamine type sialyloligosaccharides differing in branching and sialylation pattern (Table 2). In corresponding fractions of sialidosis and galactosialidosis urine, similar features in molar ratios of NeuAc α2–3/α2–6 linkages and oligosaccharide branching types were found. Moreover, as in galactosialidosis fractions the presence of oligo-*N*-acetyllactosamine units in sialidosis fractions 1–4 was observed.

After purification by reversed-phase chromatography, the fractions 2–5 were separated by anion-exchange chromatography on Mono Q (Figure 2), resulting in series of subfractions. Fraction 1 could not be subfractionated on Mono Q under the applied conditions. The isolated and desalted sialidosis Mono Q fractions were

**Table 2 Schematic results of 500-MHz $^1$H-NMR spectroscopy of the sialidosis Bio-Gel P-6 fractions 1–5, compared with those of galactosialidosis urine (Van Pelt *et al.*, 1989)**

| | *Fraction* | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *Sialidosis* | | | | | |
| α2-3 : α2-6 | 1 : 0 | 1 : 0.3 | 1 : 1 | 1 : 3 | 1 : 6 |
| Type[a] | Unknown | Tetra | Tri | Di | Mono |
| *Galactosialidosis* | | | | | |
| α2-3 : α2-6 | 1 : 0 | 1 : 0.2 | 1 : 1 | 1 : 2 | 1 : 4 |
| Type[a] | Unknown | Tetra | Tri | Di | Mono |

[a] Type indicates sialylation and branching degree

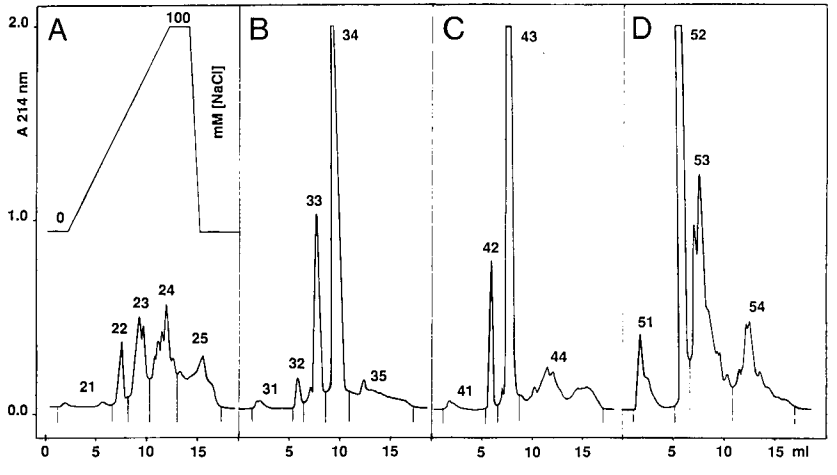



**Figure 2** Mono Q elution profiles of the Bio-Gel P-6 fractions 2–5 monitored at 214 nm. (A) Fraction 2. (B) Fraction 3. (C) Fraction 4. (D) Fraction 5. The applied linear NaCl gradient is shown in (A). The Mono Q subfractions were pooled and designated as indicated

subjected to sugar analysis (Table 3), 500-MHz $^1$H-NMR spectroscopy and analytical HPLC, which revealed a striking resemblance with galactosialidosis results (Van Pelt *et al.*, 1989). Therefore, experimental data are discussed only briefly. Molar ratios of structures occurring in the Mono Q subfractions were estimated by measurement of the UV 205-nm peak areas at analytical HPLC in combination with the signal intensities of the various structural reporter groups at $^1$H-NMR spectroscopy. The analytical HPLC method was calibrated by use of relevant sialyloligosaccharides isolated from galactosialidosis placenta and urine (Van Pelt *et al.*, 1988c, 1989). The calibration of the Lichrosorb-$NH_2$ column with three disialylated di-antennary *N*-glycosidic oligosaccharides is shown in Figure 3.

*Fraction 5:* Bio-Gel fraction 5 was separated on Mono Q (Figure 2D) in a series of subfractions 51–54 of which subfraction 52 contained almost all carbohydrate material (Table 3). $^1$H-NMR spectroscopy of subfraction 52 in combination with analytical HPLC on Lichrosorb-$NH_2$, eluted with acetonitrile–phosphate buffer, pH 4.7, gave evidence for the presence of five different, monosialylated structures. Molar ratios of the structures occurring, given below in parentheses (S), differed to some extent from those obtained from galactosialidosis urine, which are given in brackets [GS]. The chemical shift data matched completely those of a mixture of reference structures *21, 22, 23, 35* and *35** (Vliegenthart *et al.*, 1983). The subfractions 53 and 54 contained mainly *O*-glycosidic glycopeptides, but structural analysis was not possible, owing to heterogeneity of the peptide part.

**Table 3 Sugar analysis data and total amounts of Man of the relevant Mono Q subfractions**

| | *Fraction* | | | | | | |
|---|---|---|---|---|---|---|---|
| *Monosaccharide* | 22 | 23 | 24 | 32 | 33 | 34 | 35 |
| Man[a] | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Gal | 5.6 | 4.4 | 5.2 | 4.3 | 3.0 | 3.0 | 5.7 |
| GalNAc | — | — | — | + | 0.4 | — | — |
| GlcNAc | 7.0 | 6.2 | 6.7 | 5.7 | 4.1 | 4.1 | 5.2 |
| NeuAc | 1.7 | 3.3 | 5.1 | 1.8 | 2.0 | 3.3 | 6.0 |
| nmol Man | 126 | 250 | 181 | 54 | 341 | 600 | 55 |
| | 42 | 43 | 44 | 51 | 52 | 53 | 54 |
| Fuc | — | — | — | 1.8 | — | — | — |
| Man[a] | 3 | 3 | 3 | 2 | 2 | 2 | 2 |
| Gal | 2.5 | 2.0 | 3.9 | 2.2 | 1.1 | 4.1 | 10.9 |
| GalNAc | 0.6 | — | 2.2 | 0.3 | — | 1.9 | 4.2 |
| GlcNAc | 4.1 | 2.9 | 5.7 | 1.0 | 2.2 | 1.9 | + |
| NeuAc | 1.5 | 2.0 | 7.1 | 0.3 | 1.3 | 3.3 | 11.1 |
| nmol Man | 338 | 2500 | 286 | 250 | 10000 | 213 | 45 |

[a] The data are expressed as molar ratios relative to 3 Man or to 2 Man in subfractions 51–54

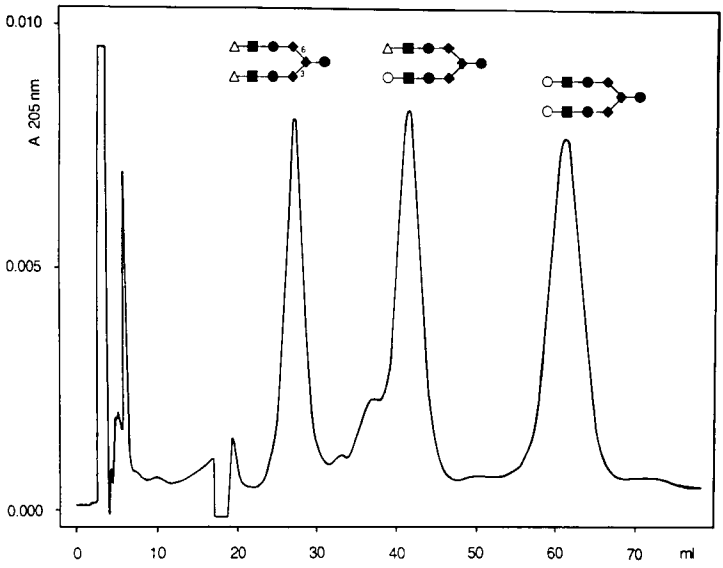



**Figure 3** Analytical HPLC of three reference, disialylated, di-antennary, *N*-glycosidic oligosaccharides, monitored at 205nm. The Lichrosorb-$NH_2$ column was eluted isocratically with acetonitrile – 30 mmol/L $KH_2PO_4$ – $K_2HPO_4$, pH 7.0 (5:3, v/v) at a flow rate of 2.0 ml/min. The structures are represented by short-hand notation: ● = GlcNAc; ◇ = Man; ■ = Gal; ○ = NeuAcα2-6; △ = NeuAcα2-3

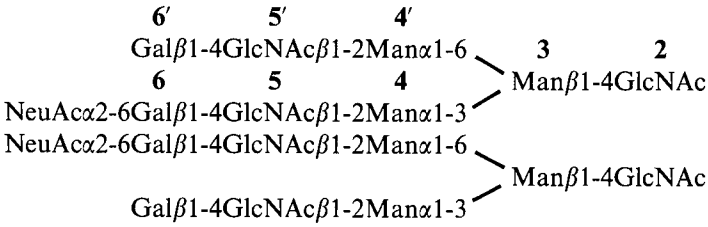
*Sialidosis fraction* 52

| | | |
|---|---|---|
| NeuAcα2-6Galβ1-4GlcNAcβ1-2Manα1-3 — Manβ1-4GlcNAc | (10) | [5] |
| NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1-3 — Manβ1-4GlcNAc | (1) | [1] |
| NeuAcα2-6Galβ1-4GlcNAcβ1-2Manα1-6 ⟍ Manβ1-4GlcNAc | (1) | [0.6] |
| NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1-6 ⟍ Manβ1-4GlcNAc | (0.2) | [0.2] |
| Manα1-6 ⟍ Manβ1-4GlcNAc<br>NeuAcα2-6Galβ1-4GlcNAcβ1-2Manα1-3 ⟋ | (0.1) | [0.6] |

*Fraction 4:* Fraction 4 was separated on Mono Q (Figure 2C) into one minor (42) and one major subfraction (43), at elution positions which suggested the presence of one and two NeuAc residues, respectively (Van Pelt *et al.*, 1987).

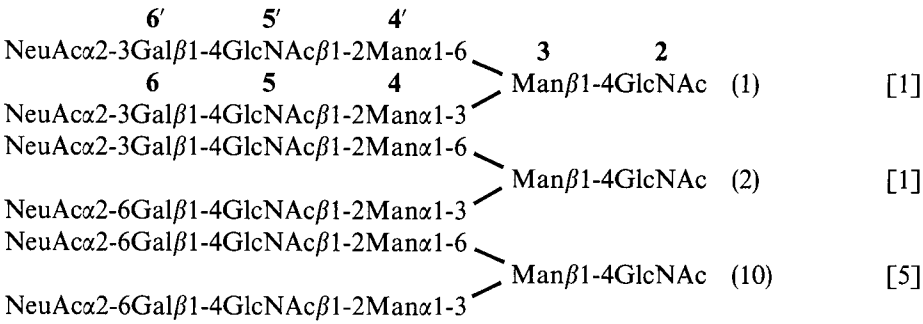
$^1$H-NMR spectroscopy of subfraction 42 pointed to the presence of monosialylated di-antennary compounds with NeuAc in α2-6 linkage at Gal-6 or at Gal-6′, together with one or more analogues with NeuAc in α2-3 linkage in relatively small amounts. The NMR data of the di-antennary compounds matched those of a mixture of reference compounds *27* and *28* (Vliegenthart *et al.*, 1983).

*Sialidosis fraction* 42




$^1$H-NMR spectroscopy of fraction 43 showed a mixture of three disialylated di-antennary structures. The chemical shift values matched completely those of a mixture of reference structures *29, 38* and *39* (Vliegenthart *et al.*, 1983). The molar ratios were obtained by $^1$H-NMR spectroscopy and analytical HPLC on Lichrosorb-$NH_2$, eluted with acetonitrile–phosphate buffer, pH 7.0 (see Figure 3). The ratios indicated below in parentheses differed from those of the same compounds from galactosialidosis urine, which are given in brackets.

*Sialidosis fraction* 43




*Fraction 3:* Separation of Bio-Gel fraction 3 on Mono Q (Figure 2B) resulted in a major (34) and a minor subfraction (33) at elution positions suggesting tri- and di-sialylated compounds, respectively (Van Pelt *et al.*, 1987). The very small fraction 32, present in an elution position indicating one NeuAc residue, contained too little material for further investigation.

The $^1$H-NMR spectrum of subfraction 33 showed the occurrence of disialylated di-antennary compounds with di-*N*-acetyllactosamine units in one of the branches. The di-antennary nature of the main component of subfraction 33 was demonstrated by the H-2 signals of the Man residues, while the NeuAc H-3a and H-3e regions proved the presence of NeuAc α2-3 and α2-6 linkages in a molar ratio of 1 : 1. As well as these compounds, the $^1$H-NMR spectrum indicated the presence of other compounds of which the structural characterization was not completed.

$^1$H-NMR spectroscopy of subfraction 34 revealed a trisialylated tri-antennary compound of which the chemical shift values matched those of reference structure *41* (Vliegenthart *et al.*, 1983) as the major component. However, all three branches

were substituted by NeuAc residues in both α2-3 and α2-6 linkages. The chemical shift values of the sets of minor signals were compared to those of reference trisialylated tri-antennary structures isolated from galactosialidosis urine and placenta (Van Pelt *et al.*, 1988c, 1989). The molar ratios of the various trisialylated tri-antennary compounds were estimated by analytical HPLC on Lichrosorb-$NH_2$. The structures indicated below (the numerals in one column representing one structure) and their ratios were similar to those observed in galactosialidosis urine. Also a tri'-antennary structure containing three NeuAc residues in α2-3 linkage was present in a molar ratio of 0.6.

*Sialidosis fraction* 34

NeuAc substitution pattern:

| | | | | | | 6′ | 5′ | 4′ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gal-**6′** | 3 | 3 | 6 | 6 | NeuAcα2-3/6Galβ1-4GlcNAcβ1-2Manα1-6 | | | | **3** | **2** |
| | | | | | | **6** | **5** | **4** | | Manβ1- |
| | | | | | | | | | | 4GlcNAc |
| Gal-**6** | 3 | 6 | 6 | 6 | NeuAcα2-3/6Galβ1-4GlcNAcβ1-2Manα1-3 | | | | | |
| | | | | | | **8** | **7** | | | |
| Gal-**8** | 3 | 3 | 3 | 6 | NeuAcα2-3/6Galβ1-4GlcNAcβ1-4 | | | | | |

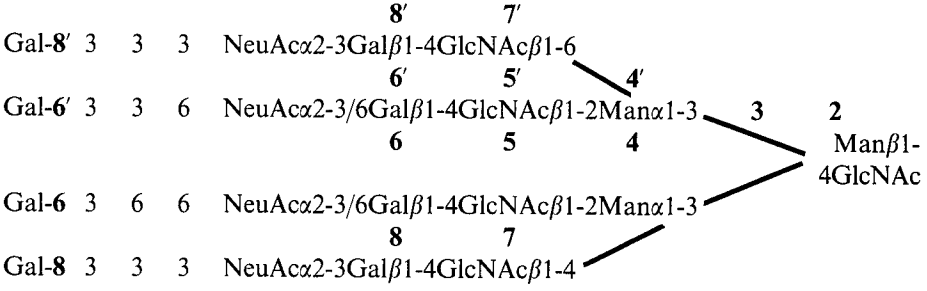
Ratio 0.6 : 1.5 : 4.0 : 0.7

*Fraction 2:* The separation procedure on Mono Q of Bio-Gel fraction 2 (Figure 2A) resulted in three subfractions 22, 23 and 24 at elution positions suggesting the presence of di-, tri- and tetrasialylated compounds, respectively (Van Pelt *et al.*, 1987). Fraction 22 contained probably disialylated di-antennary compounds, bearing several oligo-*N*-acetyllactosamine units in the branches, but the amount of material was very low. The $^1$H-NMR spectrum of 23 showed spectral features of a trisialylated tri-antennary compound bearing a di-*N*-acetyllactosamine unit. The tri-antennary character followed from the H-2 signals of the Man residues, while the NeuAc H-3a and H-3e regions showed the presence of NeuAc α2-6 and α2-3 linkages in a molar ratio of 1 : 2, respectively.

$^1$H-NMR spectroscopy of subfraction 24 showed the occurrence of tetrasialylated tetra-antennary compounds. NeuAc was linked to Gal-6 and Gal-6′ in either α2-3 or α2-6 linkage, while Gal-8 and Gal-8′ were substituted by NeuAc in α2-3 linkage. The chemical shift values of subfraction 24 matched those of a mixture of tretrasialylated tetra-antennary compounds, isolated from galactosialidosis placenta and urine (Van Pelt *et al.*, 1988c, 1989). The structures of the compounds indicated below (the numerals in one column representing one structure) and their molar ratios obtained by analytical HPLC on Lichrosorb-$NH_2$, were similar to those observed in the corresponding galactosialidosis fraction.

*Sialidosis fraction* 24

NeuAc substitution pattern:




Ratio 0.3 : 0.7 : 1.0

## DISCUSSION

Sialidosis urine was fractionated by gel filtration and anion-exchange chromatography, resulting in mixtures of sialyloligosaccharides being heterogeneous in respect to the sialic acid substitution pattern. These oligosaccharide mixtures could be characterized by $^1$H-NMR spectroscopy, sugar analysis and analytical HPLC on Lichrosorb-$NH_2$, because of the detailed knowledge of series of pure sialic acid-containing carbohydrates, isolated from galactosialidosis placenta and urine (Van Pelt *et al.*, 1988c, 1989). This analytical approach revealed the presence of the same sialyloligosaccharides in sialidosis urine as in galactosialidosis urine, including mono-, bi-, tri- and tetrasialylated compounds. The resemblance included also the occurrence of sialylated compounds with one or more repeating oligo-*N*-acetyllactosamine units. Therefore, it could be concluded that both sialidosis and galactosialidosis presented identically concerning the excreted carbohydrates. Apparently, the additional β-galactosidase deficiency in galactosialidosis did not contribute to the nature of urinary oligosaccharides and the sialidase deficiency solely determined the phenotype of the defective catabolism of the carbohydrate chains of glycoproteins in both diseases. However, some minor differences were also observed. In the mono- and di-antennary compounds from sialidosis urine, the molar ratio of α2-3/α2-6 linked NeuAc residues is smaller than in the same compounds from galactosialidosis urine. Consistently, the same difference was found in a comparative study of the sialic acid-containing storage material from sialidosis and galactosialidosis fibroblasts (Van Pelt *et al.*, 1988a,b). The reason for these differences is not known, but it may be caused by variations in activities of sialyltransferases or by minor differences in residual activities or specificities of the deficient sialidases towards NeuAcα2-3 and NeuAcα2-6 linkages. The latter possibility is attractive as sialidosis and galactosialidosis have a distinct genetic origin (Mueller *et al.*, 1986). Sialidosis seems to be caused by a defective synthesis of sialidase (O'Brien, 1982), while galactosialidosis is caused by a defective synthesis of a 32-kD protective protein necessary for expression of sialidase activity (Hoogeveen *et al.*, 1981; D'Azzo *et al.*, 1982; Verheijen *et al.*, 1985).

## ACKNOWLEDGEMENT

This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for Scientific Research (NWO) and by the Foundation for Clinical Genetics, Rotterdam, The Netherlands.

## REFERENCES

Cantz, M. Sialidoses. *Cell Biol. Monogr.* 10 (1982) 307–320

D'Azzo, A., Hoogeveen, A. T., Reuser, A. J. J., Robinson, D. and Galjaard, H. Molecular defect in combined β-galactosidase and neuraminidase deficiency in man. *Proc. Natl. Acad. Sci. USA* 79 (1982) 4535–4539

Dorland, L., Haverkamp, J., Vliegenthart, J. F. G., Strecker, G., Michalski, J.-C., Fournet, B., Spik, G. and Montreuil, J. 360-MHz $^1$H nuclear-magnetic-resonance spectroscopy of sialyl-oligosaccharides from patients with sialidosis (mucolipidosis I and II). *Eur. J. Biochem.* 87 (1978) 323–329

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. Colorimetric method for determining of sugars and related substances. *Anal. Chem.* 28 (1956) 350–356

Hoogeveen, A. T., D'Azzo, A., Brossmer, R. and Galjaard, H. Correction of combined β-galactosidase/neuraminidase deficiency in human fibroblasts. *Biochem. Biophys. Res. Commun.* 103 (1981) 292–299

Kamerling, J. P. and Vliegenthart, J. F. G. Gas-liquid chromatography and mass spectrometry of sialic acids. *Cell Biol. Monogr.* 10 (1982) 95–125

Koseki, M. and Tsurumi, K. Structure of a novel sialooligosaccharide from the urine of a patient with mucolipidosis. *Tohoku J. Exp. Med.* 124 (1978) 361–366

Koseki, M. and Tsurumi, K. The structure of a new sialic acid-containing decasaccharide from the urine of a patient with mucolipidosis. *Tohoku J. Exp. Med.* 128 (1979) 39–49

Kuriyama, M., Ariga, T., Ando, S., Suzuki, M., Yamada, T. and Miyatake, T. Four positional isomers of sialyloligosaccharides isolated from the urine of a patient with sialidosis. *J. Biol. Chem.* 256 (1981) 12316–12321

Kuriyama, M., Ariga, T., Ando, S., Suzuki, M., Yamada, T., Miyatake, T. and Igata, A. Two positional isomers of sialylheptasaccharides isolated from the urine of a patient with sialidosis. *J. Biochem.* 98 (1985) 1049–1054

Lowden, J. A. and O'Brien, J. S. Sialidosis: a review of human neuraminidase deficiency. *Am. J. Hum. Genet.* 31 (1979) 1–18

Mueller, O. T., Henry, W. M., Haley, L. L., Byers, M. G., Eddy, R. L. and Shows, T. B. Sialidosis and galactosialidosis: Chromosomal assignment of two genes associated with neuraminidase deficiency disorders. *Proc. Natl. Acad. Sci. USA* 83 (1986) 1817–1821

O'Brien, J. S. Sialidosis. In Durand, P. and O'Brien, J. S. (eds.) *Genetic Errors of Glycoprotein Metabolism*, Ermes, Milano, 1982, pp. 33–48

Schauer, R. and Corfield, A. P. Colorimetry and thin-layer chromatography of sialic acids. *Cell Biol. Monogr.* 10 (1982) 77–94

Strecker, G., Peers, M.-C., Michalski, J.-C., Hondi-Assah, T., Fournet, B., Spik, G., Montreuil, J., Farriaux, J.-P., Maroteaux, P. and Durand, P. Structures of nine sialyloligosaccharides accumulated in urine of eleven patients with three different types of sialidosis. Mucolipidosis II and two new types of mucolipidosis. *Eur. J. Biochem.* 75 (1977) 391–403

Van Diggelen, O. P., Hoogeveen, A. T., Smith, P. J., Reuser, A. J. J. and Galjaard, H. Enhanced proteolytic degradation of normal β-galactosidase in the lysosomal storage disease with combined β-galactosidase and neuraminidase deficiency. *Biochim. Biophys. Acta* 703 (1982) 69–76

Van Pelt, J., Damm, J. B. L., Kamerling, J. P. and Vliegenthart, J. F. G. Separation of sialyl-oligosaccharides by medium-pressure anion-exchange chromatography on Mono Q. *Carbohydrate Res.* 169 (1987) 43–51

Van Pelt, J., Kamerling, J. P., Vliegenthart, J. F. G., Verheijen, F. W. and Galjaard, H. Isolation and structural characterization of sialic acid-containing storage material from mucolipidosis I (sialidosis) fibroblasts. *Biochim. Biophys. Acta* 965 (1988a) 36–45

Van Pelt, J., Kamerling, J. P., Vliegenthart, J. F. G., Hoogeveen, A. T. and Galjaard, H. A comparative study of the accumulated sialic acid-containing oligosaccharides from cultured human galactosialidosis and sialidosis fibroblasts. *Clin. Chim. Acta* 174 (1988b) 325–336

Van Pelt, J., Van Kuik, J. A., Kamerling, J. P., Vliegenthart, J. F. G., Van Diggelen, O. P. and Galjaard, H. Storage of sialic acid-containing carbohydrates in the placenta of a human galactosialidosis fetus. Isolation and structural characterization of 16 sialyloligosaccharides. *Eur. J. Biochem.* 177 (1988c) 327–338

Van Pelt, J., Hård, K., Kamerling, J. P., Vliegenthart, J. F. G., Reuser, A. J. J. and Galjaard, H. Isolation and structural characterization of twenty-one sialyloligosaccharides from galactosialidosis urine. An intact *N,N'*-diacetylchitobiose unit at the reducing end of a diantennary structure. *Biol. Chem. Hoppe-Seyler* 370 (1989) 191–203

Verheijen, F. W., Palmeri, S., Hoogeveen, A. T. and Galjaard, H. Human placental neuraminidase. Activation, stabilization and association with β-galactosidase and its 'protective' enzyme. *Eur. J. Biochem.* 149 (1985) 315–321

Vliegenthart, J. F. G., Dorland, L. and Van Halbeek, H. High-resolution $^1$H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins. *Adv. Carbohydrate Chem. Biochem.* 41 (1983) 209–374