The Structure of a New Sialic Acid-Containing Decasaccharide from the Urine of a Patient with Mucolipidosis

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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., 1979, 128 (1), 39-49 — A new sialic acid-containing oligosaccharide has been isolated from urine of a patient with a type of mucolipidosis newly recognized by Orii et al. (1972). This compound was found to be composed of galactose (2 moles), mannose (3 moles), N-acetylglucosamine (3 moles) and sialic acid (2 moles). On the basis of the results of sequential glycosidase digestion, of methylation analysis, and of the Smith degradation, the structure of this oligosaccharide was elucidated as follows: NeuAcα2-6Galβ1-4GlcNAcβ1-2Manβ1-3[NeuAcα2-6Galβ1-4GlcNAcβ1-2Manβ1-6]Manβ1-4GlcNAc. The increased excretion of this oligosaccharide may be caused by the impaired metabolism of glycoproteins having N-glycosidic linkage.

Mucolipidosis (ML) is a heritable disorder of metabolism, manifesting similar clinical features to mucopolysaccharidosis but lacking the marked mucopolysacchariduria (Spranger et al. 1968; Spranger and Wiedemann 1970; Spranger 1975). Cultured fibroblasts from the patients with this disease contain numerous inclusion bodies which have been considered to represent lysosomes distended by storage materials (Leroy and DeMars 1967; Spranger et al. 1968; Spranger and Wiedemann 1970; Kelly 1976).

In 1972 Orii and his coworkers reported a new type of metabolic disease which showed clinical features similar to ML type I which had been reported by Spranger et al. (1968). In the previous study, the authors reported abnormal excretion of sialic acid-containing complex carbohydrates in the urine of this patient (Koseki et al. 1978) and found that one of the major components of the sialic acid-containing substances was a hexasaccharide closely related to the carbohydrate side chains of many glycoproteins with an N-glycosidic linkage (Koseki and Tsurumi 1978).

The present investigation deals with the isolation and the characterization of another major oligosaccharide excreted in the urine of this patient.

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MATERIALS AND METHODS

Materials. Urine (5,000 ml) from the patient with ML, who showed clinical features similar to ML type I, low β-galactosidase activity, and hyperglycopeptiduria (Orii et al. 1972), was collected through the courtesy of Dr. Orii of the Department of Pediatrics, Sapporo Medical College, Sapporo. Sialooligosaccharide fraction (Fr. SG-1-III-0.1M-C) used in the present study was isolated from the urine by the procedure described in the previous paper (Koseki et al. 1978). N-Acetyleneuraminic acid (NeuAc) was prepared in our laboratory from human colostrum, and N-glycolyneuraminic acid (NeuGc) was purchased from Sigma Chemical, Co., Ltd., St. Louis, Mo., U.S.A. Disialyl-lacto-N-tetraose (DSLNT) was isolated from human colostrum as described in another paper (Koseki 1976). Arthrobacter ureafeciens β-galactosidase [EC 3.2.1. 18] was kindly supplied by Dr. Uchida, Kyoto Research Laboratories, Marukin Shoyu, Co., Ltd., Kyoto. Charonia lampanas β-galactosidase [EC 3.2.1. 23], Turbo cornutus α-mannosidase [EC 3.2.1. 24] and β-N-acetylhexosaminidase [EC 3.2.1. 30] were purchased from Seikagaku Kogyo, Co., Tokyo. Other chemicals used in the present study were obtained from Wako Pure Chemical Industries, Ltd., Osaka.

Analytical methods. Total sialic acid was determined by the orcinol method of Böhm et al. (1954), and free sialic acid by the thiobarbituric acid method of Warren (1959). The results were expressed as NeuAc. Total hexosamine was determined by the modified Elson-Morgan method of Bliz (1948), using glucosamine hydrochloride as a standard. The samples for the assay were hydrolyzed with 2 N hydrochloric acid at 100°C for 14 hr. The ratio of glucosamine to galactosamine was determined on a JLC-5AH automatic amino acid analyzer (Japan Electron Optics Laboratories Co., Ltd., Tokyo). The sample for the assay was hydrolyzed with 2 N hydrochloric acid at 100°C for 14 hr. N-Acetylgalactosamine was determined by the modified Morgan-Elson method described by Reissig et al. (1955), using N-acetylgalactosamine as a standard. Neutral sugars were determined by gas-liquid chromatography as their alditol trifluoroacetates on a glass column (3 mm x 200 cm) packed with 3% XF–1105 on Gaschrom Q (80–100 mesh). The column temperature was programmed from 115°C to 145°C at a rate of 1°C per min, then to 175°C at a rate of 2°C per min. Samples for the assay were hydrolyzed with 2.5 N trifluoroacetic acid at 100°C for 6 hr. Monosaccharides in the hydrolyzates were converted to their alditols by treatment with sodium borohydride and trifluoroacetylated according to the method described by Tamura et al. (1968).

Glycosidase digestion. The conditions for the enzymatic digestion were as follows: β-Galactosidase digestion — samples corresponding to 1.8 μmoles of galactose were incubated at 37°C for 24 hr with 0.1 unit of β-galactosidase in 110 μl of 0.2 M acetate buffer (pH 4.0) containing 0.5 M sodium chloride. α-Mannosidase digestion — the conditions for α-mannosidase digestion were essentially identical with those of β-galactosidase treatment except that incubation period was extended to 72 hr with successive addition of 0.1 unit of the enzyme every 24 hr. Released monosaccharides were determined as their alditol trifluoroacetates. β-N-Acetylhexosaminidase digestion — the digestion with β-N-acetylhexosaminidase was carried out under the same conditions as those of β-galactosidase treatment except that 0.2 M citrate-phosphate buffer (pH 4.0) containing 0.5 M sodium chloride was used instead of acetate buffer. Released free N-acetylgalactosamine was determined by the Morgan-Elson method of Reissig et al. (1955). Neuraminidase digestion — samples corresponding to 400 μg of NeuAc were incubated with 0.2 unit of neuraminidase in 220 μl of 0.05 M acetate buffer (pH 5.0) at 37°C. Free sialic acid was isolated by the column method described by Svennerholm (1958) and determined by the thiobarbituric acid method of Warren (1959).

Methylation analysis. Oligosaccharides were permethylated by the method of Hakomori as described by Sandford and Conrad (1966). Methylated oligosaccharides were recovered by repeated extraction with chloroform and subjected to acid hydrolysis (dissolved in cold 72% sulfuric acid then diluted with 6 volumes of distilled water and heated at 100°C for 6 hr) followed by reduction with sodium borohydride and acetylation as
A New Sialic Acid-Containing Decasaccharide described by Björndal et al. (1967). The compositions of partially methylated alditol acetates in the samples were determined by gas-liquid chromatography on a glass column (3 mm x 200 cm) packed with 3% ECNSS-M on Gaschrom Q (80–100 mesh). The column temperature was maintained at 155°C for 30 min and then was raised to 205°C.

Smith degradation. Periodate oxidation was carried out under the conditions described by Kuhn and Gauhe (1965). Aqueous solution (1.0 ml) containing about 6 μmoles of sialooligosaccharide was adjusted to pH 4.0 with 0.5 ml of 0.5 M acetate buffer (pH 4.0), and the buffered solution was mixed with 0.5 ml of 0.2 M sodium metaperiodate under ice-cooling. The reaction mixture was stored in a dark place at 4°C, and the consumption of periodate was determined by the method of Dixon and Lipkin (1954). After oxidation for 24 hr, the reaction was terminated by the addition of two drops of ethylene glycol, and the resulting polyaldehyde was reduced with sodium borohydride. The excess borohydride was destroyed by acidifying the reaction mixture with glacial acetic acid, and boric acid was removed by gel filtration on a Bio Gel P-2 column (1.5 cm x 80 cm). The chemical composition of the oxidation product was determined by colorimetry and by gas-liquid chromatography. Gas-liquid chromatographic analysis of glycerol produced by the Smith degradation was performed on a glass column (3 mm x 200 cm) packed with 3% ECNSS-M on Gaschrom Q (80–100 mesh). The column temperature was programmed from 100°C to 145°C at a rate of 1°C per min. The sample for the assay was hydrolyzed with 2.5 N trifluoroacetic acid at 100°C for 4 hr and acetylated as described under "methylation analysis."

General method. Gel filtration was carried out on a column of Bio Gel P-2. Either 0.1 M pyridinium acetate (pH 5.3) or distilled water was used as an eluent. Ion-exchange chromatography was performed on a column of Dowex 1 x 2 (200–400 mesh, acetate form), using pyridinium acetate (pH 5.3) as an eluent. Analytical high voltage paper electrophoresis was carried out on Toyo Roshi No. 50 filter paper at a potential gradient of 40 V/cm. A mixture of acetic acid, pyridine, and distilled water (10:1:89, by vol.) was used as a buffer for the electrophoresis.

Filter paper, Toyo Roshi No. 51A was used for analytical paper chromatography, and the following solvents were used for development: solvent A, ethylacetate-pyridine-acetic acid-water (5:5:1:3, by vol.); solvent B, butane-1-ol-propane-1-ol-0.1 N hydrochloric acid (1:2:1, by vol.). Paper chromatograms and paper electrophoretograms were stained with the aniline hydrogen phthalate reagent.

EXPERIMENTS AND RESULTS

Isolation of a sialic acid-containing decasaccharide

Aqueous solution (20 ml) containing 760 mg of Fr. SG-1–III–0.1M–C was applied to a Dowex 1 x 2 column (1.6 cm x 79 cm), and after washing the column with three column-volumes of distilled water, sialic acid-containing substances were eluted carefully with 0.1 M pyridinium acetate (pH 5.3). Ten ml fractions were collected, and the sialic acid concentration of the fractions was determined by the orcinol method. The result is shown in Fig. 1. The major fractions (shaded area in Fig. 1) were combined and concentrated to a small volume, and the sialic acid-containing substance was recovered by precipitation with ethanol and ether (yield, 500 mg).

In paper chromatography using solvent A, the purified sialic acid-containing substance showed a single reducing spot with a relative mobility of 0.15 to DSLNT (referred to as Rsct 0.15 oligosaccharide). In high voltage paper electrophoresis it showed a single reducing band which migrated slightly slower.
than DSLNT. Fig. 2 shows the paper chromatograms of R_{DSLNT}0.15 oligosaccharide and the hexasaccharide reported in the previous paper (Koseki and Tsurumi 1978).

The chemical compositions of R_{DSLNT}0.15 oligosaccharide and its reduced derivative are shown in Table 1. As shown in the table, the sialooligosaccharide isolated in the present study was composed of 2 moles each of galactose and sialic acid and 3 moles each of mannose and N-acetylglucosamine, with N-acetylglucosamine at the reducing end of the molecule. The intact oligosaccharide showed a negative reaction to the Morgan-Elson reagent of Reissig et al. (1955), suggesting that the N-acetylglucosamine residue at the reducing end was substituted at C-4.

**Preparation of the asialo-derivative and identification of sialic acid**

Aqueous solution containing 50 mg of R_{DSLNT}0.15 oligosaccharide was mixed with an equal volume of 0.1 N sulfuric acid and heated at 80°C for 60 min. The acid hydrolyzate was applied to a Dowex 1 × 2 column (0.8 cm × 15 cm), and the column was washed with 30 ml of distilled water. The washings were combined and concentrated to a small volume, and the asialo-derivative of the intact oligosaccharide was recovered by precipitation with ethanol and ether.
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Fig. 2. Paper chromatograms of the purified sialooligosaccharides isolated from the urine of a patient with mucolipidosis. DSLNT, Oligo. A, and Oligo. B stand for disialyl-lacto-N-tetraose, $R_{DSLN'T}1.40$ oligosaccharide, and $R_{DSLN'T}0.15$ oligosaccharide, respectively.

Table 1. Carbohydrate compositions of purified sialooligosaccharides (mmoles/100 g)

<table>
<thead>
<tr>
<th></th>
<th>Galactose</th>
<th>Mannose</th>
<th>Glucosamine</th>
<th>Sialic acid</th>
<th>Morgan-Elson</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{DSLN'T}$ 0.15 oligosaccharide</td>
<td>100.2</td>
<td>150.4</td>
<td>154.2</td>
<td>108.4</td>
<td>(-)</td>
</tr>
<tr>
<td>Reduce $R_{DSLN'T}$ 0.15 oligosaccharide</td>
<td>96.7</td>
<td>159.0</td>
<td>86.0</td>
<td>106.5</td>
<td></td>
</tr>
<tr>
<td>Gal$_2$Man$_2$GlcNAc$_2$SA$_2$*</td>
<td>98.9</td>
<td>148.3</td>
<td>148.6</td>
<td>99.0</td>
<td></td>
</tr>
</tbody>
</table>

* Theoretical values of the oligosaccharides with the given compositions.

Sialic acid retained on the column was eluted with 30 ml of 0.5 M pyridinium acetate (pH 5.3) and subjected to paper chromatography using solvent B. The paper chromatogram was stained with the thiobarbituric acid reagent of Warren (1960), and a single spot with the same mobility as that of NeuAc was observed in the chromatogram of sialic acid derived from $R_{DSLN'T}0.15$ oligosaccharide.

Sequential glycosidase digestion

Sialic acid contained in $R_{DSLN'T}0.15$ oligosaccharide was completely released by the treatment with neuraminidase for 3 hr, but the intact oligosaccharide was resistant to the action of $\beta$-galactosidase, $\alpha$-mannosidase, and $\beta$-N-acetylhexosaminidase. On the other hand, the asialo-derivative of the intact oligosaccharide released 2 moles of galactose on incubation with $\beta$-galactosidase but was resistant to the action of $\alpha$-mannosidase and $\beta$-N-acetylhexosaminidase. The asialo-derivative,
which had been treated with β-galactosidase, released 2 moles of N-acetylglucosamine on incubation with β-N-acetylhexosaminidase but was resistant to the action of α-mannosidase. The asialo-derivative, which had been treated successively with β-galactosidase and β-N-acetylhexosaminidase, released 1.3 moles of mannose on incubation with α-mannosidase. The release of mannose was a little smaller than the expected value (2 moles). It is quite reasonable, however, to consider that 2 moles of mannose was susceptible to the action of α-mannosidase, since the amount of free mannose gradually increased with prolonged incubation with α-mannosidase. The results of enzymatic digestion are listed in Table 2.

### Table 2. Effect of exo-glycosidases on the asialo-derivative of urinary sialooligosaccharide

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gal</th>
<th>Man</th>
<th>GlcNAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase</td>
<td>1.5</td>
<td>—</td>
<td>0.2</td>
</tr>
<tr>
<td>β-N-Acetylhexosaminidase</td>
<td>—</td>
<td>—</td>
<td>tr*</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>1.9</td>
<td>—</td>
<td>2.1</td>
</tr>
<tr>
<td>then β-N-acetylhexosaminidase</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>1.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>then α-mannosidase</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>1.8</td>
<td>1.3</td>
<td>2.2</td>
</tr>
<tr>
<td>then β-N-acetylhexosaminidase</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>then α-mannosidase</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed as moles of monosaccharides released per mole of the oligosaccharide.

* tr stands for “trace amount”.

From these results, it is deduced that RDSLNT0.15 oligosaccharide possessed the following sequence and anomeric configuration: (NeuAcβ2-)2 (Galβ1-)2 (GlcNAcβ1-)2 (Manα1-)2 Manβ1-GlcNAc.

**Permethylatation analysis**

RDSLNT0.15 oligosaccharide (5 mg) and its derivatives (5 mg) were permethylated according to the method of Sandford and Conrad (1966), and the compositions of partially methylated monosaccharides in the samples were determined by gas-liquid chromatography. The results are shown in Fig. 3 and Table 3.

As shown in the table, 2,3,4,6-tetra-O-methyl-D-galactose (2 moles), 3,4,6-tri-O-methyl-D-mannose (2 moles), and 2,4-di-O-methyl-D-mannose (1 mole) were contained in the acid hydrolyzate of asialo-RDSLNT0.15 oligosaccharide. The result obtained with the intact oligosaccharide showed that two sialyl residues were linked, separately, to C-6 position of two galactosyl residues in the asialo-RDSLNT0.15 oligosaccharide. Trimannosyl-N-acetylgalactosamine, which had been prepared by sequential digestion of the asialooligosaccharide with β-galactosidase and β-N-acetylhexosaminidase, produced 2 moles of 2,3,4,6-tetra-O-methyl-D-mannose and 1 mole of 2,4-di-O-methyl-D-mannose. These data, in combination with the results of chemical analysis and of glycosidase digestion, indicate that the
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Fig. 3. Gas-liquid chromatograms of partially methylated alditol acetates obtained from R_DS\_NT0.15 oligosaccharide (A), asialo-R_DS\_NT0.15 oligosaccharide (B), and trimannosyl-N-acetylglucosamine which was obtained by sequential digestion of asialo-R_DS\_NT0.15 oligosaccharide with β-galactosidase and β-N-acetylhexosaminidase (C). Peak 1, 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-mannitol; peak 2, 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-galactitol; peak 3, 3,4,6-tri-O-methyl-1,2,5-tri-O-acetyl-D-mannitol; peak 4, 2,4,6-tri-O-methyl-1,3,5-tri-O-acetyl-D-mannitol; peak 5, 2,3,4-tri-O-methyl-1,5,6-tri-O-acetyl-D-galactitol; peak 6, 2,4-di-O-methyl-1,3,5,6-tetra-O-acetyl-D-mannitol; peak 7, N-methyl-2-acetamido-2-deoxy-3,6-di-O-methyl-1,4,5-tri-O-acetyl-D-glucitol.

The asialooligosaccharide isolated in the present study possessed the following structure:


Smith degradation

Smith degradation was carried out under the conditions described by Kuhn and Gauhe (1965). The consumption of periodate reached a plateau after 24 hr,
TABLE 3. Partially methylated alditols obtained by methylation analysis

<table>
<thead>
<tr>
<th></th>
<th>R_PSLNT 0.15 oligosaccharide</th>
<th>Asialo-R_PSLNT 0.15 oligosaccharide</th>
<th>Man₆GleNAc*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-Mₑ₂-Gal-OH</td>
<td></td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>2,3,4-Mₑ₂-Gal-OH</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-Mₑ₄-Man-OH</td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>3,4,6-Mₑ₃-Man-OH</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>2,4-Mₑ₂-Man-OH</td>
<td>1.2</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>3,6-Mₑ₂-N-Me-GlcN-OH</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as molar ratios.
* Tetrasaccharide obtained by sequential digestion of the asialooligosaccharide with β-galactosidase and β-N-acetylhexosaminidase.

TABLE 4. Analysis of the Smith degradation product of the sialooligosaccharide

<table>
<thead>
<tr>
<th></th>
<th>Periodate-consumption (moles/mole)</th>
<th>Galactose</th>
<th>Mannose</th>
<th>Glucosamine</th>
<th>N-Acetyleneuraminic acid</th>
<th>Glucosaminol</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.7 (10.0)</td>
<td>n.d.*</td>
<td>0.9 (1.0)</td>
<td>2.0 (2.0)</td>
<td>n.d.</td>
<td>+ (1.0)</td>
<td>3.0 (4.0)</td>
</tr>
</tbody>
</table>

Values are expressed as molar ratios to glucosamine, and those in parentheses are theoretical values.
* n.d. stands for "not detectable".

which corresponded to 10 moles of periodate per mole of the sialooligosaccharide. Carbohydrate composition of the degradation product was determined by colorimetric analysis and by gas-liquid chromatography. The results are shown in Table 4.

Although the production of glycerol was a little smaller than the expected value (75% of theoretical value), these data were fairly compatible with the results of glycosidase digestion and methylation analysis.

DISCUSSION

The sialooligosaccharide isolated in the present study possessed a chemical structure closely resembling the carbohydrate side chains of many glycoproteins with N-glycosidic linkage such as those of α₁-acid glycoprotein (Schwarzmann et al. 1974; Hatcher and Jeanloz 1974), immunoglobulins (Baenziger 1975), transferrin (Spik et al. 1975), erythrocyte membrane glycoprotein (Thomas and Winzler 1971), etc.

From this reason, we refer to this oligosaccharide as "Disialyl-oroso-N-octaose" and propose a name "Monosialyl-oroso-N-pentaose" for the hexasaccharide reported in the previous paper (Koseki and Tsurumi 1978).

Although there is no evidence for the presence of mammalian endo-β-N-acetylhexosaminidase responsible for the cleavage of di-N-acetylchitobiosyl bond
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ontained in glycoproteins with N-glycosidic linkage, it is fairly probable that these oligosaccharides originate from these glycoproteins by the action of the enzyme having this activity. This assumption was supported by the fact that the sialooligosaccharides isolated in the present study possessed a common mannosyl-\(l\)-acetylglucosamine core.

Some evidences have been reported that an alteration in the metabolism of sialic acid-containing substances is involved in mucolipidosis. Spranger and his coworkers (1977) proposed that the primary lesion of ML type I lay in the deficiency of an acid sialidase, and in the course of their investigation on this disorder, they found an abnormal urinary excretion of low-molecular sialic acid-containing substances.

On the other hand, ML type II has been regarded as an inborn error of metabolism characterized by the abnormal localization of lysosomal enzymes (Hickman and Neufeld 1972; Hickman et al. 1974). It has been confirmed by several workers (Vladutiu and Rattazzi 1975; Thomas et al. 1976; Strecker et al. 1976, 1977) that this abnormality is caused by the presence of additional sialic acid on carbohydrate side chains of the lysosomal enzymes due to the deficiency of a specific sialidase. As a consequence of this defect, an abnormally large amount of sialic acid-containing oligosaccharides is excreted in the urine of patients with this disease (Strecker et al. 1977).

There is no conclusive evidence into which type of ML the present case should be classified. However, the patient investigated in the present study could be regarded as a variant of ML type I, since clinical and ultrastructural features closely resemble those of ML type I (Orii et al. 1972). Thus the observations described in the present study strongly suggest that ML type I could be regarded as an inborn error of metabolism of sialic acid-containing substances.

It is highly important, however, to note that the sialic acid-containing substances which contribute to the increased excretion of urinary sialic acid were confined, exclusively, to the oligosaccharides with close structural similarity to the carbohydrate side chains of glycoproteins with N-glycosidic linkage. According to the study of Huttunen (1966), two isomers of sialyllactose and sialyl-N-acetyllactosamine are excreted in normal human urine. In the course of the present study, we have also observed the excretion of these oligosaccharides (Koseki et al. 1978). However, in contrast to the oligosaccharides described

<table>
<thead>
<tr>
<th>Table 5. Proposed structure for the sialooligosaccharides isolated from the urine of a patient with mucolipidosis</th>
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<tbody>
<tr>
<td>Monosialyl-orosyl-N-pentaose</td>
</tr>
<tr>
<td>NeuAca2-6Galβ1-4GlcNAcα1-2Manα1-3Manα1-4GlcNAc</td>
</tr>
<tr>
<td>Disialyl-orosyl-N-octasose</td>
</tr>
<tr>
<td>NeuAca2-6Galβ1-4GlcNAcβ1-2Manα1-3</td>
</tr>
<tr>
<td>NeuAca2-6Galβ1-4GlcNAcβ1-2Manα1-6</td>
</tr>
</tbody>
</table>

Manα1-4GlcNAc
here, there was no detectable change in the excretion levels of sialyllactose-containing fraction in all urine specimens examined (Koseki et al. 1978). Thus the increased urinary excretion of “oroso” type oligosaccharides would be the consequence of the defect or reduced activity of one of the isozymes of sialidase responsible for the degradation of acidic glycoproteins with N-glycosidic linkage.

Acknowledgments

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References


14) Kuhn, R. & Gauhe, A. (1965) Bestimmung der Bindungsstelle von Sialinsäureresten


