Structure of a Novel Sialooligosaccharide from the Urine of a Patient with Mucopolipidosis

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KOSEKI, M. and TSURUMI, K. *Structure of a Novel Sialooligosaccharide from the Urine of a Patient with Mucopolipidosis*. Tohoku J. exp. Med., 1978, **124** (4), 361-366 — A mannose-containing sialooligosaccharide has been isolated from the urine of a patient with a newly recognized mucopolipidosis which showed a low liver β -galactosidase activity and hyperglycopeptiduria. The chemical structure has been determined by glycosidase digestion, Smith degradation and permethylation studies. The following structure has been given for the oligosaccharide: NANA α 2-6Gal β 1-4GlcNAc β 1-2Man β 1-3Man β 1-4GlcNAc. This compound appears to be derived from an incomplete catabolism of glycoproteins with aspartylglucosylamine type side chains. ——— sialooligosaccharide; mucopolipidosis; lysosomal storage disease; sialoglucide

Although it has been widely known that mucopolipidosis is one of the lysosomal storage diseases (Spranger and Wiedemann 1970), the biochemical defect of mucopolipidosis has not yet been fully characterized except for mucopolipidosis I (lipomucopolysaccharidosis) and mucopolipidosis II (I-cell disease) (Hickman and Neufeld 1972; Neufeld et al. 1976).

In the previous study (Koseki et al. 1978) it was demonstrated that an abnormally large amount of mannose-containing sialoglucide was excreted in the urine of a patient with a newly recognized mucopolipidosis reported by Orii et al. (1972). This patient showed a low β -galactosidase activity and hyperglycopeptiduria according to Orii et al. (1972).

In the present paper the authors report the isolation and characterization of a novel mannose-containing sialooligosaccharide excreted in an abnormal amount in the urine of this patient.

MATERIALS AND METHODS

Sialoglucide fraction of the urine was isolated from the pooled urine sample (5,000 ml) from the patient with a new type of mucopolipidosis by the following procedure. A 5,000 ml urine was applied to an activated charcoal column (5 cm $\times$ 60 cm), and the column was washed thoroughly with distilled water. The adsorbed materials were eluted with the mixture of ethanol, pyridine and water (2:1:2, by volume). The eluate, after the removal of organic solvent, was applied to a Dowex 50 (hydrogen form, 200-400 mesh) column (2.5 cm $\times$ 30 cm) mounted upon a Dowex 1 $\times$ 2 (acetate form, 200-400 mesh) column (2.5 cm $\times$ 40 cm). After washing the columns with distilled water, sialoglucides which had been

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retained on the Dowex 1×2 column were eluted with 1.0 M pyridinium acetate, pH 5.3. The eluate was concentrated to a small volume, then applied to a Sephadex G-25 column (2.5 cm $\times$ 90 cm) and fractionated with 0.1 M pyridinium acetate, pH 5.3. Fractions of 10 ml were collected and the elution profile was monitored by the orcinol method. Three sialic acid-containing fractions were obtained (SG-1, SG-1' and SG-2). The fraction SG-1' was rechromatographed on a Sephadex G-25 column and purified further by linear gradient elution chromatography (0 to 0.1 M pyridinium acetate) followed by preparative paper chromatography on Toyo Roshi No. 131 filter paper. The filter paper was developed in ethylacetate-pyridine-acetic acid-water (5:5:1:3, by volume) for three days in descending way.

Chemical composition of the purified sialooligosaccharide was examined by the conventional colorimetric analyses and by gas-liquid chromatography. The samples for the assay were hydrolyzed in 2.5 N trifluoroacetic acid at 100°C for 6 hr. Gas chromatographic analysis was performed under the condition described by Tamura et al. (1968). Sequential degradation study was carried out by the use of glycosidases according to the method of Fukuda and Egami (1971). The glycosidases used were *Arthrobacter ureafaciens* sialidase (Uchida et al. 1977), *Charonia lampas* β -galactosidase, *Turbo cornutus* β -N-acetylhexosaminidase and α -mannosidase. The Smith degradation was applied to the oligosaccharide according to the method described by Kuhn and Gauhe (1965). The intact sialooligosaccharide and its asialo-derivative and dimannosyl-N-acetylglucosamine, which had been obtained by sequential digestion of the asialooligosaccharide with β -galactosidase and β -N-acetylhexosaminidase, were subjected to permethylation analysis according to the method of Hakomori as described by Sandford and Conrad (1966). The analysis of partially methylated alditol acetates was performed by gas-liquid chromatography according to Björndal et al. (1967).

RESULTS

The elution profile of the urinary sialoglucide from the Sephadex G-25 column showed three overlapping peaks (Fig. 1). The intermediate fraction (SG-1') was purified further by repeated gel filtration on a Sephadex G-25 column, ion exchange chromatography and preparative paper chromatography. The yield was 254 mg from 5,000 ml of the urine. Fig. 2 shows the chromatogram of the sialooligosaccharide fraction on a Dowex 1×2 column.

Chemical analysis of the purified sialooligosaccharide revealed that it was a hexasaccharide consisted of N-acetylneuraminic acid (1.0), galactose (1.0), mannose (2.0) and N-acetylglucosamine (2.0). Analytical evidence is as follows: Borohydride treatment of the purified material resulted in the production of one mole of N-acetylglucosaminitol, showing that the reducing end of this oligosaccharide is occupied by N-acetylglucosamine residue. Table 1 shows the analytical data.

An attempt to digest the intact oligosaccharide with β -galactosidase, β -N-acetylhexosaminidase and α -mannosidase was unsuccessful. But the asialo-derivative of the sialooligosaccharide released one mole each of galactose, N-acetylglucosamine and mannose on sequential incubation with above mentioned glycosidases. The results of the enzymatic experiment are shown in Table 2.

The results of the permethylation study of the intact and the asialooligosaccharide and dimannosyl-N-acetylglucosamine are shown in Table 3 and Fig. 3. The data of permethylation analysis, together with the results obtained by the digestion study and the chemical analysis, show that the asialo-derivative of the

Fig. 1

Fig. 2

Fig. 1. Elution pattern of the crude urinary sialoglucide on a Sephadex G-25 column.

Fig. 2. Elution profile of the crude sialooligosaccharide fraction (SG-1') in Dowex 1×2 ion exchange chromatography in a linear gradient system from 0 (mixing chamber, 400 ml) to 0.1 M pyridinium acetate, pH. 5.3 (reservoir, 400 ml). Solid line, absorbance at 560 nm; dotted line, concentration of pyridinium acetate buffer.

TABLE 1. Carbohydrate composition of the purified urinary sialooligosaccharide (mmoles/100 g)

Sample	Total hexose	Galactose	Mannose	Glucosamine	Sialic acid	Morgan-Elson
Urinary sialooligosaccharide	254.4	86.1	168.3	175.4	84.5	-
Borohydride-reduced urinary sialooligosaccharide	242.4	81.6	159.7	72.4	84.4	n.d.†
Gal ₁ Man ₂ GlcNAc ₂ NANA*	249.8	83.3	166.6	166.5	83.3	-

* Theoretical values of the oligosaccharide with the given composition.

† n.d.: not determined.

TABLE 2. Effects of *exo*-glycosidases on the asialo-derivative of the urinary sialooligosaccharide (moles per mole of the oligosaccharide)

Enzyme applied	Galactose	Mannose	N-Acetylglucosamine
β-Galactosidase	1.0	-	-
β-N-Acetylhexosaminidase	-	-	tr.
α-Mannosidase	-	-	-
β-Galactosidase then β-N-acetylhexosaminidase	0.9	-	1.0
β-Galactosidase then α-mannosidase	1.0	-	-
β-Galactosidase then β-N-acetylhexosaminidase then α-mannosidase	1.2	1.0	1.4

tr.; trace amount.

TABLE 3. *Methyl ethers of the alditols obtained in the methylation analysis*

Sample	Methyl ether obtained					
	2,3,4,6-Me ₄ -Gal-OH	2,3,4-Me ₃ -Gal-OH	2,3,4,6-Me ₄ -Man-OH	2,4,6-Me ₃ -Man-OH	3,4,6-Me ₃ -Man-OH	3,6-Me ₂ -N-Me-GlcN-OH
Intact sialooligosaccharide	—	0.8	—	1.1	1.0	+
Asialo-derivative of the sialooligosaccharide	1.0	—	—	0.9	1.0	+
Dimannosyl-N-acetylglucosamine	—	—	0.9	1.0	—	+

Fig. 3. Gas-liquid chromatograms of partially methylated alditol acetates obtained in the permethylation analysis of urinary sialooligosaccharide and its derivatives.

(A), intact sialooligosaccharide; (B), asialo-derivative of the sialooligosaccharide; (C), Dimannosyl-N-acetylglucosamine obtained by sequential digestion of asialooligosaccharide with β -galactosidase and β -N-acetylhexosaminidase.

Peak 1, 2,3,4,6-tetra-O-methyl-D-mannose; peak 2, 2,3,4,6-tetra-O-methyl-D-galactose; peak 3, 3,4,6-tri-O-methyl-D-mannose; peak 4, 2,4,6-tri-O-methyl-D-mannose; peak 5, 2,3,4-tri-O-methyl-D-galactose; peak 6, 2,4-di-O-methyl-D-mannose; peak 7, 3,6-di-O-methyl-N-methyl-D-glucosamine.

sialooligosaccharide possesses the following sequence and configuration of sugar constituents: Gal β 1-4GlcNAc β 1-2or3Man- α 1-3or2Man β 1-4GlcNAc.

The gas chromatogram of the intact oligosaccharide was essentially identical with that of the asialo-derivative except that a peak corresponding to 2,3,4-tri-O-methyl-D-galactose was produced instead of a peak corresponding to 2,3,4,6-tetra-O-methyl-D-galactose. This observation suggests that the sialyl residue should be linked to the C-6 position of terminal galactose residue in the asialo-derivative of the sialooligosaccharide. Dimannosyl-N-acetylglucosamine, which had been obtained by the sequential digestion of the asialo-derivative of the sialooligosaccharide with β -galactosidase and β -N-acetylhexosaminidase, produced an equimolar amount of 2,3,4,6-tetra-O-methyl-D-mannose and 2,4,6-tri-O-methyl-D-mannose. This result indicates that the trisaccharide has the following structure: Man α 1-3Man β 1-4GlcNAc.

The data obtained by the permethylation analysis, together with other results, indicate that the urinary sialooligosaccharide possesses the following structure:

The Smith degradation of the sialooligosaccharide revealed that the consumption of periodate reached a plateau after oxidation for 24 hr, which corresponded to 5 moles of periodate per mole of the sialooligosaccharide. The sugar composition of the Smith degradation product is listed in Table 4. The results are fairly compatible with the results obtained by the enzymatic study, and by the methylation analysis.

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

Periodate consumption (moles/mole)	5.2(5.0)
Galactose	n.d.*
Mannose	0.9(1.0)
Glucosamine	1.0(1.0)
NANA	n.d.
Glucosaminitol	+ (1.0)
Glycerol	2.1(2.0)

Values are expressed as molar ratio to glucosamine.

Values in the parentheses are theoretical values.

* not detectable.

DISCUSSION

It is clear from the study described here that the sialooligosaccharide isolated from the urine of a patient with "mucopolipidosis" has the chemical structure closely resembling the carbohydrate side chains of glycoproteins with aspartylglucosylamine type linkage region. So it is highly possible that the oligosaccharide described here would be derived from incomplete catabolism of such glycoproteins due to the disturbance of the enzyme activity responsible for the degradation of glycoproteins.

Some investigators reported that an alteration in the metabolism of sialic acid-containing materials was involved in mucopolipidosis. Dawson et al. (1972) described the accumulation of sialic acid-containing glycolipids in mucopolipidosis II, and Thomas et al. (1976) observed the high level of sialic acid content in cultured fibroblasts from a patient with mucopolipidosis II. Berman et al. (1974) isolated sialic acid-containing materials from the urine of patients with mucopolipidosis III and they also observed a high content of sialic acid in the extract of leukocytes from the patients with this disease. These reports strongly indicate that mucopolipidosis could be regarded as a metabolic disorder of sialic acid-containing substances. This assumption would be in agreement with the suggestion presented by Vladutiu and Rattazzi (1975) that the higher negative charge of the isozymes of β -N-acetylhexosaminidase, which are excreted by cultured fibroblasts derived from patients with I-cell disease, would be due to the presence of additional sialic acid not present on the normal enzyme molecule.

Observations obtained by the present study are fairly coincident with the above-mentioned previous works, and the chemical structure of the sialooligosaccharide identified in the present study strongly suggests that the mucopolipidosis described here can be classified biochemically as a new type of lysosomal storage disease characterized by an abnormal metabolism of glycoproteins having aspartylglucosylamine type side chains.

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References

- 1) Berman, E.R., Kohn, G., Yatziv, S. & Stein, H. (1974) Acid hydrolase deficiencies and abnormal glycoproteins in mucopolipidosis III (Pseudo-Hurler polydystrophy). *Clin. chim. Acta*, **52**, 115–124.
- 2) Björndal, H., Lindberg, B. & Svensson, S. (1967) Gas-liquid chromatography of partially methylated alditols as their acetates. *Acta chem. scand.*, **21**, 1801–1804.
- 3) Dawson, G., Matalon, R. & Dorfman, A. (1972) Glycosphingolipids in cultured human skin fibroblasts. II. Characterization and metabolism in fibroblasts from patients with inborn errors of glycosphingolipid and mucopolysaccharide metabolism. *J. biol. Chem.*, **247**, 5951–5958.
- 4) Fukuda, M. & Egami, F. (1971) The structure of a glycopeptide purified from porcine thyroglobulin. *Biochem. J.*, **123**, 415–420.
- 5) Hickman, S. & Neufeld, E.F. (1972) A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. *Biochem. biophys. Res. Commun.*, **49**, 992–999.
- 6) Koseki, M., Wu, J.-Y., Tsurumi, K. & Nagai, (1978) Sialogluciduria in lysosomal diseases. *Tohoku J. exp. Med.*, **124**, 351–360.
- 7) Kuhn, R. & Gauhe, A. (1965) Bestimmung der Bindungsstelle von Sialinsäureresten in Oligosacchariden mit Hilfe von Periodat. *Chem. Ber.*, **98**, 395–413.
- 8) Neufeld, E.F., Lim, T.W. & Shapiro, L.J. (1976) Inherited disorders of lysosomal metabolism. *Ann. Rev. Biochem.*, **44**, 357–376.
- 9) Orii, T., Minami, R., Sukegawa, K., Sato, S., Tsugawa, S., Horino, K., Miura, R. & Nakao, T. (1972) A new type of mucopolipidosis with β -galactosidase deficiency and glycopeptiduria. *Tohoku J. exp. Med.*, **107**, 303–315.
- 10) Sanford, P.A. & Conrad, H.E. (1966) The structure of the *Aerobacter aerogenes* A3 (SI) polysaccharide. I. A reexamination using improved procedure for methylation analysis. *Biochemistry*, **5**, 1508–1517.
- 11) Spranger, J.W. & Wiedemann, H.-R. (1970) The genetic mucopolipidoses. Diagnosis and differential diagnosis. *Humangenetik*, **9**, 113–139.
- 12) Tamura, Z., Imanari, T. & Arakawa, Y. (1968) Gas chromatographic analysis of sugars in glycoproteins. Ikagaku Symposium (Proc. of the Symposium on chem. Physiol. and Pathol.), **8**, 72–75. (in Japanese with English summary)
- 13) Thomas, G.H., Tiller, G.E., Jr., Reynolds, L.W., Miller, C.S. & Bace, J.W. (1976) Increased level of sialic acid associated with a sialidase deficiency in I-cell disease (mucopolipidosis II) fibroblasts. *Biochem. biophys. Res. Commun.*, **71**, 188–195.
- 14) Uchida, Y., Tsukada, Y. & Sugimori, T. (1974) Distribution of neuraminidase in *Arthrobacter* and its purification by affinity chromatography. *J. Biochem.*, **82**, 1425–1433.
- 15) Vladutiu, G.D. & Rattazzi, M.C. (1975) Abnormal lysosomal hydrolases excreted by cultured fibroblasts in I-cell disease (mucopolipidosis II). *Biochem. biophys. Res. Commun.*, **67**, 956–964.