# STRUCTURES OF SIALYL-OLIGOSACCHARIDES EXCRETED IN THE URINE OF A PATIENT WITH MUCOLIPIDOSIS I

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## 1. Introduction

Mucolipidosis I is a rare congenital disorder of complex carbohydrate metabolism, characterized clinically by coarse facial features, skeletal dysplasia, neurodegeneration, cherry-red macular spot, mental retardation, and early death [1–3]. An abnormal accumulation of sialic acid-containing compounds in cultured fibroblasts and leukocytes, and a profoundly diminished activity of an α-neuraminidase (sialidase) in the former suggested a defect in the catabolism of sialoglycopeptides and/or gangliosides as the metabolic basis of this disease [3,4]. In this communication, we report on the excessive excretion and the structure of urinary sialyl-oligosaccharides in a patient with mucolipidosis I.

## 2. Materials and methods

Sialyl-oligosaccharides were fractionated as described in previous papers [5,6]. Their structures were determined by methylation, periodate oxidation, hydrazinolysis-nitrous deamination, and NMR spectroscopy. The chemical investigations will be developed in a following article [7].

## 3. Results

As shown in fig.1, more than 10 sialyl-oligosaccharides were identified in the urine of patient D. F. by paper chromatography of fractions eluted from a Dowex 1 × 2 column. The quantitation and carbohydrate composition of the 10 major oligosaccharides are presented in table 1. When compared to the normal, the excretion of these compounds is increased from about 80–800-fold. Figure 2 shows the complete structures of these sialyl-oligosaccharides, which closely resemble the glycan portions found in many glycoproteins. Invariably, the *N*-acetylneuraminic acid residues are located at a non-reducing terminus in either α 2–6 or α 2–3 linkage to galactose. The reducing termini consist of *N*-acetylglucosamine, suggesting that each saccharide results from the action of an endo-β-*N*-acetylglucosaminidase on the parent sialoglycopeptide.

## 4. Discussion

The excessive urinary excretion of these sialyl-oligosaccharides must arise from the α-neuraminidase deficiency in the patients tissues, which causes a block

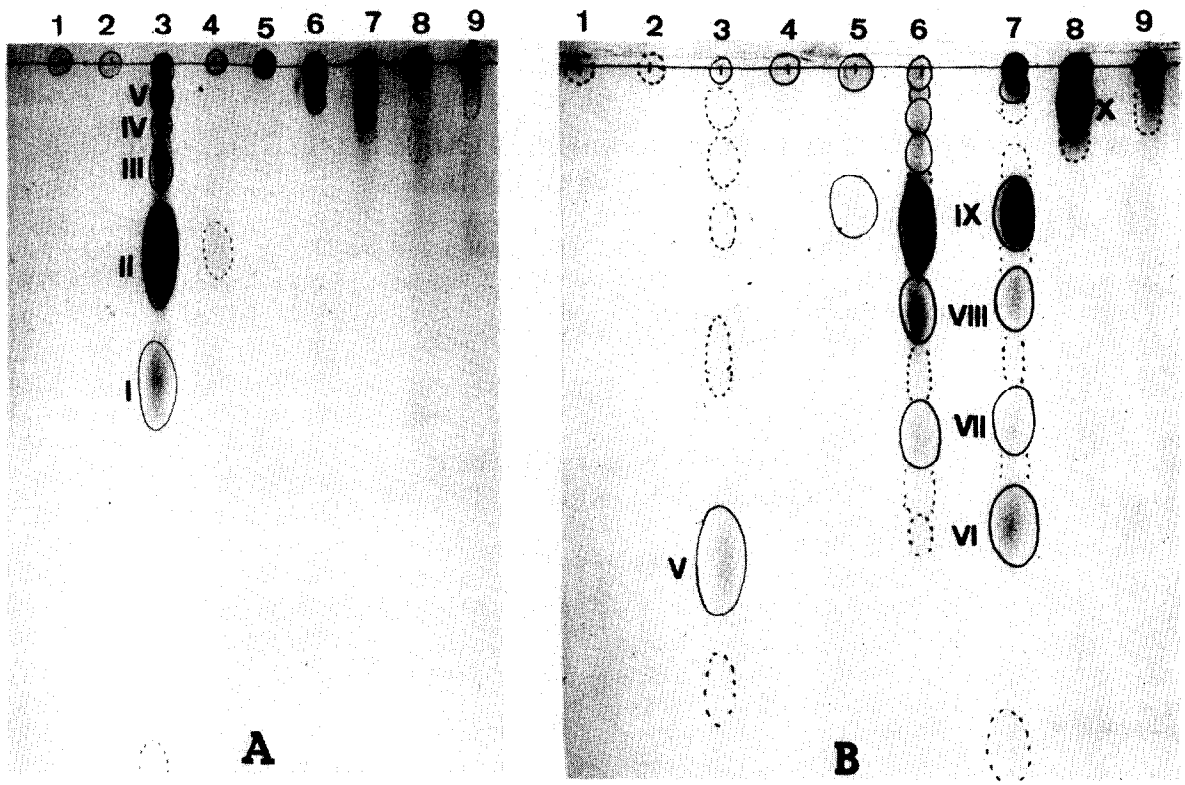


Fig.1. Paper chromatography of sialyl-oligosaccharides eluted from Dowex 1 × 2 (200–400 mesh; acetate form) by a discontinuous gradient of pyridine acetate (pH 5.4); 1 : 1 mM; 2 : 2 mM; 3 : 5 mM; 4 : 10 mM; 5 : 20 mM; 6 : 50 mM; 7 : 100 mM; 8 : 200 mM; 9 : 500 mM. Paper Whatman No. 3. Solvent: ethyl acetate/pyridine/acetic acid/water (5:5:1:3), developed during 5 days (A) or 40 days (B). Chromatograms were stained with alanine oxalat reagent.

Table 1
Carbohydrate analysis of urinary sialyl-oligosaccharides

| | Quantity (mg/l) | | Molar ratios | | | |
|---|---|---|---|---|---|---|
| | Mucolipidosis I | Normal urine | Gal | Man | GlcNAc | Ac Neu |
| I | 16 | 0.1–0.3 | 0.96 | 2 | 1.84 | 0.86 |
| II | 185 | 0.2–0.3 | 1.12 | 2 | 1.94 | 0.92 |
| III | 22 | 0.1–0.3 | 0.86 | 3 | 1.85 | 0.94 |
| IV | 18 | < 0.1 | 2.16 | 3 | 3.06 | 0.99 |
| V | 32 | 0.1–0.5 | 1.84 | 3 | 2.76 | 0.84 |
| VI | 38 | < 0.1 | 1.91 | 3 | 2.84 | 2.04 |
| VII | 11 | < 0.1 | 1.84 | 3 | 2.96 | 1.96 |
| VIII | 6 | < 0.1 | 2.05 | 3 | 3.04 | 1.94 |
| IX | 225 | 0.1–1.0 | 3.00 | 3 | 3.02 | 2.05 |
| X | 112 | ? | 3.21 | 3 | 3.95 | 2.78 |

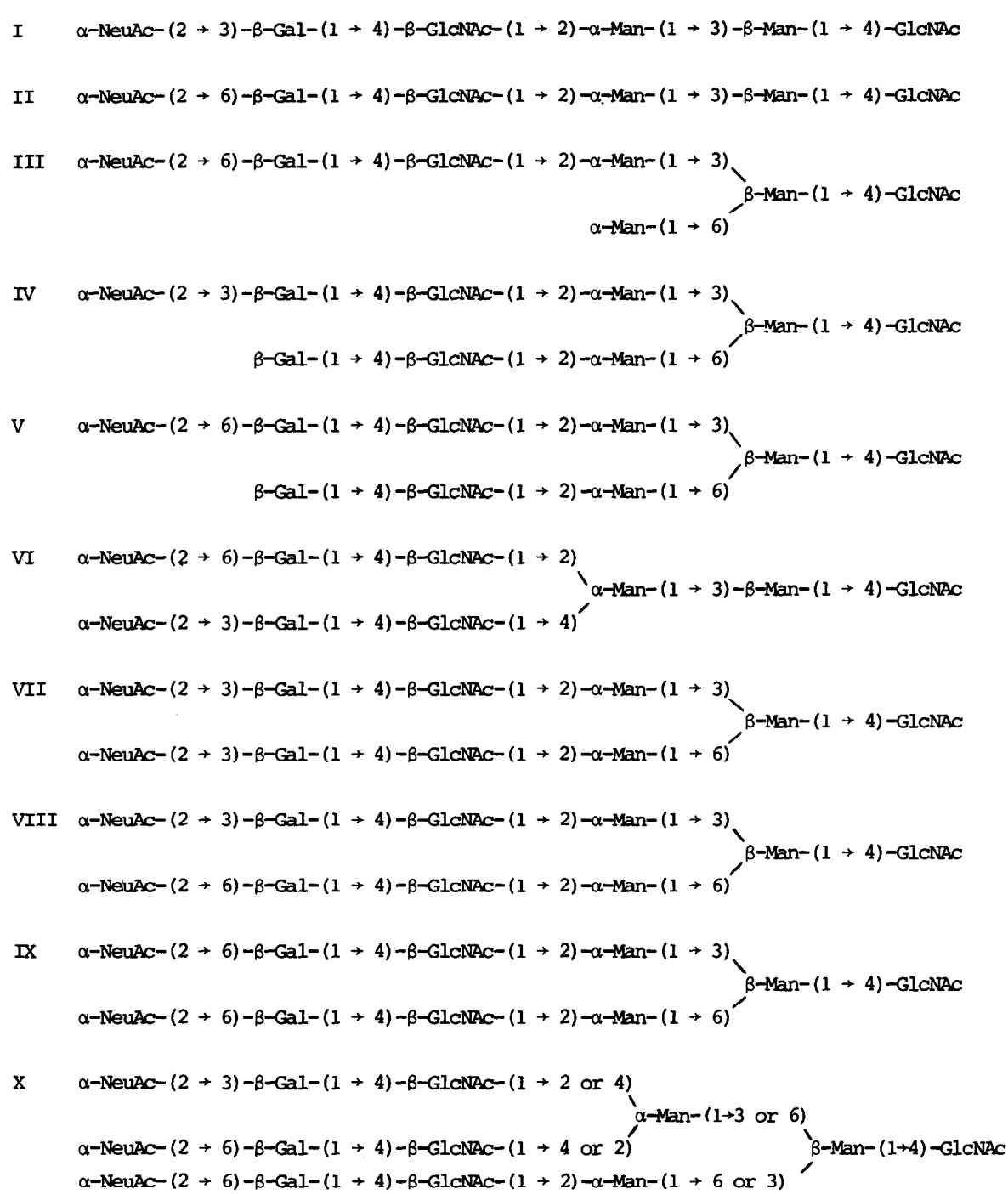


Fig.2. Structure of the 10 major sialyl-oligosaccharides isolated from MLP I urine. The structure of compound X here described results from preliminary investigations.

in the further catabolism of the chains by exoglycosidases. Urinary oligosaccharides of identical structures have been observed in three other genetic disorders with deficiency of an α-neuraminidase: mucolipidosis II and two 'new' types of mucolipidosis [6,8–10]. In mucolipidosis II, the neuraminidase deficiency probably results from a defect in lysosomal enzyme localization involving many such hydrolases [11].

In mucolipidosis I and the other two forms of mucolipidosis, however, the neuraminidase deficiency is singular, suggesting the mutation of a gene which directly codes for the expression of a neuraminidase. At present, it cannot be decided whether the marked differences in phenotype between these latter types of mucolipidoses are due to allelic mutations at a single neuraminidase gene, to a deficiency of different neuraminidases, or to still another mechanism.

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