References

Radial and Linear Thin-Layer Chromatographic Procedures Compared for Screening Urines to Detect Oligosaccharidases

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We describe a circular (radial) thin-layer chromatographic procedure for separating urinary oligosaccharides. Results were better than those obtained by a single linear development. Bands and specific patterns were finely resolved for various known oligosaccharidoses. The procedure provides a simple means of screening for these disorders.

Additional Keyphrases: inherited disorders - lysosomal storage disease

The term "oligosaccharidoses" refers to a group of genetic disorders in which there is a large excretion of oligosaccharides in the urine (1). The basic metabolic defects lie in a deficiency of lysosomal enzymes that degrade the carbohydrate moiety of glycoproteins and glycolipids (2–4). Some of these biochemical abnormalities can be readily demonstrated by thin-layer chromatographic (TLC) screening (5). Patients affected with fucosidosis, mannosidosis, and GM1-gangliosidosis have atypical and unique urinary oligosaccharide patterns, allowing these disorders to be distinguished from one another. Recently, Strecker and his associates have characterized the structures of many oligosaccharides found in the urine of patients affected with fucosidosis, sialidosis, and Sandhoff's disease (6–8). More recently, an improved TLC procedure for detection of mannosidosis has appeared (9).

In this communication, we compare the radial TLC technique with the linear development technique for separating urinary oligosaccharides. Characteristic patterns were observed with urine of patients affected with GM1 gangliosidosis (infantile and adult types), fucosidosis, mannosidosis, sialidosis (types 1 and 2), mucolipidosis II (I-cell disease), and aspartylglucosaminuria.

Materials and Methods

Samples

Untimed urine samples were collected from eight normal children two to eight years old, from three ostensibly normal adults, and from patients with the various lysosomal storage diseases described. In all cases the biochemical defects were established. All urine samples were stored at -20 °C until chromatography.
Fig. 1. Linear TLC for urinary oligosaccharides (single solvent development)
Lane widths, 1.5 cm. Lanes 1 and 2—sialidosis type 2 (mucolipidosis II); 3 and 4—sialidosis type 1 (cherry-red spot with myoclonus syndrome); 5 and 6—mucolipidosis II; 7 and 8—mannosidosis. Lanes 1, 4, 6, and 8 contain lactose marker

Materials for Thin-Layer Chromatography
Commercially available pre-coated TLC plates (E. Merck; silica gel 60 on glass, 20 × 20 cm or 10 × 20 cm, 0.25 mm in thickness, without fluorescent indicator) were used without prior preparation. The TLC plates were obtained from Scientific Products, Irvine, CA 92714. For linear chromatography we used a rectangular glass tank, and for radial chromatography the “Selecta Sol,” with wicks W-2 (Schleicher & Schuell, Keene, NH 03431).

The TLC solvent consisted of reagent-grade n-butanol/glacial acetic acid/H₂O, 2/1/1 by vol. The detection reagent was a mixture of 0.1 g of orcinol, 0.1 g of resorcinol, 4.4 mL of concd. H₂SO₄, and 35.6 mL of 95% (non-anhydrous) ethanol.

Procedure
Linear development: Portions, 10 to 40 µL, of untreated urine were mixed with lactose (2 to 5 µg) as a standard marker and applied from a capillary onto lanes 0.5 cm in width, with drying in a current of warm air between spotings. The spotting lanes were 2 cm above the bottom edge of the plate. In some cases, 1–2 cm lanes were used and sample volumes were adjusted accordingly. The application regions must be thoroughly dried (with a hair dryer) before development. Some plates were developed once in 40 mL of solvent (generally 6 h), allowed to dry overnight, and sprayed. Other plates were developed twice, once on each of two successive days. The developed, dried plates were sprayed and heated at 110 °C for 10 min, cooled, and photographed in transmitted light from an x-ray view box (no. 240050; Picker, Burbank, CA 91504).

Radial (circular) development: A circle 2 cm in diameter was drawn with a compass in the center of a 20 × 20 cm TLC plate, with care not to scratch the silica gel where the samples would be applied. Eight to 10 urine samples could be applied around the perimeter of the circle. We applied 5–20 µL aliquots containing lactose (2 to 5 µg), as a marker, as small spots and thoroughly dried the spots in a current of hot air. We placed 5 to 6 mL of solvent in the center well of the Selecta Sol and used a spacer gasket 170 mm in diameter, along with a W-2 polyethylene wick. The TLC plate was placed in the Selecta Sol holder, adsorbent side down, so that the wick transmitted the solvent to the center of the plate. Development usually took 6 h, or until the solvent front reached the edge of the spacer gasket. In some cases two developments in the solvent system were used on successive days, the better to separate oligosaccharides with low Rf values. The plates were dried, sprayed, and heated as in linear development.

Results
Figure 1 illustrates a chromatogram for urine from patients with sialidosis type 2 (mucolipidosis II), sialidosis type 1 (cherry-red spot with myoclonus syndrome), mucolipidosis II, or mannosidosis. The nomenclature for classifying sialidosis is given by Lowden and O’Brien (10). Each sample was spotted in duplicate, with and without lactose as a marker, and developed only once (5). It has been assumed that bands migrating more slowly than lactose represent compounds having two or more sugar residues, and are of primary interest. Outstanding strong bands at and above the origin were found in sialidosis type 2. Strong bands at the origin were also found in sialidosis type 1 and mucolipidosis II. Multiple bands were found in mannosidosis. In contrast, urines from the eight normal children showed fewer bands, with lesser intensity, between the lactose region and the origin (figure not shown). The strongly reactive material present at the origin probably represents oligosaccharides containing many more sugar residues, or charged groups, or both. Under our experimental conditions there were many bands in patterns from cases of mannosidosis, but they were not well separated, prompting us to try a second development, to enhance resolution.

Figure 2 illustrates the better resolution obtained from two
consecutive developments of a TLC plate in the same solvent as in Figure 1. Not only are the oligosaccharide bands in mannosidosis better resolved but also two different patients with the same disease showed essentially identical patterns (lanes 3 and 4). This TLC plate also shows the bands characteristic of different glycoprotein storage diseases. Lane 1 represents the pattern of the infantile form of GM1 gangliosidosis, lane 2 that of the adult form. In spite of the similar deficiency in β-galactosidase (EC 3.2.1.23) the oligosaccharides excreted appear to differ. Lane 5 is for a urine from a patient with sialidosis type 2 (ML-I); three strong bands were observed (including a heavy band at the origin). Lanes 6 and 7 represent two different patients having 1-cell disease and illustrate the variations in intensity of oligosaccharide bands with urine concentration. Lane 8 represents a patient with sialidosis type 1 (cherry-red spot with myoclonus syndrome). The pattern is similar to that of sialidosis type 2. Interestingly, patients represented by lanes 5–8 have low to absent sialidase activity. Lane 9 is the pattern for a dilute urine from a patient with fucosidosis. Lane 10 is representative of the pattern seen for a normal adult.

Figure 3 shows patterns of additional patients with known lysosomal storage diseases; some of the patients previously presented are also included, for comparison. The banding pattern of a known Krabbe's disease patient is shown in lane 11. Lanes 12–16 represent urines from four patients with fucosidosis (lanes 13 and 14 are two urine samples collected from the same patient on different dates). Note that the urines from all fucosidosis patients show a band just above the origin, as do all patients with neuraminidase (EC 3.2.1.18) deficiency.

Figure 4 shows the characteristic bands observed in the case of two patients with aspartylglucosaminuria (lanes 4 and 5). Identical bands with $R_f$ values below that of lactose are demonstrated. Lanes 3 and 6 are from two patients suspected of having lysosomal storage diseases. Although the diagnosis of the patient on lane 3 is not known, we have diagnosed the patient on lane 6 as a mucopolysaccharidosis with substantial dermatan sulfate excretion. Two strong oligosaccharide bands were present in the urine from each of these two patients. Whether these compounds are of any significance or are related to the actual biochemical defects is not known. Lanes 7 and 8 are urines from patients referred for oligosaccharide studies but whose patterns appear normal.

Figures 5 and 6 are patterns of oligosaccharides obtained by radial chromatography with one and two developments, respectively. The lane numbers on the TLC plates shown in Figures 2, 5, and 6 represent urines from the same patients. Evidently the bands observed for each patient with linear TLC (Figure 2) are quite comparable to those observed by circular TLC (Figures 5 and 6). However, a single development by the circular technique gives better results than does a single linear development (Figure 1). This is particularly evident for patients with mannosidosis. A second development with circular TLC is very useful for resolving bands with very low $R_f$, i.e., just above the origin, as in GM1 gangliosidosis and ML-I.

**Discussion**

Our results demonstrate that, in linear TLC, two developments improve the resolution. In addition, we conclude that circular TLC has the following advantages over the linear TLC: a single circular development is about as useful as two linear developments in resolving bands; the ultimate resolution by circular TLC appears to be better than for linear TLC; and two developments provide increased resolution of bands.

![Fig. 4. Linear TLC of urinary oligosaccharides with two developments in solvent](image)

**Figure 4.** Linear TLC of urinary oligosaccharides with two developments in solvent
Lane widths, 0.5 cm; all urine samples contain lactose marker. Lane 1—normal adult; 2—mannosidosis; 3—unknown defect; 4 and 5—two patients with aspartylglucosaminuria; 6—patient with mucopolysaccharidosis with dermatan sulfate accumulation; 7 and 8—two patients with apparently normal oligosaccharide patterns.

![Fig. 5. Circular TLC of urinary oligosaccharides, with single development in solvent](image)

**Figure 5.** Circular TLC of urinary oligosaccharides, with single development in solvent
Lane 1 (numbering clockwise from top in Figs. 5 and 6)—infantile form of GM1 gangliosidosis; 2—adult form of GM1 gangliosidosis; 3 and 4—two patients with mannosidosis; 5—sialidosis type 2 (ML-I); 6 and 7—two patients with I-cell disease (ML-II); 8—sialidosis type 1 (myoclonus); 9—fucosidosis; 10—normal adult

![Fig. 6. Circular TLC of urinary oligosaccharides, with two developments in solvent](image)

**Figure 6.** Circular TLC of urinary oligosaccharides, with two developments in solvent
Lane 1—infantile form of GM1 gangliosidosis; 2—adult form of GM1 gangliosidosis; 3 and 4—two patients with mannosidosis; 5—sialidosis type 2 (ML-I); 6 and 7—two patients with I-cell disease (ML-II); 8—sialidosis type 1 (myoclonus); 9—fucosidosis; 10—normal adult
near the origin. To the best of our knowledge, use of the circular technique for separation of urinary oligosaccharides has not been previously published.

The banding patterns appear to be very reproducible for a given disease. Specific patterns have been demonstrated in the following lysosomal storage diseases: GM$_1$ gangliosidosis (infantile form and adult form), sialidosis type 1 (myoclonus syndrome), sialidosis type 2 (ML-I), mucolipidosis II (I-cell disease), mannosidosis, fucosidosis, and aspartylglucosaminuria. The small volume required for such a simple TLC technique (as little as 20 μL of urine for diagnosis) is a tremendous advantage in screening for abnormalities of oligosaccharide metabolism. An abnormal pattern can be compared with patterns for known disease states, thereby narrowing the number of different enzyme-assay procedures required for the elucidation of the biochemical defect.

We made no attempt to differentiate those oligosaccharides that contain neuraminic acid. However, this can be done by using more selective spraying reagents, such as orcinol or resorcinol alone. Also, the mobilities of oligosaccharides containing many sugar moieties can be increased by changing the solvent composition, for example, the solvent system propanol/acetic acid/H$_2$O (3/2/2) used by O'Brien (11).

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References