Thin-Layer Chromatography of Urinary Neutral Oligosaccharides: The Detection of Blood Group-Related Oligosaccharides and Screening for Lysosomal Storage Disease

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KURIYAMA, M., HIWATARI, R. and IGATA, A. Thin-Layer Chromatography of Urinary Neutral Oligosaccharides: The Detection of Blood Group-Related Oligosaccharides and Screening for Lysosomal Storage Disease. Tohoku J. Exp. Med., 1990, 161(4), 335-341 —— We devised an improved technique of thin layer chromatography, which permitted the high resolution of urinary neutral oligosaccharides and the qualitative determination of blood group related oligosaccharides as well as oligosaccharides pathologically secreted in lysosomal storage diseases. This procedure can be used in screening for disorders associated with abnormal excretion of oligosaccharides, as well as in the purification of oligosaccharides. urinary oligosaccharides; lysosomal storage disease; blood group-related oligosaccharides; urinary screening

Many patients with inherited lysosomal storage diseases show massive urinary excretion of oligosaccharides, which results from impaired catabolism of glycoproteins or glycogen due to deficiencies of lysosomal enzymes (Hallgren et al. 1974; Strecker 1981). Investigations of the oligosaccharides accumulated in these diseases can provide valuable information on their pathogenesis and the reasons for the variety of clinical phenotypes, as well as a better understanding of the normal metabolism of various glycoproteins or glycogen. Thin-layer chromatography (TLC) analysis of urinary oligosaccharides is of value in screening for these diseases, because of its simplicity and rapidity.

Palo and Savolainen (1972) first described a TLC procedure for the detection of urinary oligosaccharides in aspartylglucosaminuria. Humbel and Collart
designed a simple method using untreated urine samples and overnight develop-
ment in a single solvent system to screen for mannosidosis, fucosidosis, Gm1
gangliosidosis and aspartlyglucosaminuria (Humbel and Collart 1975). Ther-
after, several other authors have devised sensitive and rapid techniques, including
desalting of the urine using an ion-exchange resin (Friedman et al. 1978; Sewell
et al. 1980; Blom et al. 1983), development of the chromatogram in two steps with
different solvents (Sewell 1979; Tsai and Marshall 1979), and the use of radial
TLC plates (McLaren and Ng 1979). However, most of these methods do not
provide adequate resolution of oligosaccharides, especially of the long chain
oligosaccharides.

All humans physiologically excrete various monosaccharides, disaccharides,
and oligosaccharides in their urine. Many blood group-related oligosaccharides
have been isolated from urine and their structures have been determined (Lundb-
lad 1968; Bjorndal and Lundblad 1970; Lundblad and Svensson 1973; Lundb-
lad et al. 1973; Hallgren et al. 1974; Hallgren and Lundblad 1977a, b). Thus, it
is important to distinguish the pathological oligosaccharides from physiologically
excreted oligosaccharides, and especially from blood group-related oligosacchar-
ides. However, no previous reports have described the TLC characteristics of
blood group–related oligosaccharides. In the course of purifying and characteriz-
ing the urinary oligosaccharides in sialidosis and glycogen storage disease type II
(GSD II) (Kuriyama et al. 1981; Kuriyama et al. 1985a, b), we developed a TLC
procedure which permitted the qualitative analysis of oligosaccharides. This
article describes the procedure, which provides a significant improvement in the
resolution of oligosaccharides.

MATERIALS AND METHODS

Urine specimens were obtained from 1 patient with adult type GSD II (blood group A),
1 patient with Gm1 gangliosidosis type 1 (blood group A), and 11 normal control subjects
belonging to different blood groups (A, n = 5; B, n = 2; O, n = 4). The GSD II patient
was a 23-year-old male who had muscle weakness, wasting of all extremities, and scoliosis
(Nakagawa et al. 1982). The Gm1 gangliosidosis patient was a 3-year-old boy with
psychomotor deterioration and bilateral pyramidal tract signs. The diagnosis in both cases
was based on the clinical manifestations, the morphological findings in biopsy samples, and
the biochemical demonstration of acid α-glucosidase deficiency and acid β-galactosidase
deficiency, respectively. The GSD II patient subsequently died at the age of 28 and the
Gm1 gangliosidosis patient died at the age of 9.

Secretor status was determined by Lewis blood type. Urinary oligosaccharides were
isolated by the method of Strecker et al. (1977) with slight modifications (Kuriyama et al.
1981, 1985a). Twenty-five milliliters of urine were applied directly to an activated charcoal
column (2 x 5 cm; Nakarai-tesque Ltd, Tokyo). The column was washed with 50 ml of
distilled water and the adsorbed materials were eluted with 100 ml of 50% ethanol under
suction. The ethanol solution was concentrated to dryness in vacuo and redissolved in 25
ml of distilled water. This was then applied to a Dowex 50–X2 (200 to 400 mesh, H+ from,
The Dow Chemical Company, Midland, MI, USA) column (1.5 x 5 cm) connected in series
with a Dowex 1–X2 (200 to 400 mesh, CH3COO– from, the Dow Chemical Company) column
(1.5 x 5 cm). The columns were washed with 25 ml of distilled water. Neutral oligosac-
TLC of Urinary Oligosaccharides

Charides were obtained in the fraction which passed through both columns. This fraction was concentrated to dryness in vacuo, and redissolved in 1 ml of distilled water, and subjected to high performance thin layer chromatography (HPTLC). HPTLC plates, precoated with Silica Gel 60 (10 X 20 cm, E. Merck Co., Darmstadt, FRG) were developed with 1-butanol/acetic acid/water (3:3:2, v/v/v). Prior to use, the HPTLC plates were washed by developing 3 times with the solvent in the eventual separating direction to remove substances which could interfere with chromatography. The plates were dried at room temperature and stored in a dessicator until use. TLC was usually performed in two ascents, but 3 to 4 ascents were used to resolve longer chain oligosaccharides. The separated neutral oligosaccharides were located by spraying the plates with anthrone-sulfuric acid reagent (Scott and Melvin 1953). Bands were analyzed by a Shimadzu Chromatoscanner SC-910 equipped with a Shimadzu Chromatopac C-R1A (Shimadzu Inc., Kyoto). The total amount of neutral oligosaccharides was determined on a separate aliquot of the neutral fraction of urine with the anthrone-sulfuric acid reagent, using glucose as a standard (Scott and Melvin 1953). All other chemicals were of reagent grade and were purchased from commercial sources.

RESULTS

Table 1 shows the total amount of neutral oligosaccharides, the ABO(H) and Lewis blood groups, and the secretor status of the subjects. The total amount of neutral oligosaccharides was increased about 6.6-fold in urine from the adult type GSD II patient, and about 6.0-fold in urine from the G_M1 gangliosidosis patient, compared with normal control subjects. Fig. 1 shows the HPTLC patterns of the neutral oligosaccharides of urine from individuals belonging to the blood groups

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Blood group</th>
<th>Secretor or non-secretor</th>
<th>Total amount of oligosaccharides mg/24 h urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>A</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>A</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>A</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>A</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>B</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>O</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>O</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>O</td>
<td>Le(a-, b+)</td>
<td>Secretor</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>A</td>
<td>Le(a+, b-)</td>
<td>Non-secretor</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>B</td>
<td>Le(a+, b-)</td>
<td>Non-secretor</td>
</tr>
<tr>
<td>11</td>
<td>19</td>
<td>O</td>
<td>Le(a+, b-)</td>
<td>Non-secretor</td>
</tr>
<tr>
<td>Normal controls (mean±s.d.)</td>
<td></td>
<td></td>
<td></td>
<td>55.6±10.7</td>
</tr>
</tbody>
</table>

Table 1. Total amounts of urinary neutral oligosaccharides and blood groups in G_M1 gangliosidosis, glycogen storage disease type II (GSD II), and normal control subjects
Fig. 1. Thin layer chromatogram of neutral oligosaccharides in the urine from control subjects belonging to different blood groups. The TLC plate was developed in two ascents. Some bands appeared to be ABO(H) blood group-related oligosaccharides (a, b and c), or Lewis blood group-related oligosaccharides (d). L, lactose.

Fig. 2. Thin layer chromatograms of total urinary neural oligosaccharides (A) and their densitograms of the TLC patterns (B) in the patients with lysosomal diseases. Oligosaccharides were visualized by spraying with anthrone-sulfuric acid. Lane 1, partial hydrolysate of dextran which was obtained by mild acid hydrolysis; lane 2, glycogen storage disease type II (adult type Pompe's disease); lane 3, GM1 gangliosidosis, type 1; lane 4, control subject, a blood group O(H) secretor; lane 5, control subject, a blood group A secretor. G, glucose; L, lactose. D.P., degree of polymerisation.
A, B and O(H), for both secretors and non-secretors. The TLC plate was developed in two ascents. Some differences could be recognized among the A, B and O(H) secretors. Bands a, b, and c visualized in the secretors are probably oligosaccharides related to ABO(H) blood groups. There were no differences of the TLC patterns seen in A, B and O(H) non-secretors. Band a could not be separated into further bands, when the plate was developed in four ascents. Band d may represent oligosaccharides related to the Lewis blood group. Fig. 2 shows the TLC patterns of the neutral oligosaccharides and their densitograms for the patients and for two normal controls (A and O(H) secretors). Band a in Fig. 2 may be oligosaccharides related to blood group A. Band x is a tetrassaccharide, the structure of which was previously determined as to be Glc\textsubscript{a}1→6Glc\textsubscript{a}1→4Glc\textsubscript{a}1→4Glc (Kuriyama et al. 1985a).

**DISCUSSION**

Our procedure allowed the oligosaccharides to be highly resolved by TLC for the following reasons: 1) Salt and acid sialyloligosaccharides were removed by charcoal and ion-exchange resin chromatography. 2) Substances in the silica gel which interfere with chromatography were removed by washing the plate with solvent before applying the samples. 3) The plates used were HPTLC grade. With this method, compounds shorter than decasaccharides could be resolved, as shown by the separation of a partial hydrolysate of dextran (Fig. 2). In the urine of the G\textsubscript{M\textsubscript{1}} gangliosidosis patient, some longer oligosaccharides could be also visualized as distinct bands (Fig. 2). In addition, the percentage distribution of neutral oligosaccharides could be quantitatively estimated by densitometric scanning, as these oligosaccharides were clearly separated. The densitometric scanning pattern of urinary neutral oligosaccharides in the adult type GSD II patient was very similar to the elution profile obtained by gel filtration (Bio-Gel P-2, 3 x 130 cm), as described previously (Kuriyama et al. 1985a).

Both storage disease patients excreted excess neutral oligosaccharides of different sizes in their urine. The oligosaccharides were clearly detected and the patterns on the TLC plates were characteristic for each disease. We thus confirmed potential usefulness of TLC analysis of urinary oligosaccharides in screening for GSD II and G\textsubscript{M\textsubscript{1}} gangliosidosis. Furthermore, we could also obtain the total sialyloligosaccharides with this system by elution of the charged species from the Dowex 1 x 2 column with 1.0 M pyridine-acetate buffer, pH 5.4. Thus, the TLC analysis can also be applied to screening for sialidosis and mucolipidoses (Kuriyama et al. 1981).

In about 80% of all individuals, the A, B, and O(H) antigens can be detected into saliva and other exocrine secretions. Such people are therefore called secretors while the other 20% are called non-secretors. Secretor status can be determined by the Lewis blood group (Le(a-, b+) for secretors and Le(a+, b-) or Le(a-, b-) for non-secretors). Blood group active tetrassaccharides and
pentasaccharides have been isolated from the urine of ABO(H)-secretors (Lundblad 1968; Björndal and Lundblad 1970; Lundblad and Svensson 1973). Additional excretion of blood group active disaccharides or trisaccharides in the urine of ABO(H)-secretors can be induced by food intake, especially the ingestion of galactose or lactose (Lundblad et al. 1973). Several kinds of Lewis antigen-related oligosaccharides were also isolated from the urine of both secretors and non-secretors (Hallgren and Lundblad 1977a, b). The TLC patterns we detected suggested that several bands might be blood group-related oligosaccharides (Fig. 1). It seems possible that band a was a blood group A active pentasaccharide (GalNAcα1→3(Fucα1→2)Galβ1→4(Fucα1→3)Glc) or a blood group B active pentasaccharide (Galα1→3(Fucα1→2)Galβ1→4(Fucα1→3)Glc), which could not be separated on TLC. Bands b, c and d may represent the B active trisaccharide, Galα1→3(Fucα1→2)Gal, the O(H) active tetrasaccharide, Fucα1→2Galβ1→4(Fucα1→3)Glc, and a Leα antigen related oligosaccharide, respectively. Blood group related oligosaccharides (band a), could also be detected by TLC of the pathological urine (Fig. 2).

In conclusion, this improved TLC procedure permits high resolution of urinary oligosaccharides and the quantitative estimation of amounts of secreted oligosaccharides. This procedure can be used in screening for disorders associated with abnormal excretion of oligosaccharides, as well as in the purification of oligosaccharides.

Acknowledgments

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References

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