ISOLATION AND CHARACTERIZATION OF A BLOOD GROUP A-SPECIFIC URINARY TETRASACCHARIDE

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1. Introduction

Blood group A-, B- and H-active tetra- en pentasaccharides have been isolated from urine of starved ABH secretors [1,2]. Ingestion of free galactose or a glycoside of galactose (lactose) induces the formation and excretion of blood group specific di- and trisaccharides [3,4] and small amounts of hexa- and heptasaccharides [4]. The serological activities of some A- and B-specific oligosaccharides have been studied and compared with those of oligosaccharides from soluble blood group A and B substances [3,5]. When urine from individuals of different ABO blood group and secretor status was analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) for the content of various oligosaccharides, a new component was discovered in some, but not in all, urines of blood group A secretors. We now report the isolation, characterization and serological characterization of this new component.

2. Experimental

Urine produced during 6 h following 12 h starvation was collected from 10 healthy individuals of different ABO blood group and secretor status, after fasting or oral ingestion of an aqueous solution of galactose (0.35 g/kg body wt) or lactose (0.70 g/kg body wt). Water was allowed ad libitum. (20 l) was also collected from a blood group A secretor without any dietary restrictions. All urines were stored at −20°C until required. The 6 h urine portions were deionized by passage through a mixed-bed ion-exchange resin. To 20 ml of the desalted urine 200 μg isomaltotetraose were added as internal standard and the sample was reduced and permethylated. The permethylated oligosaccharides were analyzed by GLC–MS [6].

Isolation of the A-active tetrasaccharide was carried out using gel chromatography on Sephadex G-15 (for experimental details see legend to fig.1), and preparative paper chromatography was done on Whatman no. 3 papers using the developing systems (a) ethyl acetate/acetic acid/water 3/1/1 (by vol.) and (b) ethyl acetate/pyridine/water 10/4/3 (by vol.). Colorimetric methods were used for the determination of total hexose [7] and deoxyhexose [8]. The structure of the isolated oligosaccharide was deduced from results obtained from optical rotation, sugar and methylation analyses [1,2] and determination of serological activity [3,5,9]. The instrumentation and experimental conditions have been detailed in [10].

3. Results

Urine (20 l) from an A secretor was desalted, concentrated (rotatory evaporation) and fractionated by gel chromatography (fig.1a). The tetra-pentasaccharide region (II) was pooled as indicated, concentrated and further fractionated by preparative paper chromatography (fig.1b). Two main fractions were obtained (II_1,II_2). The latter had a mobility identical to urine A-pentasaccharide (for structure see table 1). Fraction II_1 was run preparatively also in solvent system b

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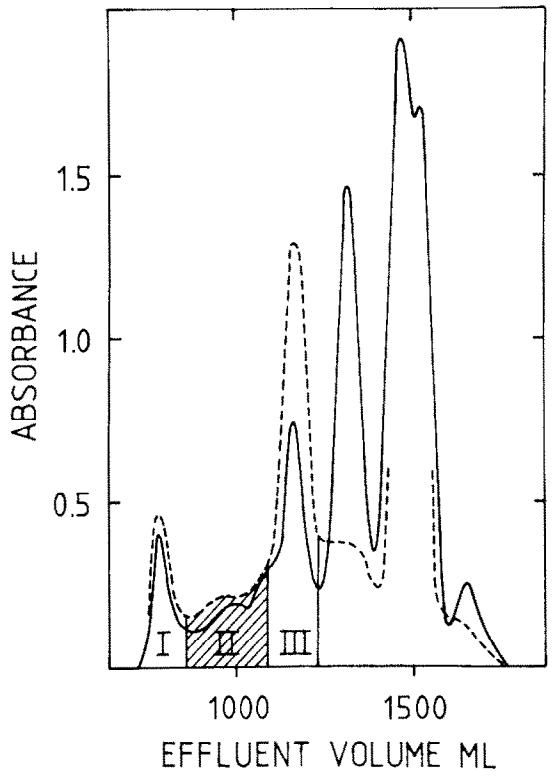

a

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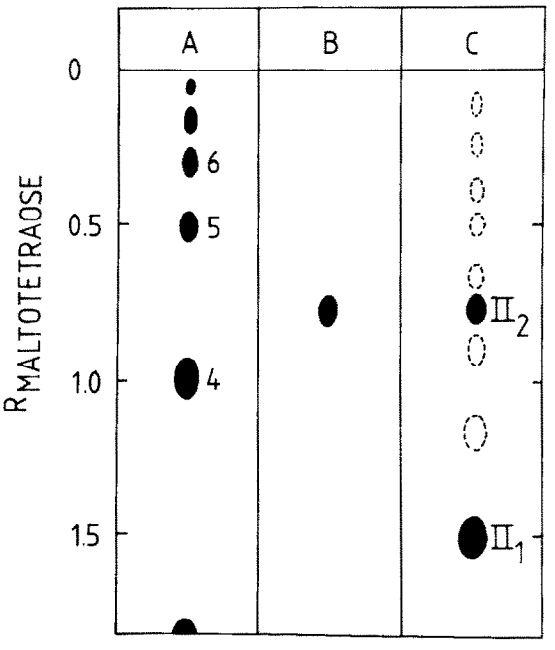


Table 1

Blood group A-active oligosaccharides

Trivial name (abbreviation)	Structure	Ref
A trisaccharide (A tri)	GalNAc($1\xrightarrow{\alpha}3$)Gal α^2_1 Fuc	(3)
A tetrasaccharide (A tetra)	GalNAc($1\xrightarrow{\alpha}3$)Gal($1\xrightarrow{\beta}4$)Glc α^2_1 Fuc	
A pentasaccharide (A penta)	GalNAc($1\xrightarrow{\alpha}3$)Gal($1\xrightarrow{\beta}4$)Glc α^2_1 α^3_1 Fuc Fuc	(1)
A heptasaccharide (A hepta)	GalNAc($1\xrightarrow{\alpha}3$)Gal($1\xrightarrow{\beta}3$)GlcNAc($1\xrightarrow{\beta}4$)Gal α^2_1 α^4_1 α^6_1 Fuc Fuc Fuc	(4)
MSS AR_L O 52	GalNAc($1\xrightarrow{\alpha}3$)Gal($1\xrightarrow{\beta}4$)GlcNAc($1\xrightarrow{\beta}6$)-R α^2_1 Fuc	(11)

and was thereafter found homogeneous in 5 additional solvent systems. The yield was 2.8 mg/l urine.

Purified fraction II_1 ($[\alpha]_D^{20} + 52.5°$) yielded on sugar analysis D-glucose, D-galactose, D-fucose, and *N*-acetyl-D-galactosamine in the relative molar proportions 1.0:0.9:0.9:1.1. These sugars accounted for >85% of the material. Small amounts of D-mannose were also present. By methylation analysis, the following partially methylated sugar derivatives were identified in about equimolar proportions: 2,3,4-tri-*O*-methyl-L-fucitol, 1,2,3,5,6-penta-*O*-methyl-D-glucitol-1-*d*, and 4,6,-di-*O*-methyl-D-galactose. Two derivatives of hexosamine were found: 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-galactitol; and 2-(*N*-methyl)-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-galactitol.

The blood group A activity of compound II_1 was determined (fig.2). The activity was found to be almost as high as the most active oligosaccharide

Fig.1a. Gel chromatographic distribution of hexose- and deoxyhexose(fucose)-containing material in urine from a blood group A secretor. Concentrated urine (20 ml) was applied. The fractionation was performed on a Sephadex G-15 column (5 × 130 cm; V_o 800 ml) elution rate, 70 ml/h; eluent, distilled water. (——) Total hexose; (– – –) fucose.

Fig.1b. Fractionation by paper chromatography in solvent (a). (A) Partial hydrolysate of starch: 4, maltotetraose; 5, maltopentaose; etc. (B) Urine A pentasaccharide. (C) Gel chromatographic fraction II.

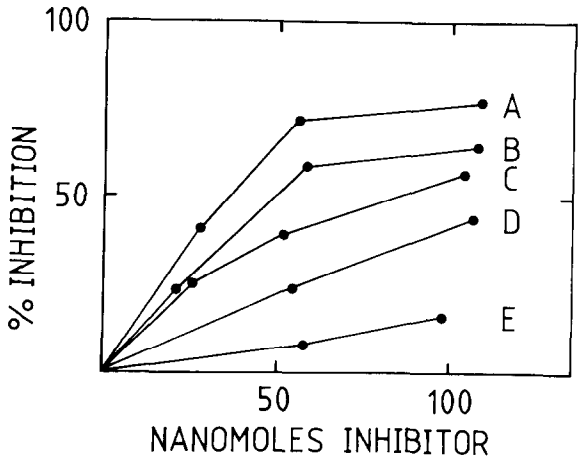


Fig.2. Inhibition of precipitin reaction by different A-active oligosaccharides. Increasing amounts of oligosaccharide was added to 0.2 ml anti-A serum (ortho) and 12.2 μg human A substance in 375 μl a total vol. 0.15 M NaCl. (A) MSS AR_L 0.52. (B) Urine A tetrasaccharide. (C) Urine A trisaccharide. (D) Urine A pentasaccharide. (E) Urine A heptasaccharide.

(MSS AR_L 0.52) isolated from soluble blood group A substance and more active than any of the previously isolated A-active urinary oligosaccharides (table 1).

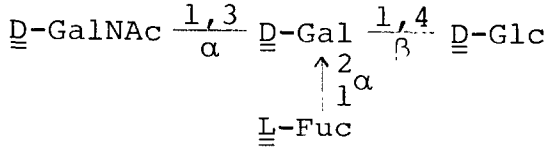
The results obtained are compatible with the structure given for urine A tetrasaccharide (table 1, fig.3).

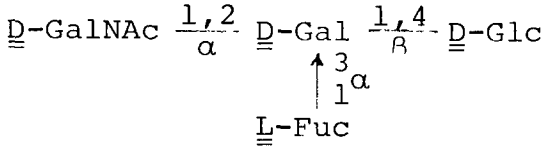
Quantitation of the urine A tetrasaccharide as well as of the urine A trisaccharide was done by GLC–MS.

The mass spectrum and primary fragmentation of reduced and permethylated A tetrasaccharide are given in fig.3. Results from quantitation are seen in table 2. The A tetrasaccharide was present only in one out of four A secretors studied. The excretion rate increased slightly after ingestion of galactose or lactose but not to the same extent as is the case for the A trisaccharide.

4. Discussion

The data from sugar and methylation analyses of urine A tetrasaccharide are compatible with two different structures:





The high blood group A activity found makes the second structure highly unlikely, since the structure

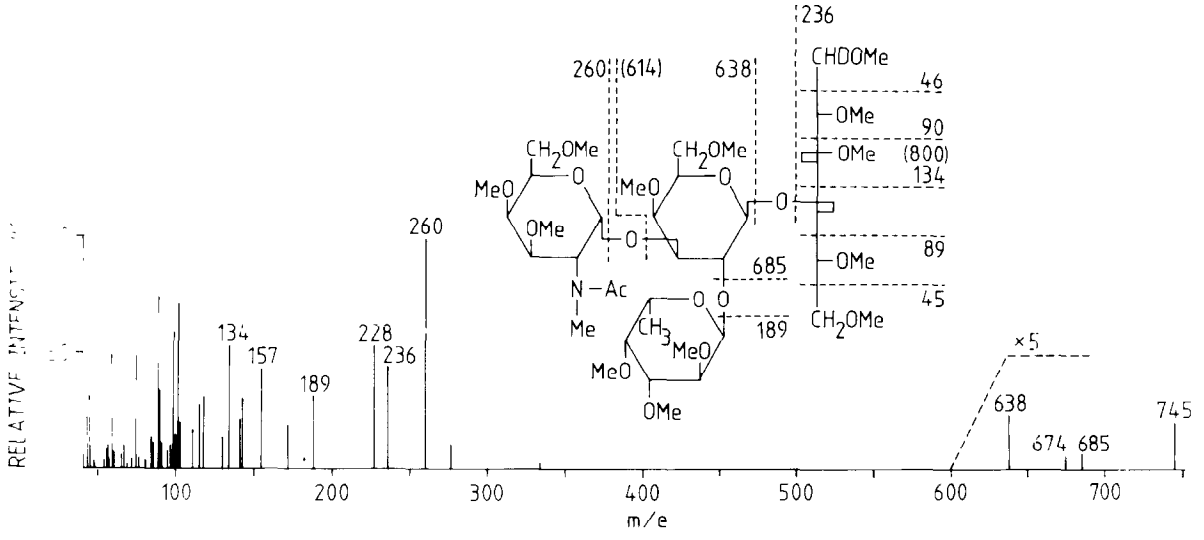


Fig.3. Mass spectrum and some important primary fragments of urine A tetrasaccharide.

Table 2
Excretion rate[a] of 'A tri' and 'A tetra' in urine of four blood group A individuals[b]

Subject	Secretor status	Subgroup of A	Diet	'A tri' (mg/6 h)	'A tetra' (mg/6 h)
A.E.	+	A_1	Starved	0	0.5
A.E.	+	A_1	Oral galactose	74	2.0
A.E.	+	A_1	Oral lactose	37	1.2
A.M.	+	A_1	Oral lactose	49	0
A.L.	+	A_1	Oral lactose	130	0
G.L.	–	A_2	Oral lactose	0	0

[a] Identification based on identical retention times and mass spectra as compared to those of authentic samples
[b] 'A tri' and 'A tetra' were both absent in urines from individuals of other ABO blood groups

of the blood group A hapten is so well established that results from serological tests can serve as a structural proof. The quantitation data reveal a slight dependence in excretion rate on oral galactose or lactose intake, and in this respect 'A tetra' behaves similarly as 'A penta' but is completely different from 'A tri' which is absent in urine of starved A secretor urine but becomes the predominant oligosaccharide in normal urine after galactose or lactose ingestion.

The biosynthetic site for 'A tetra' as well as 'A penta' is unknown whereas the intestine is the most probable place for biosynthesis of the 'A tri', by analogy with what was shown for B-trisaccharide [12,13].

Analogous compounds might be expected to occu in urine of B and O(H) secretors, but have so far not been discovered with the following exceptions. In O(H) secretors the analogous compound would be 2′-fucosyllactose. This compound, which is a characteristic milk oligosaccharide has been found in urine of pregnant and lactating women [10] and is also present in urine of newborns receiving human milk (unpublished).

The reason why 'A tetra' is not found in all A secretors is not known although individual variability in the activity of different fucosyltransferases is a possibility.

Acknowledgements

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