AN α-L-FUCOPYRANOSYL-MYO-INOSITOL IN NORMAL HUMAN URINE

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Summary: An α-L-fucopyranosyl-myo-inositol has been isolated from normal urine of ABH-secretors. The compound was also present in small amounts in the urine of ABH-non-secretors. After ingestion of 20 g of myo-inositol, two secretors increased their excretion of α-L-fucopyranosyl-myo-inositol three and thirteen times respectively. The same myo-inositol diet did not give rise to any increased excretion of α-L-fucopyranosyl-myo-inositol in the urine of two non-secretors.

INTRODUCTION

Galactinol (1-L-1-O-α-D-galactopyranosyl-myo-inositol) was the first glycoside of myo-inositol to be isolated (1). It was found to occur in the juice of the sugar beet. The structure was elucidated by Kabat et al. (2). From rape-seed meal a trisaccharide identified as α-D-Galp-(1→6)-α-D-Galp-(1→1) myo-inositol was isolated (3). Two other glycosides, β-D-Gal-(1→6)-myo-inositol from rat tissues (4, 5, 6) and β-D-Man-(1→6)-myo-inositol from Saccharomyces cerevisiae (7), have also been described. The first glycoside of myo-inositol in human material was found in 1970 when one of us reported on the presence of a fucosyl-myo-inositol in the urine of ABH-secretors (8). We now present further structural studies of this substance. A gas chromatographic—mass spectrometric method (9, 10) has been used to determine the urinary excretion rate of α-L-fucopyranosyl-myo-inositol in two secretors and two non-secretors on myo-inositol diet.

EXPERIMENTAL PROCEDURE

Materials. Pooled urine (5 liters) was collected from ten healthy ABH-secretors without any dietary restrictions. Urine samples produced during six hours following 12 hours of starvation and ingestion of 20 g of myo-inositol was collected from four individuals, two secretors (blood groups A and 0) and two non-secretors (blood groups A and B). Control samples were collected the same way, but omitting the myo-inositol. 30 ml of saturated phenyl mercuric nitrate solution was added per liter of urine to prevent bacterial growth. Galactinol was a gift from Dr. Clinton Ballou, University of California, Calif., U.S.A.

Isolation Procedure. The pooled urine samples were purified by ultrafiltration, concentration and gel chromatography (Sephadex G-25) as previously described (11). The disaccharide region (VI) was pooled, deionized by passage through Dowex 50 (H^+) and Bio-Gel AG3-X4A (OH^-), and fractionated by preparative descending paper chromatography on Whatman No. 1 paper (butan-1-ol - pyridine - water, 3:2:1.5, v/v) (double development) (Fig. 1). Fraction VI b, known to contain the fucosyl-myo-inositol (8), was further purified by paper chromatography using first ethyl acetate - acetic acid - water (3:1:1, v/v) (48 hours) and then propan-1-ol - ethyl acetate - water (6:1:3, v/v) (24 hours). The fucosyl-myo-inositol obtained was finally purified by passage through a Bio-Gel P-2 column. Approximately 2 mg of purified fucosyl-myo-inositol per liter of urine was isolated.

Analytical Methods. Sugar analysis was performed by gas liquid chromatography (12) and mass spectrometry (13). Optical rotations were determined using a Perkin-Elmer 2411 polarimeter. Methylation analysis was performed as previously described (14). Analysis of permethylated disaccharide was done using a glass capillary column (25 m x 0.25 mm) wall-coated with SE-30 (LKB, Stockholm, Sweden). The separations were performed at 200°C. For gas liquid chromatography—mass spectrometry, the same column as above was

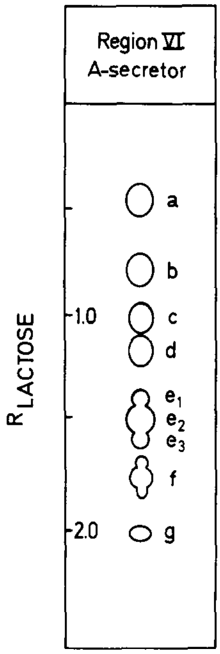

Fig. 1. Paper chromatography of gel chromatographic fraction VI (disaccharide region) from urine of a starved A-secretor. The paper was developed in butan-1-ol - pyridine - water (3:2:1.5, v/v).

introduced directly into the ion source of a Varian MAT 311 A combined gas liquid chromatography—mass spectrometry instrument. The mass spectra were recorded at an ionization potential of 70 eV, an ionization current of 3 mA and an ion source temperature of 120°C. The data were processed by on-line computer system (Spectrosystem 100 MS, Varian MAT). The procedure for quantitation of fucosyl-myo-inositol was essentially the same as previously described for other urinary oligosaccharides (9, 10, 15). Cellobiose was used as an internal standard and the retention time of its reduced and permethylated derivative was set to 1 (T=1). A linear response (k = 1.6) was obtained within the range 25-150 μg. The identification of the fucosyl-myo inositol in the different urine samples was based on identical retention time and mass spectrum as compared to those of an authentic sample.

RESULTS AND DISCUSSION

Characterization. Purified fraction VI b, $[\alpha]_D^{20}$ -70° (c 1.35, water), on acid hydrolysis yielded equimolar amounts of fucose and myo-inositol. The products in the neutralized hydrolysate were treated with sodium borohydride and acetylated and the components identified as fucitol pentaacetate and myo-inositol hexaacetate by gas liquid chromatography (co-chromatography with authentic samples) and mass spectrometry. Isomeric 6-deoxyhexitol pentaacetates and inositol hexaacetates are well separated from those obtained.

In a hydrolysed sample, the optical rotation should be due only to the fucose, as myo-inositol is optically inactive. A hydrolysate of 0.64 mg glycoside in 1 ml 0.125 M sulfuric acid showed $[\alpha]_D^{20}$ -0.027° from which the value $[\alpha]_D^{20}$ -84° for the fucose could be calculated. This is in reasonably good agreement with the value for L-fucose, $[\alpha]_D^{20}$ -76°, and demonstrates that the fucose moiety in the glycoside has the L-configuration.

The fully methylated glycoside gave a single peak on gas liquid chromatography ($T_{\text{cellobiitol}}$ = 1.14) and its mass spectrum is given in Fig. 2. Strong fragments of m/e 233 and 201 derive from the inositol moiety as indicated in Fig. 3a. A strong J_1 fragment (Fig. 3b), m/e 293, was also obtained. Only weak fragments in the A series, e.g. A_1 (m/e 189), were observed, and the reason for this is not understood. Mass spectrometry of fully methylated galactinol was, however, perfectly analogous. Hydrolysis of the methylated glycoside yielded 2,3,4-tri-O-methyl-L-fucose and a penta-O-methyl-myo-inositol, as evident by gas liquid chromatography—mass spectrometry of the product obtained after borohydride reduction and acetylation. The results above consequently demonstrate that the L-fucosyl group in the glycoside is pyranosidic and, from the negative value of the optical rotation, α-linked.

The O-acetyl-penta-O-methyl-myo-inositol obtained from the glycoside was chromatographically distinguishable from the corresponding derivative obtained on methylation analysis of galactinol. The α-L-fucopyranosyl group is therefore not linked to O-1 (O-3) of myo-inositol. The nature of this linkage is subject to further studies.

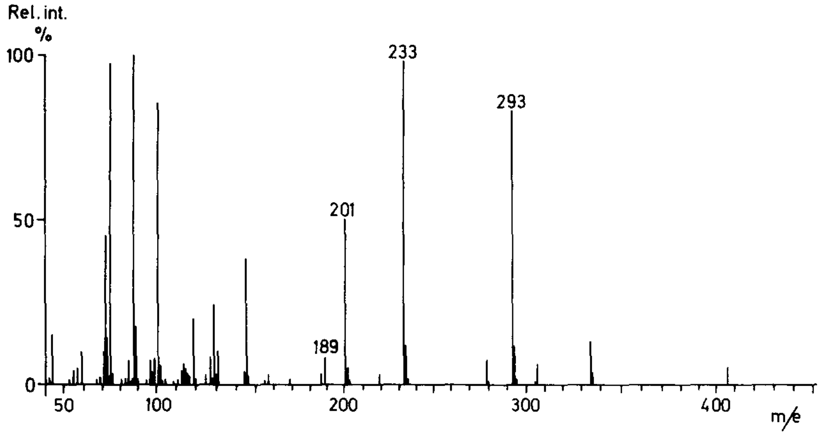

Fig. 2. Mass spectrum of α-L-fucopyranosyl-myo-inositol as its permethylated derivative.

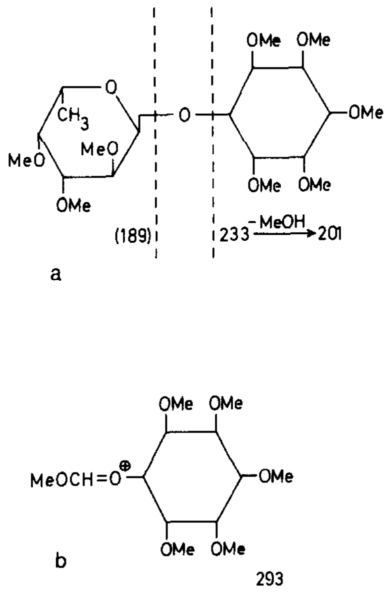

Fig. 3. a) Primary fragmentation of α-L-fucopyranosyl-myo-inositol as its permethylated derivative.
b) J_1 fragment m/e 293.

Quantitation. The content of fucosyl-myo-inositol in the disaccharide region of individual secretor and non-secretor urine samples was determined by gas liquid chromatography—mass spectrometry (Table I). Approximately

Table I

Quantitative determinations of α-L-fucopyranosyl-*myo*-inositol in different urine samples

		α-L-Fucopyranosyl-*myo*-inositol mg/24 h	
	n	Starved	Oral *myo*-inositol
A-secretor	1	1.2	16.0
0-secretor	1	3.2	10.4
Non-secretor	2	0.4	0.4

2-3 mg per 24 h was excreted by the starved secretors. A three and thirteen fold increased excretion respectively was observed after ingestion of *myo*-inositol. A small amount (approximately 0.4 mg/24 h) was excreted by two non-secretors.

Ingestion of 20 g of *myo*-inositol did not affect the excretion rate of fucosyl-*myo*-inositol in the two non-secretors. The origin and physiological role of this new component is not known. It has not as yet been possible to demonstrate the addition of fucose to either inositol or phosphatidyl inositol using α-L-fucosyltransferases from a variety of human sources (16), and therefore it is difficult to explain why the excretion rate of α-L-fucopyranosyl-*myo*-inositol is secretor dependent.

ACKNOWLEDGEMENTS

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