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Isolation and Characterization of 3-*O*-α-D-Xylopyranosyl-D-glucose and 2-*O*-α-L-Fucopyranosyl-D-glucose from Normal Human Urine†

Arne Lundblad* and Sigfrid Svensson

ABSTRACT: Two disaccharides, 3-*O*-α-D-xylopyranosyl-D-glucose and 2-*O*-α-L-fucopyranosyl-D-glucose, have been isolated from normal human urine. Their structures have been established by sugar analysis and methylation analysis. Gas–liquid chromatography and mass spectrometry of the permethylated disaccharide alditols confirmed their homogeneity and structures.

Normal human urine contains a considerable number of low molecular weight carbohydrate components (Boas, 1956; Lundblad, 1966, 1970; Lundblad and Berggård, 1962; Miettinen, 1962, 1963; Bourillon, 1970; Bourillon *et al.*, 1962; Hakomori *et al.*, 1962; Huttunen, 1966). Some of the excreted oligosaccharides are related to the ABO blood group and the secretor status of the individual (Lundblad, 1966, 1970; Björndal and Lundblad, 1970; Lundblad and Kabat, 1971). Secretors, in contrast to nonsecretors, have fucose-containing components in their urine, in addition to ABH-specific oligosaccharides. These fucose-containing components are eluted as disaccharides in gel chromatography. Previously, L-fucopyranosyl-*myo*-inositol, a new disaccharide, was isolated from human urine (Lundblad, 1970). The present study reports the isolation and characterization of two new disaccharides, 3-*O*-α-D-xylopyranosyl-D-glucose and 2-*O*-α-L-fucopyranosyl-D-glucose, from the urine of normal human ABH secretors.

Materials and Methods

Urine was collected from 14 healthy male, secretor individuals belonging to different ABO blood groups and from five healthy nonsecretors of blood group O. The urines were pooled in the following way: (I) ten O (H) secretors starved for at least 16 hr. This pool was produced during 98 hr of starvation; (II) three nonstarved secretors of blood group A, volume 8 l.; (III) one nonstarved blood group B secretor, volume 1.5 l.; and (IV) five nonsecretors of blood group O, starved for at least 16 hr. Urine was collected during 20 hr of starvation.

Preservation. Bacterial growth was prevented by the addition of phenylmercuric nitrate (30 ml of saturated solution/l. of urine).

Analytical Methods. Colorimetric methods for determination of 6-deoxyhexose and hexose and the enzymatic assay for D-glucose have been described earlier (Lundblad, 1966, 1967).

Gel chromatography, preparative zone electrophoresis, and preparative paper chromatography of oligosaccharides were performed as previously described (Lundblad, 1966, 1967) using the following buffers, solutions, and solvent mixtures: 2 M acetic acid (pH 1.9) (a), pyridine–acetic acid–water (100:6:894, v/v, pH 6.5) (b), 1-butanol–pyridine–water (3:2:1.5, v/v) (c), ethyl acetate–acetic acid–water (3:1:1, v/v) (d), 1-butanol–acetic acid–water (4:1:5, v/v) (e), and 1-butanol–formic acid–water (8:2:1, v/v) (f).

Sugar analysis was performed by gas–liquid chromatography (glc) (Sawardeker *et al.*, 1965) and mass spectrometry (Golovkina *et al.*, 1966). The absolute configuration of the sugars was determined by optical rotation.

Methylation analysis was performed as previously described (Björndal *et al.*, 1970).

Analysis of the disaccharides as permethylated alditols by glc was done using the column 5% XE-60 on Chromosorb W 80–100 mesh) at 200°. For mass spectrometry, a Perkin-Elmer 270 GLC-MS instrument fitted with the above column was used. Mass spectra were recorded at an ionization potential of 70 eV, an ionization current of 80 μA and an ion source temperature of 80°.

Results

Isolation of the Disaccharides. The four pools of urine were filtered and ultrafiltered at 4° using Visking $^{23}/_{32}$-in. dialysis tubing (Union Carbide Corp., Chicago, Ill.) and a negative pressure of 660 mm of Hg. (The tubing retains protein molecules larger than 10^4; Berggård, 1962.) The ultrafiltrates were concentrated ten times and applied to a Sephadex G-25 (fine) column (10 × 109 cm). The eluates were analyzed for 6-deoxyhexose. The elution patterns for pool I and IV are shown in Figure 1.

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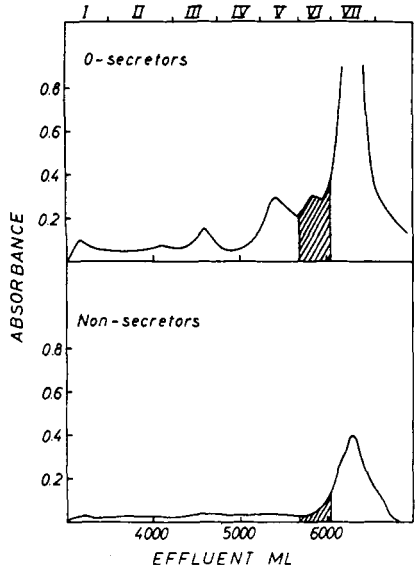

FIGURE 1: Gel chromatographic distribution of fucose-containing material in ultrafiltrates of urine from *O* secretors (pool I) and non-secretors (pool IV); 100 ml of concentrated ultrafiltrate of each pool was applied. The fractionation was performed on a Sephadex G-25 column (10 × 109 cm). Void volume indicated with arrow. Elution rate, 100 ml/hr. Eluent, distilled water.

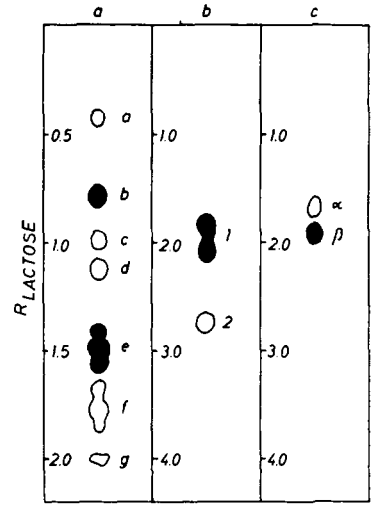

FIGURE 2: (a) *O*-secretor fraction VI from electrophoresis buffer b, further fractionated by paper chromatography (Whatman 1); solvent c. (b) *O*-secretor fraction VIe further fractionated on papers developed with system d. (c) *O*-secretor fraction VIe1 further fractionated on papers developed with system e. Papers were stained with a silver dip reagent (Smith, 1960). Fucose-containing fractions are indicated with dark spots.

The 6-deoxyhexose-containing material in region VI, which includes the characteristic secretor peak (Lundblad, 1966), was pooled, as indicated in Figure 1. This pool was purified by zone electrophoresis using buffers a and b. The 6-deoxyhexose-containing material was stationary in the electrophoresis procedures. The neutral fraction was further fractionated by preparative paper chromatography using solvent c (Figure 2a). Fractions VIb and VIe from secretors were the only 6-deoxyhexose-containing fractions obtained. No corresponding 6-deoxyhexose-containing fractions were found in region VI from nonsecretors. Further purification of fraction VIb, from secretors, yielded a pure L-fucosyl-*myo*-inositol (Lundblad, 1970). Fraction VIe, from secretors, was further fractionated by preparative paper chromatography (solvent d) (Figure 2b). The 6-deoxyhexose-containing material now present in fraction VIe1 was finally fractionated by paper chromatography (solvent e) into two apparently pure compounds VIe1α and VIe1β (Figure 2c). Fractions VIe1α and VIe1β from secretors of different ABO blood groups were pooled to give 15.6 and 14.4 mg, respectively.

Characterization of the Disaccharides. Fraction VIe1α, $[\alpha]^{20}D$ +103° (*c* 1.9, water), yielded on hydrolysis D-glucose and D-xylose in the relative proportions 1.0:1.0. These sugars constituted 98% of the dry weight of fraction VIe1α. After reduction with sodium borodeuteride VIe1α, on acid hydrolysis, released D-glucitol-*1-d* and D-xylose in equimolar proportions. Methylation of VIe1α, which had been reduced with sodium borodeuteride, gave the permethylated disaccharide alditol which was homogeneous on glc. Mass spectrometry of the permethylated, reduced disaccharide alditol had peaks at *m/e* 45 (61%), *m/e* 46 (13%), *m/e* 59 (37%), *m/e* 60 (19%), *m/e* 88 (63%), *m/e* 89 (25%), *m/e* 90 (25%), *m/e* 99 (37%), *m/e* 101 (100%), *m/e* 111 (6%), *m/e* 115 (31%), *m/e* 116 (26%), *m/e* 133 (5%), *m/e* 143 (61%), *m/e* 175 (69%), *m/e* 236 (22%), and *m/e* 296 (1%). These peaks are expected for a permethylated 3-*O*-pentapyranosylhexitol containing a deuterium atom at C-1 of the hexitol residue. The primary fragmentation routes (Kärkkäinen, 1970) are depicted in Figure 3.

Hydrolysis of reduced and permethylated VIe1α yielded 2,3,4-tri-*O*-methyl-D-xylose and 1,2,4,5,6-penta-*O*-methyl-D-glucitol-*1-d*, which were identified as their alditol acetates by gas–liquid chromatography–mass spectrometry (Björndal *et al.*, 1970). The *T* value (relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol) for the previously unknown 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-D-glucitol-*1-d* was 0.48 on the ECNSS-M column. Its mass spectrum showed peaks at *m/e* 43 (100%), *m/e* 45 (20%), *m/e* 46 (15%), *m/e* 59 (40%), *m/e* 60 (46%), *m/e* 75 (40%), *m/e* 89 (40%), *m/e* 90 (80%), *m/e* 101 (76%), *m/e* 133 (20%), *m/e* 174 (10%), and *m/e* 206 (42%). These peaks correspond to the expected fragments from a 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylhexitol-*1-d*. The primary fragmentation pattern is shown in Figure 4.

Fraction VIe1β, $[\alpha]_D^{20}$ −65.5° (*c* 1.8, water), gave equimolar proportions of L-fucose and D-glucose on acid hydrolysis. The total yield of these sugars was 96% of the dry weight. Reduction with sodium borodeuteride followed by acid hydrolysis released L-fucose and D-glucitol-*1-d* in the relative, molar proportions 1.0:1.0. Methylation of VIe1β which had been reduced with sodium borodeuteride gave a single component on glc and the permethylated derivative had a mass spectrum

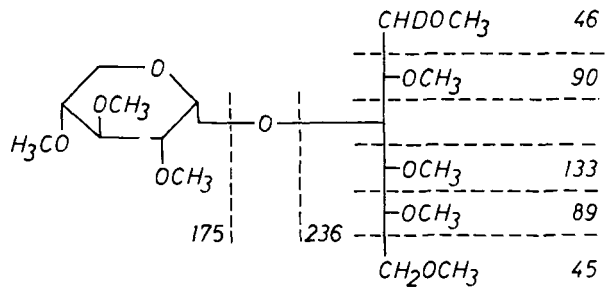

FIGURE 3: Primary fragmentation pattern for permethylated 3-*O*-α-D-xylopyranosyl-D-glucitol-*1-d*.

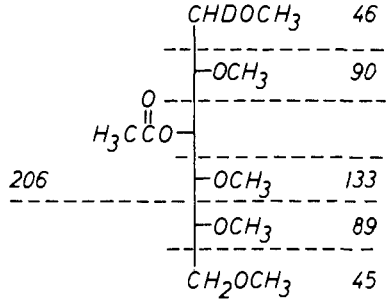

FIGURE 4: Primary fragmentation for 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-D-glucitol-*1-d*.

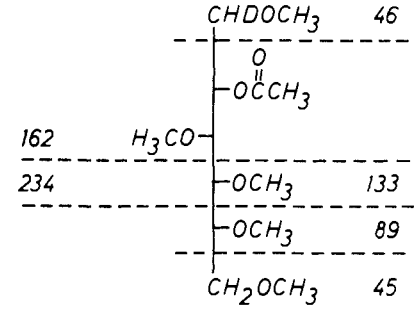

FIGURE 6: Primary fragmentation for 2-*O*-acetyl-1,3,4,5,6-penta-*O*-methyl-D-glucitol-*1-d*.

with peaks at *m/e* 45 (48%), *m/e* 46 (24%), *m/e* 59 (32%), *m/e* 88 (100%), *m/e* 89 (38%), *m/e* 99 (30%), *m/e* 101 (70%), *m/e* 113 (9%), *m/e* 115 (7%), *m/e* 116 (9%), *m/e* 125 (6%) *m/e* 129 (12%), *m/e* 131 (7%), *m/e* 133 (5%), *m/e* 145 (15%), *m/e* 157 (19%), *m/e* 172 (5%), *m/e* 175 (3%), *m/e* 177 (3%), *m/e* 189 (31%), *m/e* 236 (38%), and *m/e* 296 (1%). These peaks are expected for a 2-*O*-6-deoxyhexopyranosylhexitol with a deuterium atom at C-1 of the hexitol residue. The primary fragmentation pattern is shown in Figure 5.

Hydrolysis of the permethylated disaccharide alditol gave 2,3,4-tri-*O*-methyl-L-fucose and 1,3,4,5,6-penta-*O*-methyl-D-glucitol-*1-d* which were identified as alditol acetates by gas–liquid chromatography–mass spectrometry. The mass spectrum of the latter component (T = 0.42) had peaks at *m/e* 43 (100%), *m/e* 45 (86%), *m/e* 46 (47%), *m/e* 59 (33%), *m/e* 88 (27%), *m/e* 89 (32%), *m/e* 101 (46%), *m/e* 102 (13%), *m/e* 130 (53%), *m/e* 133 (12%), *m/e* 146 (33%), *m/e* 162 (40%), and *m/e* 206 (15%). These peaks are expected in the mass spectrum of a 2-*O*-acetyl-1,3,4,5,6-penta-*O*-methylhexitol-*1-d*. The primary fragmentation pattern is shown in Figure 6.

Discussion

Fraction VIe1α was shown to consist of a single component by paper chromatography and by glc, as permethylated alditol. Sugar analysis of the disaccharide and its alditol together with methylation analysis of the disaccharide alditol demonstrated that it was a 3-*O*-D-xylopyranosyl-D-glucose. This structure was corroborated by mass spectrometry of the permethylated disaccharide alditol. From the optical rotation, $[\alpha]_D^{20}$ +103°, it is concluded that the D-xylopyranosyl residue must be α linked to the D-glucose residue. Thus fraction VIe1α consisted of 3-*O*-α-D-xylopranosyl-D-glucose.

The component in fraction VIe1β was homogeneous by paper chromatography and by glc as permethylated alditol

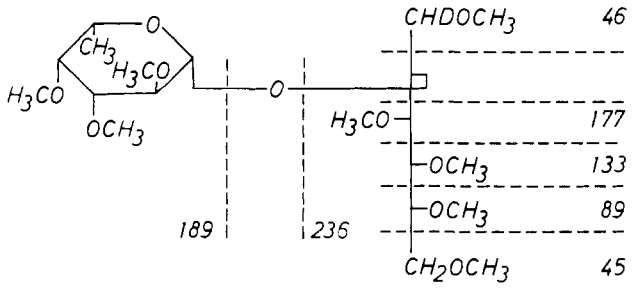

FIGURE 5: Primary fragmentation pattern for permethylated 2-*O*-α-L-fucopyranosyl-D-glucitol-1-*d*.

derivative. Sugar analysis of the disaccharide and the corresponding alditol and methylation analysis of the disaccharide alditol showed that the component was a 2-*O*-L-fucopyranosyl-D-glucose. The mass spectrum of the permethylated disaccharide alditol was in accordance with the assigned structure. The optical rotation, $[\alpha]_D^{20}$ −65.5°, demonstrated that the L-fucopyranosyl residue is α linked to the D-glucose residue. Thus the component in fraction VIe1β is 2-*O*-α-L-fucopyranosyl-D-glucose.

The two disaccharides seem to be present in the urine of both starved and nonstarved secretors. However, whether they are entirely endogenous or not cannot be established at present.

2-*O*-α-L-Fucopyranosyl-D-glucose and L-fucosyl-*myo*-inositol are apparently characteristic secretor disaccharides but, at present, nothing can be stated about 3-*O*-α-D-xylopyranosyl-D-glucose and its relationship to secretor status. The two disaccharides are new in the sense that no human or other oligosaccharide, glycoprotein, glycolipid, or glycosaminoglycan material is known to contain these particular sequences. Thus, the origin of the two compounds is unknown. One possibility is that 2-*O*-α-L-fucopyranosyl-D-glucose is a product of the secretor characteristic fucosyltransferase (Grollman and Ginsburg, 1967; Chester and Watkins, 1969) which then should be able *in vivo* to add fucose $\alpha(1\rightarrow2)$ to both glucose and galactose, and possibly also to a sterically analogous position in *myo*-inositol.

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Composition and Structure of the O-Specific Side Chain of Endotoxin from *Serratia marcescens* Bizio†

C. S. Wang and P. Alaupovic*

ABSTRACT: The endotoxin complex of *Serratia marcescens* Bizio was hydrolyzed by 1% acetic acid, and the O-specific side chain was isolated from the hydrolysate by dialysis and gel filtration on Sephadex G-100. The determinations of chemical composition and molecular weight indicated that the purified O-specific side chain was a polysaccharide consisting of equimolar quantities of D-glucose and L-rhamnose. On the basis of the evidence obtained from periodate oxidation, methylation, infrared spectroscopy, and partial acid hydrolysis, it was concluded that the O-specific side chain is a linear polysaccharide consisting of repeating units of a D-glucose–L-rhamnose disaccharide. The structure of the repeating unit was identified as →6)-β-D-Glc-(1→2)-β-L-Rha-(1→. Enzymatic hydrolysis of isolated disaccharide fractions with glucosidases and hesperidinase and nuclear magnetic resonance spectroscopy of the O-specific side chain indicated that both D-glucose and L-rhamnose have the β-anomeric configuration. The average number of repeating units in the O-specific side chain was estimated to be 43. This report presents the first structural elucidation of an O-specific side chain from genus *Serratia*. It also indicates that the O-specific side chains of Gram-negative bacteria may be composed of simple disaccharide repeating units.

Results of our recent studies (Wober and Alaupovic, 1971; Wang, 1971) have indicated that endotoxin preparations isolated by trichloroacetic acid extraction of *Serratia marcescens* 08 and *S. marcescens* Bizio consist of covalently linked polysaccharide, lipid, and protein moieties. Two fragments designated as conjugated protein and "degraded polysaccharide" were isolated from the acetic acid hydrolysates of these endotoxin preparations. Conjugated protein was characterized as an endotoxic fragment composed of intact protein and lipid moieties. The degraded polysaccharide was further fractionated by dialysis or Sephadex gel filtration (Müller-Seitz *et al.*, 1968; Fensom and Meadow, 1970; Romanowska and Lachowicz, 1970) into two fractions corresponding to the O-specific side chain and core fragments of the polysaccharide moiety. It is generally accepted (Lüderitz *et al.*, 1968; Osborn, 1969) that the macromolecular O-specific side chains are composed of a wide variety of oligosaccharide repeating units responsible for the serological specificity of each bacterial species. On the other hand, it seems that the single-unit oligosaccharide cores are limited to only a few, if not a single, compositional and structural entities characteristic of each bacterial genus (Lüderitz, 1970; Schmidt *et al.*, 1970). Isolation of these two polysaccharide fragments suggested strongly that the basic structural features of the polysaccharide moiety of endotoxins from *S. marcescens* may be similar to those from *Salmonella* (Lüderitz, 1970), *Escherichia* (Heath *et al.*, 1966), and *Shigella* (Simmons, 1969). However, in contrast to the successful elucidation of the detailed structure of the O-specific side chains and cores from some of these latter genera, commensurate information regarding the structure of polysaccharide moieties from various strains of *S. marcescens* is not available.

The results of our studies on the composition and structure of intact endotoxins from a chromogenic and a nonchromogenic strain of *S. marcescens* have indicated that the polysaccharide moieties from both bacterial strains contain a macromolecular side chain composed of repeating oligosaccharide units and a separate oligosaccharide core. In this paper, we describe the isolation and structure of the O-specific side chain from the nonchromogenic strain *S. marcescens* Bizio. Results show that this O-specific side chain consists of a unique disaccharide repeating unit of following structure: →6)-β-D-Glc-(1→2)-β-L-Rha(1→.

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