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The structural characterization of the O-polysaccharide antigen of the lipopolysaccharide of *Escherichia coli* serotype O118 and its relation to the O-antigens of *Escherichia coli* O151 and *Salmonella enterica* O47

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ABSTRACT

Mild acid hydrolysis of the lipopolysaccharide produced by *Escherichia coli* O118:H16 standard strain (NRCC 6613) afforded an O-polysaccharide (O-PS) composed of D-galactose, 2-acetamidoylamino-2,6-dideoxy-L-galactose, 2-acetamido-2-deoxy-D-glucose, ribitol, and phosphate (1:1:1:1:1). From DOC-PAGE, sugar and methylation analyses, one- and two-dimensional NMR spectroscopy, capillary electrophoresis-mass spectrometry, hydrolysis, and sequential Smith-type periodate oxidation studies, the O-PS was determined to be an unbranched linear polymer having the structure:

[6)-α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAm-(1→3)-β-D-Glc*p*NAc-(1→3)-Rib-ol-5-*P*-(O→]$_n$

The structure of the O-PS is consistent with the reported DNA data on the O-antigen gene-cluster of *E. coli* O118 and interestingly, the O-PS is similar to the structures of the O-antigens of *Salmonella enterica* O47 and *E. coli* O151:H10 reference strain 880-67, as predicted from the results of DNA sequencing of their respective O-antigen gene-clusters.

1. Introduction

Lipopolysaccharide (LPS), a macromolecular component of the outer membrane of Gram-negative bacteria, is composed of three regions: a lipid A, an oligosaccharide core, and an O-specific polysaccharide (O-antigen). The O-antigen is usually a high molecular mass polymer of a repeating oligosaccharide unit composed of two to eight monosaccharide residues. The variation in the monosaccharide residues, glycosidic linkages, anomeric configurations, and ring conformation arrangements within a given repeating unit results in the production of numerous unique structures, thus allowing LPS to be a constituent displaying many features that contribute to the antigenic, structural, and biochemical diversity of bacterial cell surfaces. Traditionally, the variability of the O-antigen has provided a basis for serotyping schemes for Gram-negative bacteria, the best known being those developed for *Escherichia coli* and *Salmonella* sp. where antisera raised in rabbits against O-antigen standard bacterial reference strains are employed in agglutination reactions to distinguish different O-serogroups. The use of these serotyping methodologies is limited due to the laborious, time consuming, and expense of the procedures but can also generate equivocal results due to serological cross-reactions. Furthermore, the serotyping antisera can only be produced in specialized laboratories that have animal facilities. Fortunately, it is now realized that more definitive typing data can be achieved using rapid and specific molecular-based techniques such as PCR that are directed to the analysis of the LPS O-antigen gene-cluster located between the *galF* and the *gnd* genes in the *E. coli* chromosome that are involved in O-PS synthesis. In the analysis of the genes involved in the biosynthesis of O-antigen, it is a distinct advantage to know the fine chemical structure of the O-antigen of the serotypes under investigation in order to define and identify the genes involved in nucleotide sugar precursor synthesis, in the sugar transfers in O-repeating unit synthesis, as well as the O-repeating unit processing genes for flippase (*wzx*) and polymerase (*wzy*).[1] Identified genes can be used as diagnostic markers for O-serogroup assignments and thus provide an alternative to traditional antibody based serotyping. Many O-antigen gene clusters in *E. coli* have already been sequenced, including among others, those of serotypes O2, O17, O26, O28, O55, O85, O91, O99, O103, O104, O111, O 113, O117, O118, O121, O126, O127, O145, O146, O150, O151, O157, O161.

The biosynthesis of bacterial monosaccharides is conserved in O-antigen gene-clusters across a wide range of species and, as such, provides a rapid method for the detection of identical or closely related O-antigenic structures occurring in different bacterial species. This is exemplified in the current work in which the O-antigen of *E. coli* O118 was revealed to be similar but not identical with that of *Salmonella enterica* O47 O-antigen[2] as well as being related to the structure of the O-antigen of *E. coli* O151 where

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Liu et al.[3] had shown from DNA sequencing that the genes located in the O-antigen gene cluster had only minor DNA variation between *E. coli* O118 and O151 serotypes. As an extension of this latter study, the structural analysis of the O-PS of *E. coli* O118 was undertaken and the results are described in this report.

2. Experimental

2.1. Bacterial cell growth and O-PS preparation

E. coli O118:H16, 31w standard reference strain (NRCC 6613)[4] was grown in 3.7% brain–heart infusion (Difco) at 37 °C under constant aeration at 20% oxygen saturation in a New Brunswick 25 L fermenter, the cells were killed with phenol (4 °C, 1 h, 1.5% final concentration) and collected by centrifugation (285 g wet paste). The cells were extracted in stirred 50% aqueous phenol at 65–70 °C for 15 min, and the water phase of the cooled (4 °C) extract was dialyzed against running tap water until free from phenol and the cleared solution was then lyophilized. The residue was dissolved in 0.02 M sodium phosphate buffer (pH 7.0) and sequentially treated with ribonuclease, deoxyribonuclease, and proteinase K (3 h each at 37 °C) and the solution, adjusted to 1% concentration of NaCl, was subjected to ultracentrifugation (105,000×*g*, 10 h, 4 °C). The precipitated gel was dissolved in distilled water and lyophilized to yield 2.14 g of LPS.

LPS samples (500 mg) were hydrolyzed in 1.5% (v/v) acetic acid (100 mL) for 2 h at 100 °C and following the removal of precipitated lipid A (162 mg) the concentrated soluble products were fractionated by Sephadex G-50 column chromatography (2.5 × 100 cm) and the lyophilized void volume fraction afforded native O-PS (223 mg). For some later NMR and chemical analyses, a modified O-polysaccharide O-PS(Mod) (conversion of acetamidoylamino to acetamido function) was prepared by treatment of native O-PS (250 mg) with 5% (v/v) aqueous Et_3N (100 mL, 3 h, 70 °C) followed by lyophilization and Sephadex G-50 chromatographic recovery (95%) of O-PS(Mod).

2.2. Monosaccharide analysis and chromatography

O-PS and oligosaccharide samples (2 mg) were hydrolyzed with either 2 M trifluoroacetic acid (115 °C, 3 h) or with 10 M hydrochloric acid (100 °C, 5 min). The concentrated residues were reduced ($NaBD_4$) and acetylated (Ac_2O, 0.5 mL, 100 °C, 1 h) and analyzed by GLC using a HP5 capillary column (30 m × 0.25 mm) with a flame ionization detector (Agilent 6850 chromatograph) in a temperature gradient from 180 to 260 °C at 4 °C/min, and by GLC–MS using a Varian 200 ion-trap instrument with the same column and conditions.

Chromatographically pure samples of aldoses were obtained by preparative paper chromatography on water-washed Whatman 3MM filter paper using 1-butanol–pyridine–water (10:3:3 v/v) mixture as the mobile phase. Detection of glycoses on excised guide strips was made with sprays of *p*-anisidine HCl (in EtOH) and 2% ninhydrin (in acetone). Quantitative phosphate analysis was made following the colorimetric method of Chen et al.[5]

2.3. Gel electrophoresis

Deoxycholate-PAGE of LPS was performed on separating gels of 14% polyacrylamide, and detection was made by silver staining after oxidation with periodate.[6]

2.4. NMR spectroscopy

^1H and ^{13}C spectra were recorded using Varian Inova 400 and 500 MHz spectrometers with samples in 99% D_2O at 25 °C and internal acetone standard reference (2.225 ppm for ^1H and 31.45 ppm for ^{13}C) employing standard DQCOSY and TOCSY (mixing times 120 ms), ROESY (mixing time 400 ms), HSQC and heteronuclear gHMBC (for 6 Hz) long-range coupling constants.

2.5. Hydrolysis of O-PS and O-PS(Mod) with 48% aqueous hydrofluoric acid

O-PS and O-PS(Mod) of *E. coli* O118 (200 mg each) were dissolved in cold (4 °C) aqueous hydrofluoric acid (12 mL) and the solutions were kept at 4 °C for 18 h and then poured into plastic Petri dishes placed in a vented dry air current. The dish residues were dissolved in 0.05 M pyridinium acetate buffer (pH 4.5, 2 mL) and the solutions were fractionated by Sephadex G-15 column chromatography (1.5 diam × 100 cm) using the same buffer as the eluent. The major fractions (95%, K_{av} 0.40) were collected and lyophilized to yield oligosaccharide **I** and **Ia** (each, 150 mg).

2.6. Smith-type periodate oxidation

Following previously described procedures[7,8] oligosaccharides **Ia** (150 mg) in water (20 mL) containing sodium metaperiodate (200 mg) was kept in the dark at 20 °C for 20 h and following the addition of glycol (0.2 mL), the carbohydrate product was reduced with $NaBD_4$ (0.12 g) and after neutralization (AcOH), was isolated by Sephadex G-15 fractionation. The product was dissolved in 2% (v/v) acetic acid (10 mL), kept at 100 °C (1 h), concentrated, and fractionated by Sephadex G-15 column chromatography to yield oligosaccharide **IIa** (K_{av} 0.50, 122 mg).

2.7. Capillary-electrophoresis-mass spectrometry

CE-MS was accomplished using a Prince CE system (Prince Technologies, Netherlands) coupled to a 4000 Q-trap mass spectrometer (Applied Biosysteme/Sciex, Concord, ON, Canada) employing a sheath solution of 2:1 isopropanol–MeOH on a 90 cm long bare fused-silica capillary tube using 15 mmol/L ammonium acetate in deionized water at pH 9.0. The electrospray-ionizing voltage (5 kV) was employed for the positive ion detection mode.

2.8. Methylation analysis

Oligosaccharide and O-PS (2–4 mg) were methylated in Me_2SO (0.5 mL) by the NaOH/MeI procedure[9] and were hydrolyzed (3 M TFA 100 °C, 2 h), reduced ($NaBD_4$), converted to their alditol-1-d derivatives, and analyzed by GLC–MS using a capillary column (30 m × 0.25 mm) and a temperature program increasing from 170 °C (94 min delay) to 260 °C at 4 °C min^{-1}. Retention time is quoted relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-galactitol (T_{GM} = 1.0).

2.9. Periodate oxidation and formaldehyde estimation

Colorimetric analysis of formaldehyde by the Nash method[10] was used to measure its time release by the periodate oxidation of terminal alditol residues of oligosaccharide, as previously described.[11]

2.10. Specific optical rotations

Specific optical rotations were determined at 20 °C using a 10 cm micotube and a Perkin–Elmer 343 polarimeter.

3. Results and discussion

Lipopolysaccharide was isolated from dried fermenter grown cells of *E. coli* O118 (standard reference strain, NRCC 6613) by a

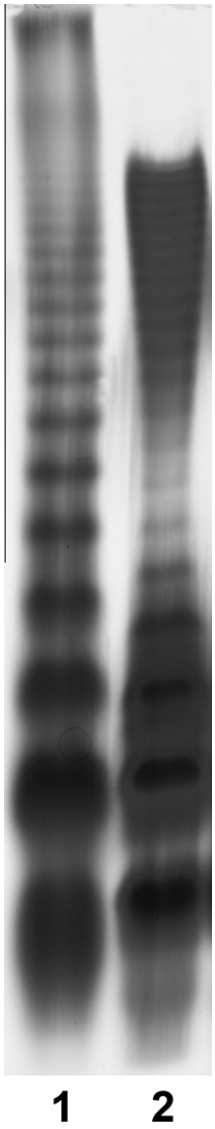


Figure 1. DOC-PAGE of (2) LPS of *Escherichia coli* O118:H16 and (1) LPS of *Salmonella milwaukee*[24] (reference LPS).

modified phenol–water procedure[12,13] followed by the collection of lipopolysaccharide (LPS) as an ultracentrifugation precipitated gel (8% yield based on dry cell wt). Deoxycholate PAGE analysis showed it to be a typical smooth type LPS (Fig. 1) with a band spacing consistent with that of an O-PS repeating tetrasaccharide unit. The O-PS was obtained by mild acid treatment of the LPS followed by Sephadex G-50 column chromatography and collection of O-PS as the lyophilized void volume fraction.

The O-PS had $[\alpha]_D$ +1.5 (*c* 1.2, water) and glycose analysis by GLC of alditol acetate derivatives of the hydrolyzed O-PS, identified anhydroribitol, galactose, 2-amino-2,6-dideoxygalactose, and 2-amino-2-deoxyglucose (1:1:1:1). The configuration of the sugars was established as D-Gal, L-FucN, and D-GlcN, by GLC analysis of their trimethylsilyated (*S*)-2-butyl glycosides[14,15] derived from the component sugars that were obtained by preparative paper chromatography. In addition to the sugars in the O-PS, colorimetric analysis[5] indicated it to contain 11.0% phosphate (Calcd for $C_{35}H_{48}O_{21}N_3P$: PO_4, 10.82) that would account for one phosphate group per subsequently defined O-PS repeating unit.

The ^{13}C NMR spectrum of the O-PS (Fig. 2, Table 1) confirmed the component glycose analysis that showed the presence of three monosaccharides and one ribitol residue. The spectrum demonstrated three anomeric carbon resonances at 97.6–102.9 ppm, a methyl carbon resonance at 16.6 ppm from C-6 of the FucN component, two nitrogen-bearing carbon resonances at 53.0 and 56.8 ppm (C-2 of FucN and GlcN), one *N*-acetyl group at 23.4 and 175.2 ppm (CH_3 and C=O acetamido group GlcNAc) inter alia with signals of an acetamidoylamino group at 20.3 ppm (CH_3) and 167.4 ppm (C=N) from the FucNAm residue.

The ^1H NMR O-PS spectrum (Fig. 3, Table 1) showed three anomeric proton signals at 5.19, 5.06, and 4.60 ppm that were, from chemical shift and $J_{1,2}$ coupling constants, subsequently assigned as α-L-Fuc*p*NAm, α-D-Gal*p*, and β-D-Glc*p*NAc residues.

Further 2D correlation NMR analyses using 2D COSY, TOCSY, ROESY, ^1H,^{13}C HSQC (Fig. 3, Table 1) and ^1H,^{31}P experiments permitted a complete structural assignment of the O-PS repeating unit. For analytical discussion, the O-PS monosaccharide components were labeled **A**, **B**, and **C** in order of their decreasing anomeric proton shifts. From the COSY experiment these three residues were identified as Fuc*p*NAm [**A**], Gal*p* [**B**], and Glc*p*NAc [**C**]. The small $J_{1,2}$ coupling constants (3–4 Hz) associated with the L-Fuc*p*NAm [**A**] and D-Gal*p* [**B**] residues identified their α- pyranosyl configuration whereas the large characteristic $J_{1,2}$ (8.5 Hz) identified a β-pyranosyl configuration for the D-Glc*p*NAc residue [**C**].

Inter-residue correlations observed from ROESY and HMBC spectra of the O-PS indicated proton connectivities from H-1**B** to H-3**A** (4.07 ppm), from H-1**A** to H-3**C** (3.72 ppm), from H-1**C** to H-3**D** (3.87 ppm), and its intraresidue NOE's to H-3**C** (3.72 ppm) and H-5**C** (3.51 ppm) confirming the β-configuration of residue **C** and defining the sequence, α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAm-(1→3)-β-D-Glc*p*NAc-(1→3)-ribitol. Consistent with the above sequence, the HMBC gave long-range correlations of C-1**A** to H-3**C** (3.72 ppm), H-1**B** to C-3**A** (78.0 ppm) and H-1**C** to C-3**D** (81.9 ppm).

The O-PS ^{13}C NMR spectrum exhibited downfield glycosylation shifts of C-3 of α-L-Fuc*p*NAm [**A**], C-6 of α-D-Gal*p* [**B**], C-3 of β-D-Glc*p*NAc [**C**] residues, and C-3 of ribitol, in comparison to their positions in the unsubstituted sugars[16] indicated their linkage positions in the O-PS, respectively, as O-3 of **A**, O-6 of **B**, O-3 of **C**, and O-3 of ribitol. The phosphate linkage was demonstrated from observed large coupling constants ($^2J_{31P,13C}$ 4–5 Hz) of C-5 of ribitol (67.6 ppm) and C-6 of D-Gal*p* (66.3 ppm) and the observed $^3J_{31P,13C}$ (7.8 Hz) of the C-4 (71.1 ppm) of ribitol, and C-5 (71.6 ppm, 7 Hz) of D-Gal. Correlation of the phosphate group location using 2D ^1H, ^{31}P HMQC spectra with observed cross-peaks of the H-6 and H-6[1] of α-D-Gal*p* [**B**] at 1.41/3.99, 4.05 ppm, and H-5,5[1] of ribitol at 1.41/3.82, 4.00 ppm confirmed that the phosphate linkage in the O-PS was between the C-6 of α-D-Gal*p* [**B**] residue and the C-5 of the ribitol residue [**D**].

The combined composition and NMR data identified the O-PS as a polymer of an unbranched repeating unit linked through a phosphate residue, and has the structure:

B A C D

[6)-α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAm-(1→3)-β-D-Glc*p*NAc-(1→3)-Rib-ol-5-*P*-(O→]$_n$

Treatment of the O-PS with cold 48% aqueous hydrofluoric acid (18 h at 4 °C) followed by column G-15 Sephadex chromatography gave a quantitative yield of a phosphate free oligosaccharide (**I**), (K_{av} 0.50) that had $[\alpha]_D$ −19 (*c* 0.26, water), that was composed of D-Gal, D-GlcNAc, L-FucNAm, and ribitol (1:1:1:1). 2D NMR analysis (Table 2), as expected, was consistent with the oligosaccharide having the structure:

B' A' C' D'

α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAm-(1→3)-β-D-Glc*p*NAc-(1→3)-Ribitol **[I]**

Further structural analyses involving CE-MS and Smith-type degradation, were made on a modified O-polysaccharide (O-PS(Mod)) in which the acetamidoylamino function in the

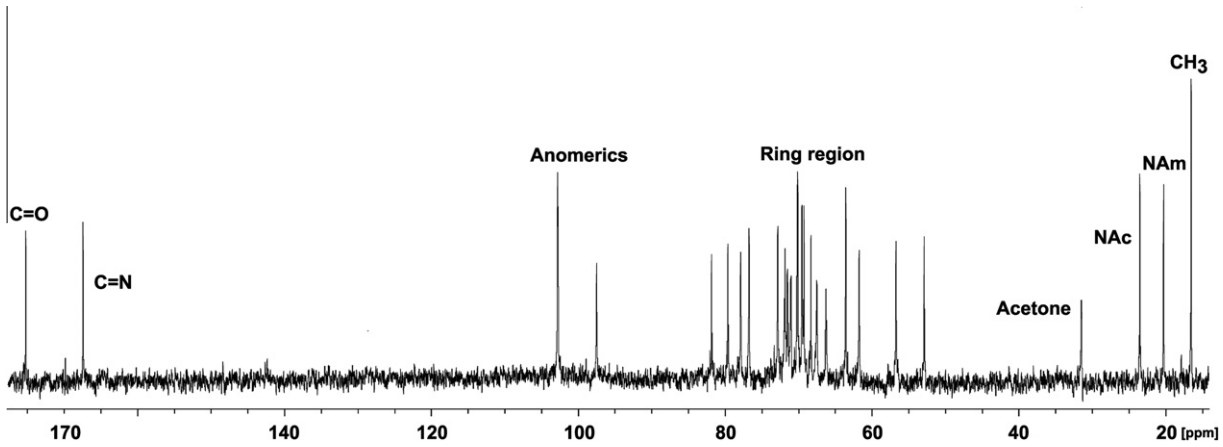


Figure 2. ^{13}C NMR spectrum of the O-polysaccharide of *Escherichia coli* O118:H16.

Table 1
Chemical shifts (ppm) of ^{1}H and ^{13}C in the NMR spectra of the native O-polysaccharide of *E. coli* O118:H16

Glycose residue		H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-6
[**A**]	→3)-α-L-Fuc*p*NAm-(1→	5.19(3.6) 97.6(176)	4.03 53.0	4.07 78.0	4.11 71.9	4.46 68.3	1.22 16.6
[**B**]	→6)-α-D-Gal*p*-(1→	5.06(4.0) 102.8(173)	3.80 69.3	3.88 70.2	4.03 70.3	4.20 71.6	3.99/4.05 66.3
[**C**]	→3)-β-D-Glc*p*NAc-(1→	4.60(8.5) 102.9(165)	3.93 56.8	3.72 79.7	3.57 69.7	3.51 76.8	3.8/3.95 61.8
[**D**]	→3)-Ribitol-(5-P-O→	3.75/3.71 63.7	3.95 72.9	3.87 81.9	3.97 71.1	4.0/3.82 67.6	

Coupling constants $J_{1,2}$ and $J_{H\text{-}1,C\text{-}1}$ in Hertz are shown in parentheses.

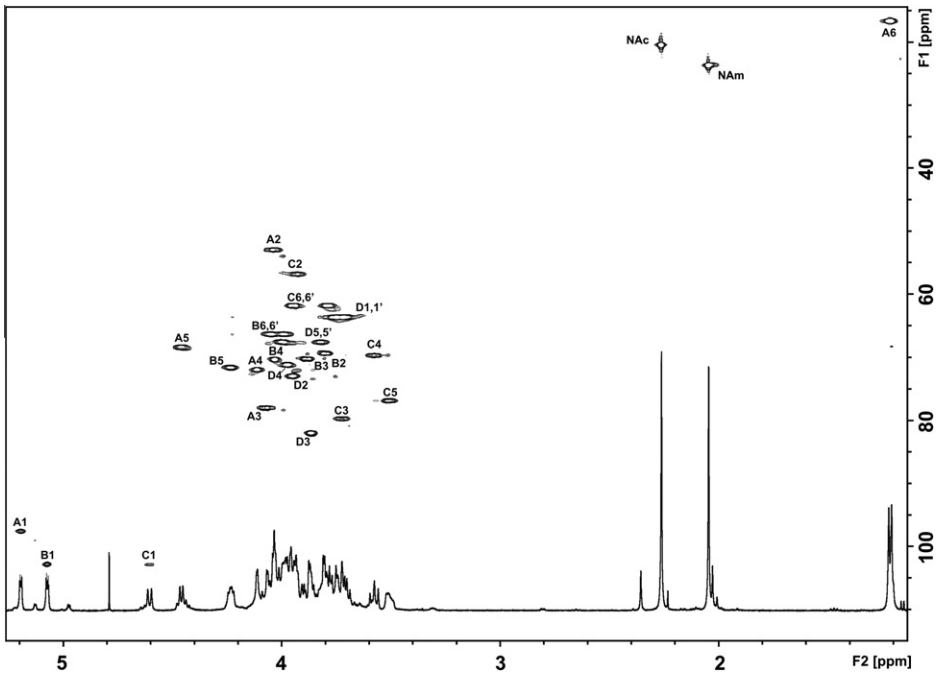


Figure 3. ^{1}H–^{13}C HSQC correlation of the O-polysaccharide of *Escherichia coli* O118:H16.

L-FucNAm residue had been quantitatively converted to an acetamido group by treatment of the native O-PS with 5% aqueous Et_3N. Thus, in the ^{13}C NMR spectra conversion of the C═N group (167.4 ppm) was shifted to C═O (175.7 ppm), the NAm proton signal at 2.26 ppm and corresponding carbon signal at 20.3 ppm were shifted to, respectively, 2.00 and 23.4 ppm in the O-PS(Mod) and the C–N signal at 53.0 ppm (NAm) was shifted to 49.5 ppm (NAc) in the O-PS(Mod).

Positive mode ESI mass spectrum analysis of the O-PS(Mod) (Fig. 4) contained fragment peaks corresponding to 377.1 amu ($HexRibPO_4$), 564.0 amu ($HexRibPO_4dHexNAc$), and a molecular ion M+1 = 767.1 Da ($HexRibPO_4dHexNAcHexNAc$) thereby confirming the proposed O-PS(Mod) structure of the repeating unit ($HexRibPO_4dHexNAcHexNAc$; calculated mass 766.1 Da).

Oligosaccharide **Ia**, obtained by aqueous HF hydrolysis of the O-PS(Mod), was methylated and GLC–MS of the reduced ($NaBD_4$) and acetylated products identified 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-galactitol-1-d (T_{GM} 1.0), 1,3,5-tri-*O*-acetyl-2-deoxy-2-(*N*-methylacetamido)-4-*O*-methyl-fucitol-1-d (T_{GM} 1.94), 1,3,5,-tri-*O*-acetyl-4,6-di-*O*-methyl-2-deoxy-2-(*N*-methylacetamido)-glucitol-1-d (T_{GM} 2.31), and 3-*O*-acetyl-1,2,4,5-tetra-*O*-methyl-ribitol (T_{GM} 0.33) consistent with the above proposed **Ia** sugar sequence and linkages as well as the glycosidic linkage of the D-GlcNAc [**C**′] to the O-3 position of the ribitol residue, and hence to the 3-O-position in the native O-PS.

Further evidence for the 1,3 linkage of the ribitol residue was afforded by the short time periodate oxidation (5 min and 20 min)[11] of **Ia** with an observed colorimetrically[10] determined production of 2.1 mol of formaldehyde per oligosaccharide moiety, consistent with the oxidation of the C1–C2 and C4–C5 1,2-diol systems of the terminal ribitol residue.

Smith-type degradation (Scheme 1) of the oligosaccharide **Ia** derived from aqueous HF hydrolyzed O-PS(Mod) involved periodate oxidation and reduction ($NaBD_4$), mild hydrolysis and G-15 Sephadex column chromatography afforded an oligosaccharide **IIa** (K_{av} 0.50) having $[\alpha]_D$ −25 (*c* 1.1, water). GLC–MS analysis of **IIa** showed it was composed of D-GlcNAc, L-FucNAc, and glycerol-1,3-d_2 (1:1:1). The identification of glycerol-1,3-d_2 (from NMR

Table 2
^1H and ^{13}C NMR data for the oligosaccharide **I** derived from the O-polysaccharide of *Escherichia coli* O118:H16

Glycose residue		H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-6
[**A**′]	→3)-α-L-Fuc*p*NAm-(1→	5.19(3.6) 97.8	4.04 53.1	4.07 77.9	4.07 72.3	4.45 68.5	1.21 16.4
[**B**′]	α-D-Gal*p*-(1→	5.06(4.0) 102.8	3.79 69.6	3.86 70.7	3.98 70.8	4.04 73.0	3.76 62.8
[**C**′]	→3)-β-D-Glc*p*NAc-(1→	4.58(8.6) 103.4	3.91 57.1	3.71 79.8	3.56 69.8	3.48 76.9	3.94/3.76 62.2
[**D**′]	→3)-Ribitol	3.7/3.5 63.8	3.90 73.1	3.80 83.2	3.87 72.7	3.70/3.72 63.9	– –

Coupling constants $J_{1,2}$ and $J_{H\text{-}1,C\text{-}1}$ in Hertz are shown in parentheses.

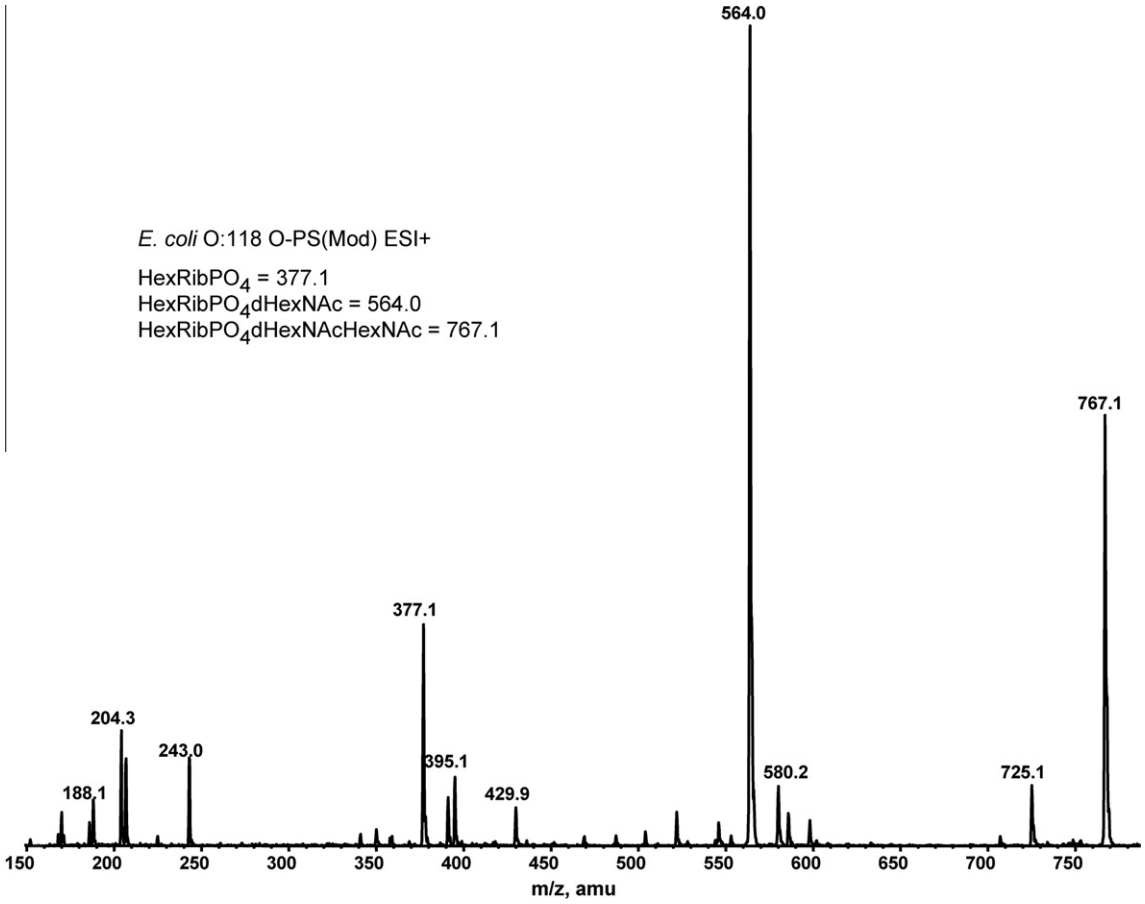
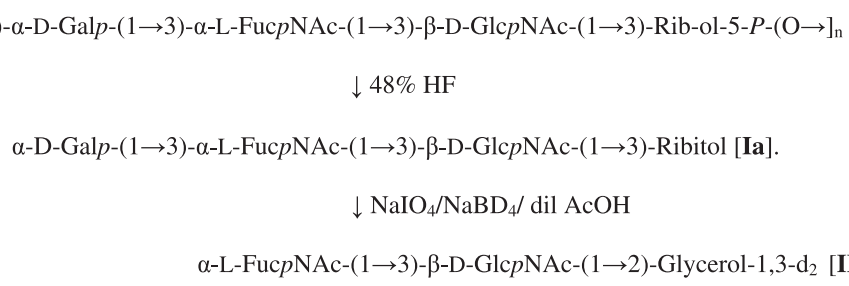


Figure 4. CE-MS analysis of the O-PS(Mod) antigen from *Escherichia coli* O118:H16.



Scheme 1. Aqueous HF hydrolysis and Smith-type degradation of O-PS(Mod) product **Ia**.

and GLC–MS analysis) was further evidence for its linkage at the O-3 position of the ribitol constituent in the oligosaccharide **Ia** and hence at O-3 in the parent O-PS(Mod). The absence of the D-Gal*p* in oligosaccharide **IIa** is consistent with its expected loss by periodate oxidation, and its location as the terminal non-reducing residue in oligosaccharide **Ia**.

The location of a low (~3%) *O*-acetyl substitution and the configuration of ribitol in the native O-PS were not determined.

The characterized structure of the O-antigen of *E. coli* O118 proved to be similar to the O-antigen of *S. enterica* O47[2] the essential difference between the two O-antigens resides in the glycoside linkage of the β-D-Glc*p*NAc to the ribitol residue that is 1,3 in the *E. coli* O118 antigen and 1,2 in the *S. enterica* O-antigen. Structural identity or close similarity of O-antigens have, among others, been identified between *E. coli* and *S. enterica* serotypes[17] as found in the present study of *E. coli* O118 O-antigen. Early examples of common antigen structures were recorded for *E. coli* O157[18] with *S. enterica* O30[19] and *Citrobacter freundii*,[20] *E. coli* O111 with *S. enterica* O35,[21] *E coli* O55 with *S. enterica* O50,[22] and *E coli* O145 with *S. enterica* O48,[23] and many new examples are being reported as a result of stimulation from the current analysis on DNA located in O-antigen gene-clusters. It has been suggested[1] that the most likely reason for the presence of identical O-antigens in these organisms is that the related O-antigen gene-clusters diverged from common ancestors.

In the case of the O-antigen gene-cluster of *E. coli* O118 antigenic diversity, only minor variation in DNA sequence was found in related O-antigen gene-cluster of *E. coli* O151 serotype.[3] The later study showed that PCR assays were specific for *E. coli* O118 and can be used as an alternative to the identification of *E. coli* O118 using antibody based serological methods. However, the PCR assays targeting the *E. coli* O118 *wzx* and *wzy* genes were also positive using *E. coli* serogroup O151 DNA. Preliminary analysis of the O-antigen of *E. coli* O151 (unreported results) suggested that it has a similar linear backbone to the herein described *E. coli* O118 O-PS but contained an additional single β-D-Glc*p*NAc residues linked to the O-4 position of β-D-GlcNAc residues in the O-PS backbone chain and the same β-D-Glc*p*NAc residue in the main chain was glycosidically linked at the 2-O-position of the ribitol residue versus the *E. coli* O118 O-PS in which the same residue was involved a 1→3 linkage to ribitol.

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