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Note

Structural analysis of the O-antigen polysaccharide from *Escherichia coli* O152

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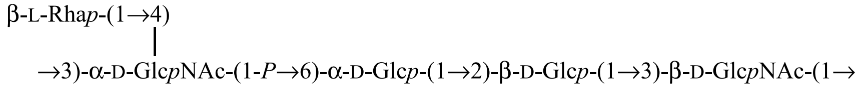
Abstract—The structure of the O-antigen polysaccharide (PS) from *Escherichia coli* O152 has been determined. Component analysis together with ^{1}H, ^{13}C and ^{31}P NMR spectroscopy were used to elucidate the structure. Inter-residue correlations were determined by ^{1}H,^{31}P COSY, ^{1}H,^{1}H NOESY and ^{1}H,^{13}C heteronuclear multiple-bond correlation experiments. The PS is composed of pentasaccharide repeating units with the following structure:



$$\begin{array}{l}\qquad\qquad\beta\text{-L-Rha}p\text{-}(1\to 4)\\ \qquad\qquad\qquad\qquad\quad |\\ \to 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\text{-}P\to 6)\text{-}\alpha\text{-D-Glc}p\text{-}(1\to 2)\text{-}\beta\text{-D-Glc}p\text{-}(1\to 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\to\end{array}$$

The structure is similar to that of the O-antigen polysaccharide from *E. coli* O173. The cross-reactivity between *E. coli* O152 and *E. coli* O3 may be explained by structural similarities in the branching region of their O-antigen polysaccharides.

Keywords: Escherichia coli; Lipopolysaccharide; O-antigen; NMR spectroscopy

Escherichia coli is a facultative anaerobic Gram-negative rod-like bacterium and a predominant species in the colonic flora of animals and man. The species is divided into serotypes based on the immunogenicity of surface structures. Thus, the strains are usually designated as O:K:H serotypes where O is the O-antigen, that is, the polysaccharide portion of the lipopolysaccharide; K is the capsular polysaccharide and H the flagella antigen. More than 170 different O-antigens and over 100 capsular polysaccharides have been identified within the species.[1] There are three common clinical syndromes that result from infections with pathogenic *E. coli*: (i) enteric/diarrhoeal, (ii) urinary tract infections and (iii) septicaemia/meningitis. Only a small number of O, K and H antigens and O:K:H serotypes are associated with pathogens in the different infections. The diarrhoeal strains can be further divided into different pathotypes based on the type of virulence factors they express and hence on the diarrhoeal disease they cause. These are: enteroinvasive *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli* and enteroaggregative *E. coli*.[2] The *E. coli* O152 is an O-serogroup that belongs to the first group[3] and has been described to be antigenically related to a provisional *Shigella* O-antigen defined by Whaten-Grady et al. as *Shigella dysenteriae* 12.[4] In addition, anti-*E. coli* O152 serum cross-react with *E. coli* O3.[5] In this study, we describe the structure of the *E. coli* O152 O-polysaccharide and discuss structural similarities to other *E. coli* O-antigen polysaccharides.

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The *E. coli* O152 was grown in a glucose containing tryptone/yeast extract medium. The LPS was isolated from the bacterial dried cells by hot phenol/water extraction and delipidated under mild acidic conditions to yield a polysaccharide (PS). A hydrolysate of the PS contained rhamnose, glucose and 2-amino-2-deoxyglucose in the ratio 21:30:44. Determination of the absolute configuration of the above components revealed that rhamnose had the L-configuration and that the other sugar residues had the D-configuration (vide infra). However, methylation analysis of the PS material was not successful (vide infra).

The ^{1}H NMR spectrum (Fig. 1) showed the presence of one signal corresponding to a methyl group of a rhamnose residue (δ 1.33, $J = 6.1$ Hz) and two methyl signals from *N*-acetyl groups (δ 2.02 and 2.12, 6H) revealing that the amino sugars are *N*-acetylated. Five anomeric resonances were present in the region between δ 4.71 and 5.37. Likewise, the $^{1}H,^{13}C$ HSQC spectrum showed five resonances from anomeric carbons in the region δ 95.0–102.2 (Fig. 2a). Furthermore, the ^{13}C NMR spectrum showed signals for the *N*-acetyl groups at 23.3, 23.5, 174.9 and 175.2 ppm. These results show that the PS consists of pentasaccharide repeating units with one L-Rha, two D-Glc and two D-GlcNAc residues. The resonance at δ_H 5.37 showed in addition to the $J_{H\text{-}1,H\text{-}2}$ value anticipated for an α-linked residue of the *gluco*-configuration a J value of 7.1 Hz, which is of a magnitude expected for a $^{3}J_{H,P}$ coupling. The ^{31}P NMR spectrum contained a signal at δ_P −1.1, indicating the presence of a phosphodiester group. Subsequently, the ^{1}H and ^{13}C NMR spectra were assigned using two-dimensional NMR techniques and the chemical shifts and selected coupling constants are compiled in Table 1, from which it can be concluded that all sugar residues have the pyranoid ring form. Problems in obtaining a permethylated material have been observed for other *E. coli* strains containing a phosphodiester group as part of the repeating unit. Therefore, the glycosylation positions were determined from the ^{13}C NMR glycosylation shifts ($\Delta\delta$).

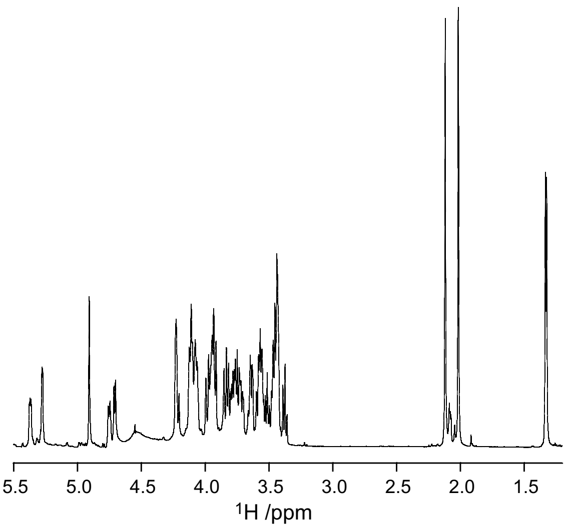


Figure 1. ^{1}H NMR spectrum of the O-antigen PS from *Escherichia coli* O152.

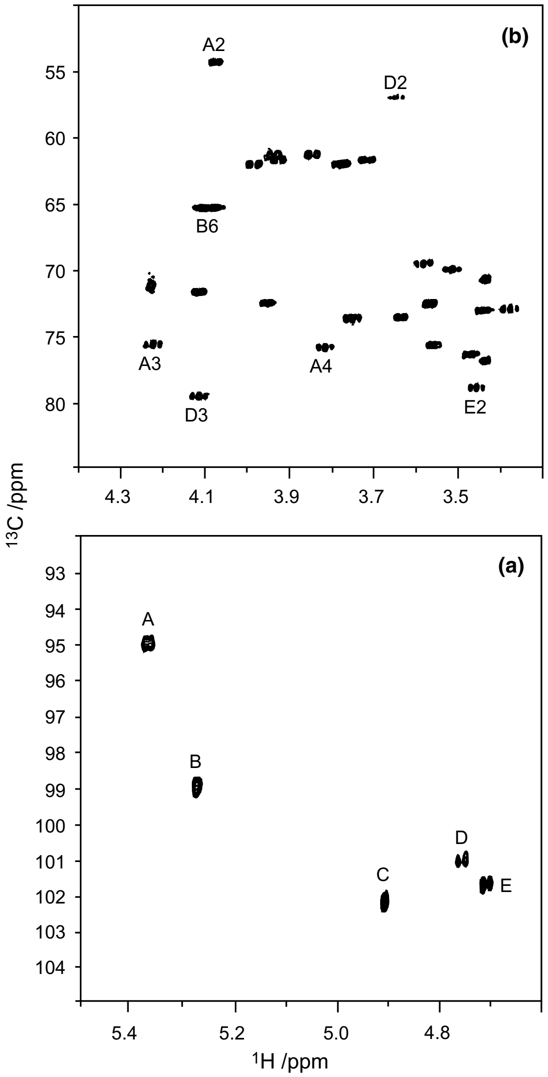


Figure 2. Sections of the $^{1}H,^{13}C$ HSQC spectrum of the O-antigen PS from *E. coli* O152 showing (a) the anomeric region and (b) the region for ring atoms and hydroxymethyl groups. Selected resonances are annotated including those from substitution positions.

The resonance at δ_H 5.37 had $J_{H1,H2} = 3.4$ Hz and its C2 resonance at δ_C 54.2 (Fig. 2b). The ^{13}C NMR glycosylation shifts in residue **A** reveal that this α-linked *N*-acetyl-glucosamine residue is 3,4-disubstituted.[6] Residue **B** has its anomeric proton resonance at δ_H 5.27 and showed $J_{H\text{-}1,H\text{-}2} = 3.8$ Hz. Thus, this residue is an α-linked glucose. The carbon-13 glycosylation shift shows that residue **B** is 6-substituted. The anomeric proton resonance of residue **C** at δ_H 4.91 had $J_{H\text{-}1,C\text{-}1} = 164$ Hz. Disentangling of the spin system showed that it

Table 1. Chemical shift (ppm) of the signals in the ^{1}H and ^{13}C NMR spectra of the *E. coli* O152 O-antigen polysaccharide and inter-residue correlations from NOESY and HMBC spectra

Sugar residue	^{1}H/^{13}C							
	1	2	3	4	5	6	NOE	HMBC
→3,4)-α-D-Glc*p*NAc-(1→	5.37 [3.4, 7.1]	4.07	4.22	3.81	3.95	3.84, 3.94		
A	(0.16)	(0.19)	(0.47)	(0.32)	(0.09)			
	95.0 {178}	54.2	75.5	75.7	72.4	61.2		
	(3.2)	(−0.8)	(3.8)	(4.4)	(−0.1)	(−0.6)		
→6)-α-D-Glc*p*-(1→	5.27 [3.8]	3.57	3.75	3.51	4.12	~4.09	**E**, H2	**E**, C2
B	(0.04)	(0.03)	(0.03)	(0.09)	(0.28)			
	98.9 {176}	72.4	73.5	69.9	71.6	65.2		**E**, H2
	(5.9)	(−0.1)	(−0.3)	(−0.8)	(−0.8)	(3.4)		
β-L-Rha*p*-(1→	4.91 [2.6][a]	4.23	3.64	3.38	3.44	1.33	**A**, H4	**A**, C4
C	(0.06)	(0.30)	(0.05)	(0.00)	(0.05)			
	102.2 {164}	71.1	73.5	73.0	73.0	17.7		**A**, H4
	(7.8)	(−1.1)	(−0.3)	(0.2)	(0.2)	(0.1)		
→3)-β-D-Glc*p*NAc-(1→	4.75 [8.2]	3.64	4.11	3.58	3.47	3.78, 3.98	**A**, H3	**A**, C3
D	(−0.03)	(−0.01)	(0.55)	(0.12)	(0.01)			
	101.0 {167}	56.9	79.5	69.3	76.2	61.9		**A**, H3
	(5.2)	(−1.0)	(4.7)	(−1.8)	(−0.6)	(0.1)		
→2)-β-D-Glc*p*-(1→	4.71 [7.7]	3.45	3.55	3.43	3.43	3.71, 3.92	**D**, H3	**D**, C3
E	(0.07)	(0.20)	(0.05)	(0.01)	(−0.03)			
	101.6 {165}	78.7	75.7	70.7	76.8	61.6		**D**, H3
	(4.8)	(3.5)	(−1.1)	(0.0)	(0.0)	(−0.2)		

$J_{\text{H-1,H-2}}$ values are given in Hz in square brackets and $J_{\text{H-1,C-1}}$ values in Hz in braces. Chemical shift displacement ($\Delta\delta$) in parenthesis, compared to the corresponding hexose residue.

[a] Signal width at half-height.

belonged to the rhamnosyl residue and consequently it is a terminal β-L-Rha*p* residue. The resonance from H1 of residue **D** at δ_H 4.75 had $J_{\text{H-1,H-2}} = 8.2$ Hz and its C2 resonance at δ_C 56.9, which together with a significant ^{13}C glycosylation shift of the C3 resonance shows that it is a 3-substituted β-linked *N*-acetyl-glucosamine residue. Finally, residue **E** showed for H1, at δ_H 4.71, $J_{\text{H-1,H-2}} = 7.7$ Hz and $\Delta\delta_{\text{C-2}} = 3.5$ ppm revealing it as a 2-substituted β-linked glucose. Thus, the five sugar units in the repeating unit have been identified, which in addition should contain a phosphodiester group.

The sequence of the sugar residues in the repeating unit was determined from ^{1}H,^{1}H NOESY and ^{1}H,^{13}C HMBC experiments. In four of the sugar residues both NOEs across the glycosidic linkage as well as each of the two heteronuclear correlations were present (Table 1). Residue **A** is connected to residue **B** since from a ^{1}H,^{31}P COSY experiment, cross-peaks were observed between the phosphorus signal and H-1 and H-2 in **A**, together with H6 in **B** (Fig. 3). In complete agreement with this result ^{13}C,^{31}P couplings were observed as follows: $J_{\text{P,C-1}\mathbf{A}} = 6.7$ Hz, $J_{\text{P,C-2}\mathbf{A}} = 7.8$ Hz, $J_{\text{P,C-5}\mathbf{B}} = 8.0$ Hz, and $J_{\text{P,C-6}\mathbf{B}} = 4$ Hz. Thus, the structure of the O-antigen polysaccharide from *E. coli* O152 is

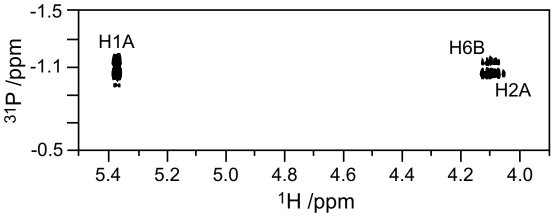
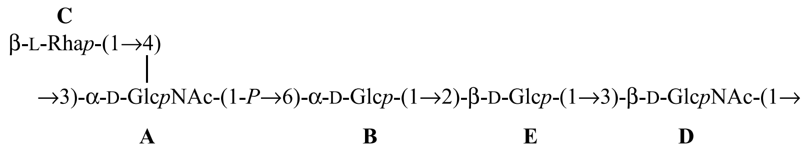


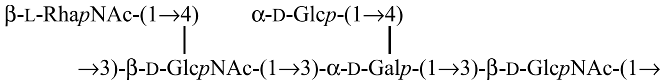
Figure 3. ^{1}H,^{31}P COSY spectrum of the O-antigen PS from *E. coli* O152.



$$\begin{array}{l} \phantom{\rightarrow 3)\text{-}}\mathbf{C} \\ \beta\text{-L-Rha}p\text{-}(1\rightarrow 4) \\ \phantom{\beta\text{-L-Rha}p\text{-}(1\rightarrow}| \\ \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\text{-}P\rightarrow 6)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 2)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow \\ \qquad\qquad\mathbf{A}\qquad\qquad\qquad\qquad\mathbf{B}\qquad\qquad\qquad\mathbf{E}\qquad\qquad\qquad\mathbf{D} \end{array}$$

The absolute configuration of the glucosyl residues as D, was further corroborated by the ^{13}C NMR glycosylation shifts of C-1 in **B** and C-1, C-2 and C-3 in **E** showing that these residues have the same absolute configuration[7] as well as the closely related chemical shifts of the same structural disaccharide component in the repeating unit of the O-antigen from *E. coli* O173.[8]

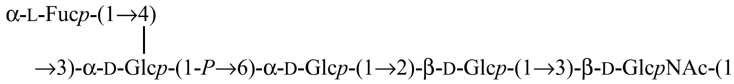
As noted above cross-reactivity is present between *E. coli* O152 and *E. coli* O3.[5] We have previously determined the structure of the O-polysaccharide from *E. coli* O3,[9] which is shown below:



$$\begin{array}{l} \qquad\qquad \beta\text{-L-Rha}p\text{NAc-}(1\to4) \qquad \alpha\text{-D-Glc}p\text{-}(1\to4) \\ \qquad\qquad\qquad\qquad\qquad\quad | \qquad\qquad\qquad\qquad\qquad | \\ \to3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\to3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\to3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\to \end{array}$$

The three-dimensional structure should be similar at the branching *N*-acetylglucosamine residue where both polysaccharides consist of trisaccharides with closely related epitopes, that is, the residues denoted –**D**[**C**]–**A**– in the *E. coli* O152 O-antigen polysaccharide.

The structure of the O-antigenic polysaccharide present in the LPS from *E. coli* O152 also has a partial similarity to the one present in *E. coli* O173,[8] shown below:



$$\begin{array}{l} \alpha\text{-L-Fuc}p\text{-}(1\to4) \\ \qquad\qquad\qquad\quad | \\ \to3)\text{-}\alpha\text{-D-Glc}p\text{-}(1\text{-}P\to6)\text{-}\alpha\text{-D-Glc}p\text{-}(1\to2)\text{-}\beta\text{-D-Glc}p\text{-}(1\to3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\to \end{array}$$

Identity is observed for the sequence –**B**–**E**–**D**– including the phosphodiester group and the differences occur at the branch-point of the repeating unit. Based on our earlier observations in which a 3-substituted β-linked *N*-acetylglucosamine residue was present at the reducing end of the biological repeating unit as determined by NMR spectroscopical methods[10,11] we herein present the repeating units in analogy with previous results. In addition, different polymerases can make different polymers based on a common oligosaccharide by suitable branching of the polysaccharide structure.[12,13] We speculate that the *E. coli* O152 and O173 O-antigens, although having only roughly similar undecaprenyl-PP-linked O-units, are polymerized in a comparable way.

1. Experimental

1.1. Bacterial strain and conditions of growth

The *E. coli* O152:K?:H? strain CCUG36521 was obtained from the Culture Collection University of Gothenburg, Sweden. Bacteria were grown in submerged cultures to late exponential phase in 15 L of a tryptone/yeast extract medium containing 1% glucose, using a 30-L fermentor (Belach AB) under constant aeration at 37 °C and pH 7.0. A preculture (1.5 L) in the same medium was used to inoculate the fermentor. All cultures were checked for purity at the end of the growth cycle. The bacteria were killed with 1% (v/v) formaldehyde. After incubation 16 h at 4 °C the cells were separated from the media by continuous-flow centrifugation using a CEPA model LE centrifuge at a cylinder speed of 29,000*g* and a flow of $25\,\text{L}\cdot\text{h}^{-1}$ (Carl Padberg Centrifugenbau, Lahr, Germany). The bacterial mass was then removed from the cylinder, washed once with NaCl/P_i (0.01 M potassium phosphate, 0.14 M NaCl, pH 7.2), centrifuged (8000*g*, 4 °C, 20 min) and finally re-suspended in distilled water.

1.2. Preparation of lipopolysaccharide and lipid-free polysaccharide

The lipopolysaccharide (LPS) was extracted by the hot phenol/water method.[14] The aqueous phase was dialyzed at 4 °C for 3–5 days against tap water, overnight against distilled water, concentrated under diminished pressure and lyophilized. Contaminating nucleic acids were removed by ultracentrifugation (100,000*g*, 4 h, 4 °C). Lipid-free polysaccharide (PS) was prepared by treatment of the LPS with 0.1 M sodium acetate, pH 4.2, at 100 °C for 5 h.[15] Lipid A was removed by centrifugation (10,000*g*, 20 min, 4 °C). The PS was further purified by gel-permeation chromatography.

1.3. Component analyses

The PS was hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h. After reduction with $NaBD_4$ and acetylation, the sample was analyzed by GLC. Alditol acetates were separated on HP-5 and DB-225 fused silica columns using a temperature program of 180 °C for 1 min followed by $3\,^{\circ}\text{C}\,\text{min}^{-1}$ to 210 °C. Hydrogen was used as carrier gas. The column was fitted to a Hewlett-Packard model 5890 series II gas chromatograph equipped with a flame ionization detector. The absolute configurations of the sugars present in the PS were determined by derivation of the sugars as their acetylated (+)-2-butyl glycosides.[16,17]

1.4. NMR spectroscopy

NMR spectra of the PS in D_2O were recorded at 50 °C using Bruker AVANCE 400 and Varian Inova 600 MHz instruments. Chemical shifts are reported in ppm relative to internal sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)propanoate (TSP, δ_H 0.00), external 1,4-dioxan in D_2O (δ_C 67.4) or external phosphoric acid (2% H_3PO_4 in D_2O, δ_P 0.00) as references. ^{13}C DEPT-135, $^1H,^1H$-correlated spectroscopy (COSY),[18] total correlation spectroscopy (TOCSY)[19] with mixing times of 30, 60 and 90 ms, $^1H,^{31}P$ COSY,[20] gradient selected heteronuclear single quantum coherence (gHSQC),[21] and gradient selected heteronuclear multiple-bond correlation (gHMBC)[21,22] experiments were used to assign signals and performed according to standard pulse sequences. For inter-residue correlations, a two-dimensional nuclear Overhauser effect spectroscopy (NOESY)[23] experiment with a mixing time of 100 ms, and an HMBC experiment with a 50 ms delay for the evolution of long-range couplings were used. The chemical shifts were compared to those of the corresponding monosaccharides.[24] Analysis of the $^1H,^{13}C$ HMBC spectrum from the *E. coli* O173 O-antigen polysaccharide,[8] related to the present work, revealed that the assignments should be reversed for the H4/C4 and H5/C5 pairs of the 6-substituted glucosyl residue.

Acknowledgements

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