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The structure of the O-specific polysaccharide from *Escherichia coli* O113 lipopolysaccharide ☆

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Abstract

The O-specific polysaccharide from *Escherichia coli* O113 lipopolysaccharide was separated from the core and lipid A by mild acid hydrolysis and purified by GPC. Methylation analysis and ^{1}H and ^{13}C NMR spectroscopic studies of the *O*-deacetylated polysaccharide allowed the determination of the structure of the pentasaccharide repeating unit of the polysaccharide which can be written as

→ 4)-α-D-Gal*p*A-(1 → 3)-α-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc-(1 → 4)-α-D-Gal*p*NAc-(1 →
3
↑
1
β-D-Gal*p*

The position of the *O*-acetyl groups was not determined.

Keywords: *Escherichia coli* O113; O-Specific polysaccharide; NMR spectroscopy; Methylation analysis; O-Antigen

1. Introduction

Escherichia coli (F. Enterobacteriaceae) have been subdivided into more than 150 different O-serogroups (O-antigens) and over 70 K-serogroups (K-antigens) [1]. The O-specific polysaccharide of the cell wall lipopolysaccharide (LPS) is composed of repeating oligosaccharides which may be neutral or acidic. Acidity is usually because of the presence of uronic acids or phosphate groups in the repeating unit although Neu5Ac has

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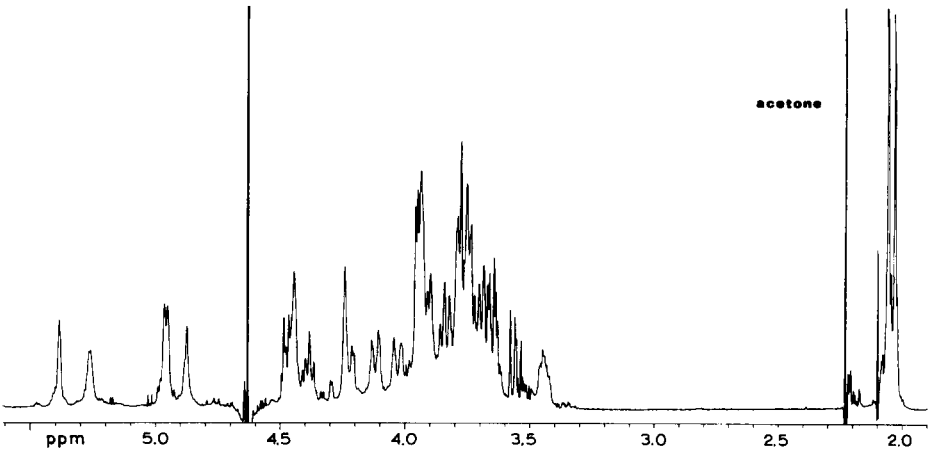


Fig. 1. ^{1}H NMR spectrum of **DPS** at 313 K.

recently been found as a component [2]. Many serological cross-reactions occur within the *E. coli* group of O-antigens and between these and the O-antigens of the Enterobacteriaceae *Shigella, Salmonella,* and *Klebsiella* [1,3], and also with others outside this group such as *Vibrio cholerae* [1]. In order to understand the basis for this cross-reactivity it is desirable that all the structures of the O-polysaccharides of *E. coli* be investigated. To date less than one-third of these are known. We now report the structure of the O-specific polysaccharide of *E. coli* O113 LPS.

2. Results and discussion

Isolation, composition, and methylation analysis.—*E. coli* O113:H21 bacteria were grown in Luria–Bertani broth at 37°C for 25 h after which the cells were killed with phenol and were isolated by centrifugation and washed. The LPS was extracted from the cells using the hot aqueous phenol method [4]. The O-polysaccharide (**PS**) was liberated from core and lipid A by mild acid hydrolysis and was purified by GPC on Sephacryl S-200. GLC analysis of the products of an acid hydrolysate of the **PS**, with and without prior carboxyl reduction, showed Gal, GalA, GalN, and GlcN in the approximate molar ratios 2.0:0.9:0.9:0.7. The sugars were all shown to be D by GLC analysis of the derived acetylated (−)-2-octyl glycosides [5]. Methylation analysis of the polysaccharide, with and without prior carboxyl reduction, showed the presence of 3-substituted Gal, terminal Gal, 4-substituted GalA, 3-substituted GlcN, and 3,4-substituted GalN, indicating that the polysaccharide is composed of a repeating pentasaccharide with a branch point at GalN and a side-chain terminated by Gal.

NMR spectroscopy of PS.—The ^{1}H NMR spectrum of the **PS** at 313K was quite complex and indicated partial and/or random *O*-acetylation. The **PS** was therefore *O*-deacetylated to give **DPS**, the spectrum of which (Fig. 1) was consistent with a polysaccharide with a regular repeating structure. Signals were observed in the anomeric region

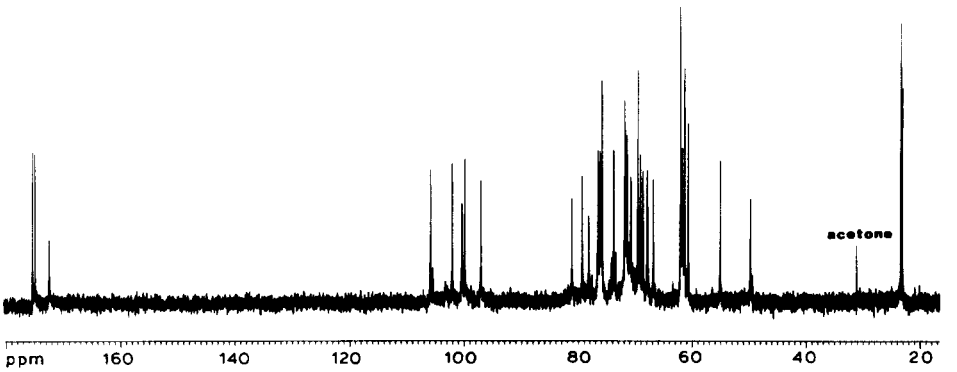


Fig. 2. ^{13}C NMR spectrum of **DPS** at 313 K.

at δ 5.39, 5.27 ($^3J \sim 3$ Hz), 4.96 (2 H), 4.88, and 4.46 (^3J 7.7 Hz), and for Me protons of *N*-acyl groups at δ 2.03 (3 H) and 2.06 (3 H). The ^{13}C NMR spectrum (Fig. 2) showed signals for five anomeric carbons at 105.85, 102.06, 100.36, 99.84, and 97.06 ppm, for two N-linked carbons at 55.01 and 49.71 ppm, for two Me carbons at 23.20 and 23.50 ppm, and for three carbonyl carbons at 172.60, 175.10, and 175.80 ppm. A DEPT experiment indicated four unsubstituted C-6 carbons at 61.29, 60.65, 61.68, and 62.06 ppm. These results are consistent with a pentasaccharide repeating unit for **DPS** containing two *N*-acetylated amino sugars and one uronic acid, and suggested that one of the signals in the anomeric region of the ^1H NMR spectrum belonged to a non-anomeric ring proton.

2D NMR studies of O113 **DPS**.—The identities, anomeric configurations, linkage positions, and sequence of the residues in the repeating unit were established using a combination of 2D NMR experiments. The ^1H and ^{13}C chemical shifts of the residues (labelled **a**–**e** in order of the decreasing chemical shift of their H-1 resonances) were assigned (Table 1) using mainly COSY [6], HOHAHA [7], HMQC [8], and HMQC-TOCSY [9] experiments with help from the intraresidue NOEs observed in the NOESY [10] spec-

Table 1
^1H and ^{13}C NMR data [a] for **DPS**

Residue	Proton or carbon							
		1	2	3	4	5	6a	6b
a	H	5.39	3.94	3.94	4.24	3.91	3.74	3.74
→ 3)-α-Gal	C	100.36	67.81	76.51 [b]	66.75	71.16	61.29	
b	H	5.27	3.95	4.12	4.45	4.88		
→ 4)-α-GalA	C	97.06	68.60	69.06	79.32	71.45	172.49	
c	H	4.96	4.22	4.03	4.44	4.39	3.79	3.64
→ 3,4)-α-GalNAc	C	99.84	49.71	78.15	76.14	70.75	60.65	
d	H	4.96	3.86	3.83	3.68	3.44	3.92	3.74
→ 3)-β-GlcNAc	C	102.06	55.01	81.16	71.73	75.86	61.68	
e	H	4.46	3.56	3.65	3.95	3.67	3.78	3.78
β-Gal	C	105.85	71.82	73.77	69.50	75.79	62.06	

[a] Chemical shifts in ppm with acetone as internal reference, δ 2.23 for ^1H and 31.07 ppm for ^{13}C, respectively.
[b] Linkage carbons underlined.

Table 2
Inter- and intra-residue NOEs for **DPS**

Proton		NOE to (ppm)
5.39	**a**1	3.94 (**a**2), 3.83 (**d**3) [a]
5.27	**b**1	4.24 (**a**4), 3.94 (**a**3), 3.95 (**b**2)
4.96	**d**1, **c**1	3.44 (**d**5), 3.83 (**d**3), 4.22 (**c**2), 4.44 (**c**4), 4.45 (**b**4)
4.88	**b**5	4.45 (**b**4), 4.12 (**b**3) 4.96 (**c**1)
4.46	**e**1	3.65 (**e**3), 3.67 (**e**5), 4.03 (**c**3)
4.45	**b**4	4.12 (**b**3)
4.44	**c**4	4.03 (**c**3), 4.39 (**c**5)
4.39	**c**5	3.64 (**c**6b), 3.79 (**c**6a), 4.03 (**c**3)
4.24	**a**4	3.74 (**a**6ab), 3.91 (**a**5), 3.94 (**a**3)
3.95	**e**4	3.65 (**e**3), 3.67 (**e**5), 3.78 (**e**6ab)

[a] Interresidue NOEs underlined.

trum (Table 2) and from the long-range three-bond intraresidue $^{1}H-^{13}C$ correlations obtained in the HMBC [11] experiment (Table 3). The following is a brief account of how the ^{1}H and ^{13}C chemical shifts were established for the individual residues of the repeating unit.

Residue **a** *[→ 3)-α-Gal].*—Assignment of the chemical shifts of the ^{1}H resonances for this residue was complicated by signal overlap within the spin system and with other spin systems. Thus only the chemical shifts for H-1 and H-2 could be assigned from the COSY spectrum. The HOHAHA spectrum showed only two cross-peaks in the H-1 track. The pattern of the cross-peak at δ 4.24 was consistent with that expected for the H-1/H-4 cross-peak of a Gal residue, suggesting that the other cross-peak contained overlapping H-1/H-2 and H-1/H-3 cross-peaks. This was confirmed in the HMQC-TOCSY spectrum in which the resonances for H-1/C-1 to H-4/C-4 were traced. The chemical shifts for H-5/C-5 were established and those for H-3/C-3 were confirmed by the long-range correlations shown between H-1 and C-3 and C-5 in the HMBC spectrum and from the $^{1}H-^{13}C$ correlation data. The chemical shifts of the remaining ^{1}H resonances were assigned from the H-4/H-5 and H-4/H-6a,b cross-peaks in the NOESY spectrum. The chemical shift of C-6 was identified from the H-5/C-6 relay in the HMQC-TOCSY spectrum.

Table 3
Three-bond $^{1}H-^{13}C$ correlations (HMBC) for **DPS**

Proton		Three-bond correlation to carbon (ppm)
5.39	**a**1	81.16 (**d**3) [a], 71.16 (**a**5), 76.51 (**a**3)
4.96	**c**1, **d**1	79.32 (**b**4), 78.15 (**c**3), 76.14 (**c**4), 70.75 (**c**5)
4.46	**e**1	78.15 (**c**3)
4.45	**b**4	99.84 (**c**1)
4.44	**c**4	102.06 (**d**1), 49.71 (**c**2)
4.03	**c**3	105.85 (**e**1), 49.71 (**c**2)
3.79	**c**6a	76.14 (**c**4)
3.74	**a**6ab	66.75 (**a**4)
3.64	**c**6b	76.14 (**c**4)

[a] Interglycosidic correlations underlined.

Residue **b** *[→ 4)-α-GalA].*—All the ^{1}H resonances for this residue were traced in the COSY and HOHAHA contour plots and the ^{13}C chemical shifts were assigned from the HMQC spectrum.

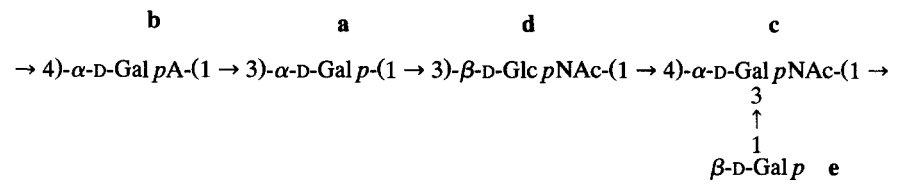
Residue **c** *[→ 3,4)-α-GalNAc].*—The chemical shifts for H-1 to H-4 were assigned from the COSY and HOHAHA spectra, and the remaining ^{1}H resonances were identified from the H-4/H-5 and H-5/H-6a and H-5/H-6b cross-peaks in the NOESY spectrum. The chemical shifts for the ^{13}C resonances followed from the HMQC data.

Residue **d** *[→ 3)-β-GlcNAc].*—Resonances for this residue were established as for residue **b**.

Residue **e** *[β-Gal].*—The resonances for H-1 to H-4 were traced in both the COSY and HOHAHA spectra while the ^{1}H and ^{13}C resonances for the entire spin system were established from the HMQC-TOCSY spectrum.

Comparison of the ^{1}H and ^{13}C chemical shift data for residues **a** to **e** with literature values for methyl glycosides [12–14] permitted the identification of the five sugar residues and their linkage positions. In agreement with the results of the methylation analysis, the resonances for C-3 of **a**, C-4 of **b**, C-3 and C-4 of **c**, and C-3 of **d** were significantly deshielded. The sequence of the residues in **DPS** was determined from the interresidue NOEs and three-bond correlations obtained from the NOESY and HMBC experiments respectively. The intermolecular NOEs observed are listed in Table 2 and the three-bond $^{1}H-^{13}C$ correlations are shown in Table 3. It was not possible to discriminate between the intermolecular NOEs from H-1 of **c** and **d** from the NOESY spectrum because of signal overlap. However, the NOEs observed were sufficient to establish the sequence of the residues in the repeating unit. The intraresidue NOEs served to confirm the anomeric configurations of the linkages, viz., H1/2 NOEs for the α- and H1/3,5 NOEs for the β-linked sugars. The structure of the pentasaccharide repeating unit is supported by the $^{1}H-^{13}C$ correlation data from the HMBC experiment. These data *inter alia* showed intermolecular correlations between C-1 of **c** and H-4 of **b**, and between C-1 of **d** and H-4 of **c**.

The above data permit the structure of the repeating unit of the *O*-deacetylated *E. coli* O113 polysaccharide to be written as



The position of the *O*-acetyl groups was not determined.

3. Experimental

General methods.—GPC was performed on a column of Sephacryl S-200 gel (2.6 × 85 cm) using 0.1 M CH_3CO_2Na buffer (pH 5.0) as eluent and a Waters R401 differential refractometer. Analytical GLC was performed with a Hewlett–Packard 5890A gas chromatograph fitted with a flame ionisation detector, a 3392A recording integrator, and

He as carrier gas. A J&W Scientific fused-silica DB-17 bonded-phase capillary column (30 m × 0.25 mm, 0.25-μm film) was used for separating alditol acetates and partially methylated alditol acetates (temperature programme 180°C for 2 min then 2°C min^{-1} to 240°C). The identity of each derivative was confirmed by GLC–MS on a Hewlett–Packard 5988A instrument, using the same column, with an ionisation energy of 70 eV and an ion-source temperature of 200°C. Hydrolysis of samples with CF_3CO_2H, carboxyl reduction of methyl esters resulting from methanolyses, determination of absolute configuration of sugars, preparation of alditol acetates, and methylation of the polysaccharide were carried out as previously described [15].

Preparation of E. coli *O113 O-specific polysaccharide.*—An authentic culture of *Escherichia coli* O113:H21 bacteria (culture number 6182-50) was obtained from Dr. I. Ørskov, Copenhagen and propagated on Luria–Bertani agar (LB). Single colonies were then transferred to liquid LB medium and the cells were grown at 37°C with shaking for 5 h after which these cultures were used to inoculate three 10-L fermentation flasks in which the bacteria were allowed to grow for 20 h with a stir rate of 200 rpm and an aeration rate of 1 L of air per min per L of broth. Dow antifoam (2 mL) was added to each flask. The cells were killed by the addition of phenol to a final concentration of 0.75%, cooled to 10°C (2 h), and centrifuged (7 500 rpm, 10°C, 20 min) in a Sorvall RC5B centrifuge using a GS-3 rotor. The cells were collected and washed twice with aq 2% NaCl and finally lyophilised from water to yield 32.5 g of dry cells. The LPS was extracted from the cells using the hot aqueous phenol method [4] and the O-polysaccharide was liberated from core and lipid A by hydrolysis with 1% AcOH at 60°C for 1.5 h and purified by GPC (yield 320 mg). Polysaccharide (50 mg) was *O*-deacetylated by treatment with 0.1 M NaOH for 1 h at 40°C and **DPS** was recovered by dialysis and lyophilisation.

NMR spectroscopy.—Samples were deuterium-exchanged by lyophilising from D_2O and were then dissolved in 99.996% D_2O with a trace of acetone added as reference (δ 2.230 and 31.07 ppm downfield from Me_4Si for ^1H and ^{13}C, respectively). Spectra were acquired at 313 K on a Bruker AMX-400 NMR spectrometer using standard Bruker software. For all ^1H dimensions the spectral width was 2000 Hz; for the HMQC and HMBC, ^{13}C dimensions were 11 067 and 20 826 Hz, respectively. All spectra were acquired with a 1-s relaxation delay. Mixing times were as follows: HOHAHA, 84 ms; HMQC-TOCSY, 25 and 84 ms; NOESY, 200 ms; HMBC delay for long-range coupling, 60 ms.

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