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Structural studies of the *Escherichia coli* O26 O-antigen polysaccharide

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Abstract

The structure of the O-specific side chain of the *E. coli* O26 lipopolysaccharide has been investigated. Based on sugar and methylation analyses, and 2D NMR spectroscopy employing HMBC experiments, it is concluded that the polysaccharide is composed of trisaccharide repeating units having the following structure:

→ 3)-α-L-Rha*p*-(1 → 4)-α-L-Fuc*p*NAc-(1 → 3)-β-D-Glc*p*NAc-(1 →

Keywords: Escherichia coli; O-Antigen; Lipopolysaccharide; Enterohemorrhagic

1. Introduction

Escherichia coli O26:H11 belongs to the group of enterohemorrhagic *E. coli* (EHEC) [1]. Previously, *E. coli* O26 was considered as a classic enteropathogenic *E. coli* serotype. EHEC strains have only recently been recognised as a cause of serious disease. Usually, pediatric diarrhoea in developed countries is not a fatal disease. Disease caused by EHEC strains, however, can cause death because of acute kidney failure (hemolytic-uremic syndrome, HUS) as a complication of the illness. In a clinicoepidemiological study in young children with HUS in Chile, it was found that next to the *E. coli* O157

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serotype, the *E. coli* O26 was the most common EHEC isolate [2]. Most EHEC strains produce a Shiga-like toxin that plays an important role in the disease. In addition, the majority of *E. coli* O26 strains produce a plasmid-mediated hemolysin [3]. The structure of the EHEC O157 serotype has been described [4]. We report here on the structure of the O-antigen polysaccharide of the second most common EHEC serotype, *E. coli* O26:H11.

2. Results and discussion

The lipopolysaccharide from *Escherichia coli* serogroup O26 was obtained by phenol–water extraction and delipidated into the corresponding polysaccharide (O26) with acid under mild conditions. Hydrolysis of the PS with 4 M HCl yielded 6-deoxymannose, 2-amino-2,6-dideoxygalactose, and 2-amino-2-deoxyglucose in the relative proportions 0.3:1:0.6. Sugars attributed to the core of the LPS were also detected in minor amounts. Analysis of the partially methylated alditol acetates obtained from the methylated PS after acid hydrolysis revealed the presence of 3-substituted 6-deoxymannose, 4-substituted 2-amino-2,6-dideoxygalactose, and 3-substituted 2-amino-2-deoxyglucose in the relative proportions 0.5:1:0.9. Determination of the absolute configuration of the sugars was performed by a modification of the method developed by Leontein et al. [5], using GLC of the acetylated (+)-2-butyl glycosides, and showed 6-deoxy-L-mannose (L-rhamnose), 2-amino-2,6-dideoxy-L-galactose (L-fucosamine), and 2-amino-2-deoxy-D-glucose. From methylation analysis and NMR spectra, discussed below, it was evident that the sugars were pyranoid.

The ^{1}H NMR spectrum of the O26 polysaccharide obtained from mild acid treatment showed, inter alia, signals at δ 5.01 ($J_{H\text{-}1,H\text{-}2}$ 3.8 Hz), 4.83 ($J_{H\text{-}1,H\text{-}2}$ 1.9 Hz), and 4.68 ($J_{H\text{-}1,H\text{-}2}$ 8.4 Hz) in the anomeric region, signals at δ 1.19 and 1.22 that were indicative of presence of 6-deoxy sugars, signals at δ 1.97, 2.04 which demonstrated the presence of two *N*-acetylated groups, and a signal at δ 2.16 which disappeared upon alkaline treatment. The latter signal was probably due to an *O*-acetyl group but as the amount was ≤ 0.2 equivalent per repeating unit and varied with different preparations, the location was not identified. All subsequent NMR data discussed originate from PS material that had been subjected to alkaline treatment. The ^{13}C NMR spectrum of the polysaccharide (Fig. 1) contained three signals in the anomeric region at δ 98.6, 102.6, and 103.5, and signals for two methyl groups at δ 16.3 and 17.4, for methyl groups of *N*-acetyl groups at δ 23.0, for two carbons carrying nitrogen at δ 50.6 and 56.5, and for two carbonyl groups at δ 175.0 and 175.1.

The chemical shifts of the ^{1}H and ^{13}C NMR spectra together with $J_{H\text{-}1,H\text{-}2}$ and $J_{C\text{-}1,H\text{-}1}$ values are given in Table 1. The assignments of the spin system for each sugar residue were performed according to standard homo- and hetero-nuclear two-dimensional techniques and showed a trisaccharide repeating unit. The sugar components are labelled **A**, **B**, and **C** with respect to decreasing chemical shift of their anomeric proton. Residues **A** and **C** have a *gluco / galacto* configuration because of their larger $J_{H\text{-}1,H\text{-}2}$ values (3.8 and 8.4 Hz, respectively), while residue **B** has a *manno* configuration because of its small coupling constant ($J_{H\text{-}1,H\text{-}2}$ 1.9 Hz). From a ^{13}C,^{1}H-coupled

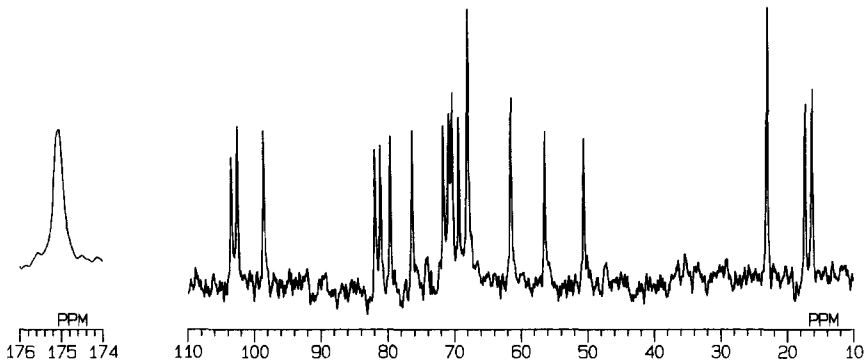

Fig. 1. The ^{13}C NMR spectrum of the *O*-deacetylated *E. coli* O26 O-antigen polysaccharide.

^{1}H-detected HMQC experiment it was possible to establish that residues **A** and **B** are α-linked ($J_{C\text{-}1,H\text{-}1}$ 174 and 169 Hz, respectively) and that residue **C** is β-linked ($J_{C\text{-}1,H\text{-}1}$ 163 Hz). The anomeric configurations of **A**–**C** are in accord with those that can be derived from $J_{H\text{-}1,H\text{-}2}$ values given above.

The identification of a spin system with a specific sugar residue and substitution pattern determined from methylation analysis was done as follows. $^{1}H,^{1}H$ COSY, relayed COSY, and $^{13}C,^{1}H$-decoupled ^{1}H-detected HMQC experiments showed that residues **A** and **B** were 6-deoxy sugars (δ_H 1.19/δ_C 16.3 for **A** and δ_H 1.22/δ_C 17.4 for **B**). The high-field chemical shifts of the C-2 signals in the **A** and **C** residues, δ 50.6 and 56.5, respectively, were diagnostic for carbons carrying nitrogen of *N*-acetyl groups. The downfield chemical shifts of the C-4 signal for residue **A** (δ 82.0) and the C-3 signals for residues **B** and **C** (δ 81.1 and 79.6, respectively) showed that **A** was a 4-substituted 2-acetamido-2,6-dideoxygalactose, **B** was a 3-substituted 6-deoxymannose, and **C** a 3-substituted 2-acetamido-2-deoxyglucose, in accordance with the methylation

Table 1
Chemical shifts (ppm) of the signals in the ^{1}H and ^{13}C NMR spectra [a] of the *O*-deacetylated *E. coli* O26 O-antigen polysaccharide

Sugar residue	H/C							
	1	2	3	4	5	6	NAc [b]	CO
→ 4)-α-L-FucpNAc-(1 →	5.01 (3.8)	4.17	3.96	3.87	4.43	1.19	1.97	
A	98.6 [174]	50.6	68.0	82.0	68.0	16.3	23.0	175.1
→ 3)-α-L-Rhap-(1 →	4.83 (1.9)	4.28	3.90	3.49	4.03	1.22		
B	102.6 [169]	70.9	81.1	71.7	70.4	17.4		
→ 3)-β-D-GlcpNAc-(1 →	4.68 (8.4)	3.89	3.70	3.55	3.47	3.77;3.89	2.04	
C	103.5 [163]	56.5	79.6	69.4	76.4	61.5	23.0	175.0

[a] $J_{H\text{-}1,H\text{-}2}$ values (Hz) are given in parentheses and $J_{C\text{-}1,H\text{-}1}$ values (Hz) in square brackets.
[b] Assignments of *N*-acetyl groups are tentative.

analysis data. Other assignments were obtained from two- and three-bond intra-residue proton–carbon correlations in the ^{1}H-detected HMBC experiment. In particular, connectivities in residue **A** for H-6 (δ_H 1.19) to C-5 (δ_C 68.0), and to C-4 (δ_C 82.0), and for C-6 (δ_C 16.3) to H-5 (δ_H 4.43) showed that these signals matched the spin system. Cross-peaks observable from H-6 (δ 1.22) to C-5 (δ 70.4) and from C-6 (δ 17.4) to H-4 (δ 3.49) showed that these signals belonged to residue **B**. From HMBC and NOESY experiments it was possible to obtain sequential information as shown below.

In the HMBC experiment, the inter-residual correlation from δ 5.01 (H-1 in residue **A**) to the carbon resonance at δ 79.6 (C-3 in residue **C**) and from δ 98.6 (C-1 in **A**) to δ 3.70 (H-3 in **C**) showed the partial sequence of **1**:

$$\rightarrow 4)\text{-}\alpha\text{-L-Fuc}^{\mathbf{A}}p\text{NAc-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}^{\mathbf{C}}p\text{NAc-}(1 \rightarrow \qquad \mathbf{1}$$

The anomeric carbon of residue **B** (δ 102.6) showed a correlation to H-4 of residue **A** (δ 3.87), and the anomeric carbon of residue **C** (δ 103.5) showed a correlation to H-3 (δ 3.90) of residue **B**. The structural element of the latter correlation was further confirmed by the NOESY experiment in which, inter alia, a cross-peak was observed from H-1 of residue **C** (δ 4.68) to H-3 (δ 3.90) of residue **B**. These correlations define the partial sequences of **2** and **3**:

$$\rightarrow 3)\text{-}\alpha\text{-L-Rha}^{\mathbf{B}}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-L-Fuc}^{\mathbf{A}}p\text{NAc-}(1 \rightarrow \qquad \mathbf{2}$$

$$\rightarrow 3)\text{-}\beta\text{-D-Glc}^{\mathbf{C}}p\text{NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}^{\mathbf{B}}p\text{-}(1 \rightarrow \qquad \mathbf{3}$$

From the combined results it is concluded that the O-antigen polysaccharide from *E. coli* serogroup O26 is composed of a trisaccharide repeating unit having the following structure **4**:

$$\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-L-Fuc}p\text{NAc-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow \qquad \mathbf{4}$$

3. Experimental

Instrumental.—Concentrations were performed under diminished pressure at < 40 °C, or under a stream of N_2 or air. For GLC, a Hewlett–Packard 5890A instrument fitted with a flame-ionisation detector was used; HP-5-V and DB225-II capillary columns were used. GC-MS was performed on a Hewlett–Packard 5890–5970 instrument equipped with an HP-5-MS capillary column. NMR experiments were performed on a JEOL GSX-270 spectrometer using standard JEOL pulse sequences.

Preparation and O-deacetylation of polysaccharide.—E. coli O26 bacteria were grown in Ty medium (30-L culture). Bacteria were killed by the addition of formaldehyde (1% final concentration) and harvested by centrifugation. The lipopolysaccharide was extracted by the hot phenol–water method [6]. It was treated with aq 2% AcOH at 100 °C for 2 h. Liberated lipid A was centrifuged and the supernatant solution was

neutralised, dialysed, and lyophilised. The product was further purified by gel permeation chromatography on Sephadex S-100 and collected in the void volume. *O*-Deacetylation of the polysaccharide was performed using 0.1 M NaOH for 16 h at room temperature.

NMR spectroscopy.—NMR spectra of solutions in D_2O were recorded at 70 °C. Chemical shifts are reported in ppm relative to sodium 4,4-dimethyl-4-sila(2,2,3,3-$^2H_4$)pentanoate (δ_H 0.00) and acetone (δ_C 31.00) as internal references. Chemical shifts were obtained from 1D spectra when possible. Assignments were obtained from proton–proton correlated spectroscopy (COSY), relayed COSY, and ^{13}C–^1H-decoupled ^1H-detected heteronuclear multiple quantum coherence (HMQC) experiments [7,8]. Relayed COSY spectra were performed using a delay time of 30 or 60 ms. Sequential information was obtained from two-dimensional nuclear Overhauser effects and heteronuclear multiple bond connectivity (HMBC) [9] experiments recorded using mixing and delay times of 300 and 45 ms, respectively.

Sugar analysis.—Hydrolysis of underivatised material was performed with 4 M HCl at 100 °C for 30 min. The sugars were then converted into alditol acetates. Unambiguous identifications of sugars were obtained by GC runs on an HP-5-V column using two temperature programs: (*a*) 180 °C (1 min), 180 °C → 210 °C at 3 °C/min; (*b*) 170 °C (1 min), 170 °C → 180 °C at 1 °C/min, 180 °C (1 min), 180 °C → 210 °C at 4 °C/min; and on a DB225-II column, using a temperature program: 180 °C (1 min), 180 °C → 210 °C at 4 °C/min, 210 °C (1 min), 210 °C → 240 °C at 3 °C/min. Retention times were compared with standards. GC-MS was performed on an HP-5-MS column, using a temperature program: 170 °C (1 min), 170 °C → 250 °C at 3 °C/min. All identifications of mass spectra were unambiguous [10,11].

Methylation analysis.—Methylation of the polysaccharide was carried out according to the methods described earlier [12,13]. The methylated material was hydrolysed with 4 M HCl at 100 °C for 30 min, then transformed into partially methylated alditol acetates and analysed by GC and GC-MS using the same conditions as described in sugar analysis [11,14].

Absolute configuration.—The absolute configurations of the sugars were determined essentially as described by Leontein et al. [5] by GC of their glycosides, using optically active 2-butanol [15]. GC was perfomed on an HP-5-V capillary column, using two temperature programs: (*a*) 170 °C (1 min), 170 °C → 180 °C at 1 °C/min, 180 °C (1 min), 180 °C → 210 °C at 4 °C/min, for detection of the 2-acetamido-2,6-dideoxy-L-galactose and the 2-acetamido-2-deoxy-D-glucose; (*b*) 145 °C (1 min), 145 °C → 160 °C at 0.5 °C/min, for detection of the 6-deoxy-L-mannose. Retention times of the derivatives were compared with those of authentic reference compounds.

Bacterial strain.—The *E. coli* O26:H11 was obtained from Dr J. Nataro, Center for Vaccine Development, Division of Geographic Medicine, Baltimore, USA.

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