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Carbohydrate Research 264 (1994) 305–311

CARBOHYDRATE RESEARCH

Structure of the O16 polysaccharide from *Escherichia coli* O16:K1: an NMR investigation

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Received 6 April 1994; accepted 17 May 1994

Abstract

The polysaccharide moiety of the O16 antigen (lipopolysaccharide) consists of D-glucopyranose, D-galactofuranose, L-rhamnopyranose, and 2-acetamido-2-deoxy-D-glucopyranose in the molar ratios 1:1:1:1. It is *O*-acetylated with one acetyl group per repeating unit. One- and two-dimensional NMR spectroscopy of the polysaccharide before and after *O*-deacetylation showed that the O16 polysaccharide has the structure

$$\rightarrow 6)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow 2)\text{-}\beta\text{-D-Gal}f\text{-}(1\rightarrow$$

with OAc at position 2 of the rhamnose residue.

Keywords: *E. coli*; O16 Antigen; Polysaccharide structure; NMR spectroscopy

1. Introduction

In excess of 160 O antigens (lipopolysaccharides, LPS) are known to occur with *Escherichia coli*. The prevalent O groups in neonatal *E. coli* meningitis are O1, O6, O7, O16, O18, and O83 (Ref [1]). With the exception of the O16 antigen, the structures of the respective O-polysaccharides are known [2–7]. Neonatal meningitis *E. coli* are encapsulated and exhibit the K1 polysaccharide (polysialic acid) [8]. Some O16 strains have the K92 polysaccharide that is a polysialic acid with a structure different from that of the K1 polysaccharide [9]. We have now elucidated the structure of the O16 polysaccharide. The

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SSDI 0008-6215(94)00202-Q

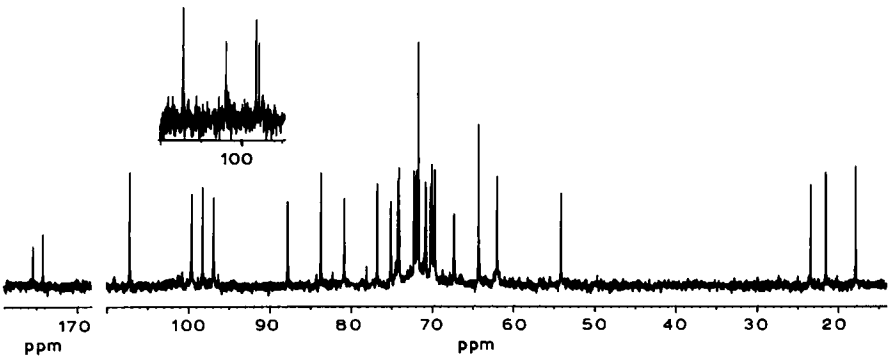


Fig. 1. 75-MHz ^{13}C NMR spectrum of the O16-specific polysasccharide, recorded in D_2O (65°C) with acetone (δ_C 31.45) as internal standard. The insert shows the anomeric region of the *O*-deacetylated polysaccharide.

studies were also prompted by the fact that *E. coli* O4 extraintestinal strains causing sepsis and/or pyelonephritis, cross-react serologically with *E. coli* O16.

2. Results and discussion

Isolation of the polysaccharide from E. coli *O16:K1*.—The LPS was obtained by extraction of the bacteria with aqueous 45% phenol and subsequent ultracentrifugation of the material from the aqueous phase [10]. The O16 polysaccharide, as obtained from the sedimented LPS by mild acid hydrolysis, was purified by gel permeation chromatography on Sephadex G-50. It was eluted with water directly after the void volume (K_D 0.9–0.95). It consisted of glucose, galactose, rhamnose, and 2-acetamido-2-deoxyglucose. As shown by ^{13}C NMR spectroscopy, the polysaccharide also contained *O*-acetyl groups. The constituents had the approximate molar ratios 1:1:1:1:1.

NMR analysis.—The ^{13}C NMR spectrum of the O16 polysaccharide (Fig. 1) contained four signals in the region of anomeric carbon atoms (δ 96.9–107.2), one signal in the region of carbon atoms bearing nitrogen (δ 54.1), and one signal typical of C-6 of 6-deoxy sugars (δ 17.8). Signals at δ 175.4, 174.2, 23.4, and 21.5 indicated the presence of *N*- and *O*-acetyl groups. The signals at δ 107.2 (anomeric carbon) and 87.8 (ring carbon) showed that the polysaccharide contained a furanose residue. An APT spectrum [11] (not shown) indicated the presence of three $-CH_2OH$ groups, two of which were unsubstituted (δ 62.2, from a pyranose, and δ 64.2, from a furanose) and one was substituted (δ 67.8). After *O*-deacetylation, the signals at δ 174.2 and 21.5 were not observed.

The ^1H NMR spectrum of the O16 polysaccharide (Fig. 2) contained five signals in the region of anomeric protons (δ 4.9–5.12). Four of these had a doublet structure typical of anomeric protons and that at δ 5.12 was a double doublet, indicative of a proton on an acetoxylated carbon. This signal was not observed in the ^1H NMR spectrum of the *O*-deacetylated polysaccharide (insert of Fig. 2). Assignments of the signals for the O16 polysaccharide and its *O*-deacetylated form (Tables 1 and 3) were obtained using 2D COSY, one-, two-, and three-step relayed coherence transfer (COSYRCT) [12,13], and with the help of 1D homonuclear double resonance in the difference mode [14]. The latter method was also used for the determinations of visual multiplicities and coupling constants.

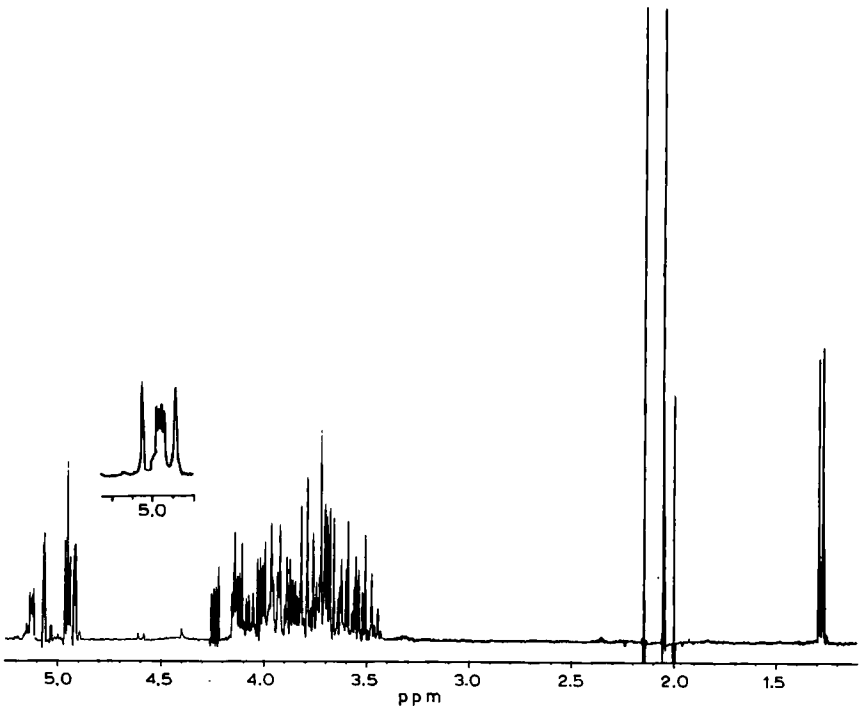


Fig. 2. 300-MHz ^{1}H NMR spectrum of the O16-specific polysaccharide, recorded in D_2O with acetone (δ_H 2.225) as internal standard. The insert shows the anomeric region of the *O*-deacetylated polysaccharide.

The signals of the ^{13}C NMR spectra were assigned (Tables 1 and 2) with 2D heteronuclear COSY spectra. The absolute configurations of all sugar residues were determined by calculating the experimental glycosylation effects [15] with D-glucose, whose absolute configuration was derived from its reactivity with D-glucose oxidase, as a basis. An analysis of coupling constants [16,17] and chemical shifts [18] revealed that the polysaccharide consisted of α-gluco-, α-rhamno-, and α-2-acetamido-2-deoxyglucopyranoses, and β-galactofuranose.

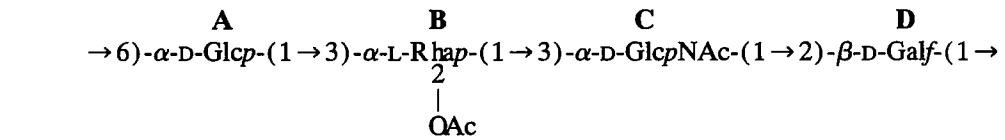
The sequence of sugar residues and the linkage positions were analysed in a series of NOE experiments with preirradiation of anomeric protons. For the polysaccharide (Table 2), the signals of H-1 of α-Glc*p* (residue **A**) and α-Glc*p*NAc (residue **C**) were too close to each other to be irradiated separately. As a consequence, two distinct responses were observed on H-3 of α-L-Rha*p*(2-OAc) (residue **B**) and on H-2 of β-D-Gal*f* (residue **D**). Preirradiation of H-1 of residue **B** resulted in an NOE effect on H-3 of residue **C** and preirradiation of H-1 of residue **D** resulted in an NOE effect on H-6a/H-6b of residue **A**. This indicated the sequence $\rightarrow$**A**$\rightarrow$**B**$\rightarrow$**C**$\rightarrow$**D**$\rightarrow$, which was supported by the results of an NOE experiment with the *O*-deacetylated polysaccharide (Table 4).

The position of the *O*-acetyl group at C-2 of rhamnose (residue **B**) was corroborated by a COSYRCT2 experiment. The spectrum showed a correlation of H-2 of rhamnose (δ5.12) with H-3, H-4, and H-5 of the same residue. These correlations, together with the absence of the signal at δ5.12 in the *O*-deacetylated polysaccharide and the appearence of the signal at δ 3.92, showed that the low-field shift of the H-2 signal of residue **B** was due to *O*-

Table 1
Assignments of the signals in the ^{1}H and ^{13}C NMR spectra of the O16 polysaccharide

Residue	Proton	δ	$J_{H,H}$	Hz	Carbon	δ	Glycosylation effect	$J_{C-1,H-1}$
→6)-α-D-Glc-(1→ (**A**)	H-1	4.95	1,2	4	C-1	96.9	+3.6	174
	H-2	3.54	2,3	10	C-2	72.9		
	H-3	3.69	3,4	10	C-3	74.2		
	H-4	3.48	4,5	10	C-4	70.8		
	H-5	3.99	5,6	2	C-5	71.7		
	H-6a	3.95	6a,6b	11	C-6	67.3		
	H-6b	3.72	5,6b					
→3)-α-L-Rha-(1→ (**B**)	H-1	4.92	1,2	2.5	C-1	99.6		171
	H-2	5.12	2,3	3	C-2	70.1		
	H-3	3.94	3,4	9.5	C-3	75.1		
	H-4	3.60	4,5	9.5	C-4	71.9	−1.6	
	H-5	4.08	5,6	6.5	C-5	70.0		
	H-6	1.28			C-6	17.8		
→3)-α-D-GlcNAc-(1→ (**C**)	H-1	4.96	1,2	3.5	C-1	98.3		171
	H-2	4.13	2,3	10	C-2	54.1		
	H-3	3.76	3,4	9	C-3	80.8		
	H-4	3.61	4,5	9	C-4	69.7		
	H-5	3.79	5,6	3.5	C-5	74.1		
	H-6a	3.95	6a,6b	12	C-6	62.0		
	H-6b	3.85	5,6b	4				
→2)-β-D-Gal*f*-(1→ (**D**)	H-1	5.07	1,2	1.5	C-1	107.2		174
	H-2	4.14	2,3	3.5	C-2	87.8		
	H-3	4.24	3,4	7	C-3	76.7		
	H-4	4.01	4,5	4	C-4	83.7		
	H-5	3.88	5,6	6.5	C-5	71.7		
	H-6a	3.73	6a,6b	11	C-6	64.2		
	H-6b	3.68	5,6b	6.5				

acetylation. Thus, the polysaccharide was *O*-acetylated at C-2 of the 3-linked L-rhamnose residue. The primary structure of the O16 polysaccharide is



The structure of the O16 polysaccharide differs so much from that of the O4 polysaccharide [19], even with respect to composition, that the serological cross-reactivity between these two antigens [1] is not at all apparent from their primary structures. Interestingly, the recombinant O4 polysaccharide obtained in genetic manipulations of *E. coli* K-12 and *E. coli* O4 strains [20] has greater structural similarity to the O16 polysaccharide. We had previously proposed that the *rfb* gene cluster of *E. coli* K-12 contains genes responsible for the formation and transfer of (activated) Gal*f* [20]. From the results presented here, it seems possible that the *rfb* gene cluster of *E. coli* K-12 contains more glycosyl transferases

Table 2
NOE data for the O16 polysaccharide

NOE observed on		Pre-irradiated proton		
Residue	Proton	**A**,H-1/C,H-1	**B**,H-1	**D**,H-1
→6)-α-D-Glc-(1→ (**A**)	H-2	+		
	H-6a			+
	H-6b			+
→3)-α-L-R h a-(1→ (**B**)	H-2		+	
2	H-3	+	+ [a]	
|				
OAc				
→3-α-D-GlcNAc-(1→ (**C**)	H-2	+		
	H-3		+	
→2)-β-D-Gal*f*-(1→ (D)	H-2	+		
	H-3			+ [a]

[a] Minor signal due to spin diffusion.

that are also operative in *E. coli* O16. A study of the genetic background for this apparent similarity would be interesting.

3. Experimental

Bacteria and cultivation.—*E. coli* F11119-41 (O16:K1:H$^-$), Freiburg strain collection number 20366, the O16 test strain, was used. The bacteria were grown to the late logarithmic phase (5–7 h) in 14-L batch cultures at 37°C in a medium containing, per L, tryptone (7.5 g), yeast extract (10 g), D-glucose (10 g), NaCl (3 g), $Na_2HPO_4 \cdot 12H_2O$ (8 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), and poly(ethylene glycol) (0.3 g). D-Glucose and the $MgSO_4$ were sterilised separately. At the end of the cultivation, the bacteria were killed with phenol (1% final concentration) and harvested by centrifugation.

Isolation of the polysaccharide.—The LPS was isolated from the bacteria with aqueous phenol at 65°C (10 min) and the material obtained from the aqueous phase was purified by repeated ultracentrifugation as described [9]. The polysaccharide was obtained from the LPS by hydrolysis in aq 1% AcOH (100°C, 90 min) and purified by chromatography on Sephadex G-50.

Analytical methods.—The sugar residues were determined as their alditol acetates by GLC using an ECNSS-M column at 170°C (neutral sugars) and Poly-A-103 at 230°C (differentiation of amino sugars) [21]. The Elson–Morgan reaction [22] was used for the determination of glucosamine. The absolute configuration of glucose was determined with D-glucose oxidase.

The ^1H and ^{13}C NMR spectra were recorded with a Bruker WM 300 spectrometer at 65°C, using acetone (δ_H 2.225; δ_C 31.45) as the internal standard in separate runs. Standard Bruker software was used for homonuclear 2D H,H COSY (COSYRCT, COSYRCT2, and

Table 3
Assignments of the signals in the ^{1}H and ^{13}C NMR spectra of the *O*-deacetylated O16 polysaccharide

Residue	Proton	δ	$J_{H,H}$	Hz	Carbon	δ
→6)-α-D-Glc-(1→ (**A**)	H-1	4.99	1,2	3.5	C-1	97.9
	H-2	3.57	2,3	10	C-2	72.5
	H-3	3.76	3,4	10	C-3	74.2
	H-4	3.49	4,5	10	C-4	70.8
	H-5	4.07	5,6	2	C-5	71.7
	H-6a	3.95	6a,6b	11	C-6	67.3
	H-6b	3.71	5,6b	5		
→3)-α-L-Rha-(1→ (**B**)	H-1	4.90	1,2	<2	C-1	102.0
	H-2	3.92	2,3	3.5	C-2	69.2
	H-3	3.76	3,4	9.5	C-3	78.3
	H-4	3.54	4,5	9.5	C-4	71.6
	H-5	4.01	5,6	6.5	C-5	70.2
	H-6	1.26			C-6	17.9
→3)-α-D-GlcNAc-(1→ (**C**)	H-1	4.96	1,2	3.5	C-1	98.2
	H-2	4.11	2,3	10	C-2	54.1
	H-3	3.78	3,4	9	C-3	80.4
	H-4	3.59	4,5	9	C-4	69.6
	H-5	3.81			C-5	74.0
	H-6a	3.87			C-6	61.9
	H-6b	3.83				
→2)-β-D-Gal*f*-(1→ (**D**)	H-1	5.06	1,2	<2	C-1	107.2
	H-2	4.14	2,3	3.5	C-2	87.8
	H-3	4.24	3,4	7	C-3	76.7
	H-4	4.00			C-4	83.6
	H-5	3.87			C-5	71.6
	H-6a	3.73			C-6	64.2
	H-6b	3.65				

Table 4
NOE data for the *O*-deacetylated O16 polysaccharide

NOE observed on		Pre-irradiated proton			
Residue	Proton	**A**,H-1	**B**,H-1	**C**,H-1	**D**,H-1
→6)-α-D-Glc-(1→ (**A**)	H-2	+			
	H-6a				+ [a]
	H-6b				+
→3)-α-L-Rha-(1→ (**B**)	H-2	+	+		
	H-3	+			
→3)-α-D-GlcNAc-(1→ (**C**)	H-2			+	
	H-3		+		
	H-4	+ [a]			
→2)-β-D-Gal*f*-(1→ (**D**)	H-2			+	+

[a] Minor signal due to spin diffusion.

COSYRCT3) and for heteronuclear 2D C,H COSY (XHCORRD). 1D NOE experiments (NOEMULT) were performed in the truncated driven (TOE) mode [23].

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