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Note

Structural studies of the O-antigenic polysaccharides from *Shigella dysenteriae* type 3 and *Escherichia coli* O124, a reinvestigation

K. Hanna M. Jonsson,[a] Andrej Weintraub[b] and Göran Widmalm[a,*]

[a]*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[b]*Karolinska Institutet, Department of Laboratory Medicine, Division of Clinical Bacteriology, Karolinska University Hospital, Huddinge, S-141 86 Stockholm, Sweden*

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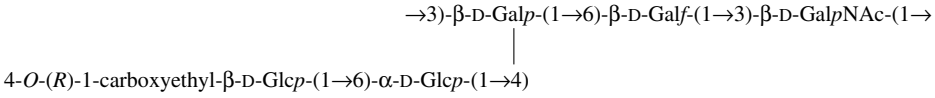
Abstract—The structures of the O-antigenic part of the lipopolysaccharides from *Shigella dysenteriae* type 3 and *Escherichia coli* O124 have been reinvestigated. ^{1}H and ^{13}C NMR spectroscopy in combination with selected 2D NMR techniques were used to determine the O-antigen pentasaccharide repeating units with the following structure:



From biosynthetic considerations this should also be the biological repeating unit. The structures of the repeating units also explain the previously observed cross-reactivity between the strains and to *E. coli* O164, which only differs in the terminal sugar residue that is lacking the (*R*)-1-carboxyethyl group.

Keywords: Cross-reactivity; *Escherichia coli* O164; Lactyl; Lipopolysaccharide; NMR

The Gram-negative bacterial species *Escherichia coli* is found in the normal flora of the human intestine where it plays many different roles. In some cases, however, it may acquire different virulence genes and become pathogenic.[1] In order to type, treat and understand its actions it is important to investigate different aspects of the species and its environment. The typing schemes are based on the O:K:H antigens, in which the O-serogroup is specified by the O-polysaccharide part of the lipopolysaccharide (LPS), which is anchored in the outer membrane of the bacterium. To date, more that 180 serogroups have been described.[2,3]

During our further development of the computer program CASPER[4] and the compilation of an *E. coli* O-antigen database,[5] we observed possible structural inconsistencies with respect to anomeric configuration of some sugar residues. The previously reported cross-reactivities[6,7] between *E. coli* O124, *Shigella dysenteriae* type 3 and *E. coli* O164 indicated structural similarities of their O-antigen polysaccharides. We therefore reinvestigated the structures of the O-polysaccharides from *E. coli* O124 and *S. dysenteriae* type 3.[8,9]

In our previous structural determination of the O-antigen of *E. coli* O164, we also showed that it is possible to prepare LPS solutions suitable for NMR spectral analysis.[10] The similarities of the O-antigens of *E. coli* O124 and *S. dysenteriae* type 3 were therefore

* Corresponding author. E-mail: gw@organ.su.se

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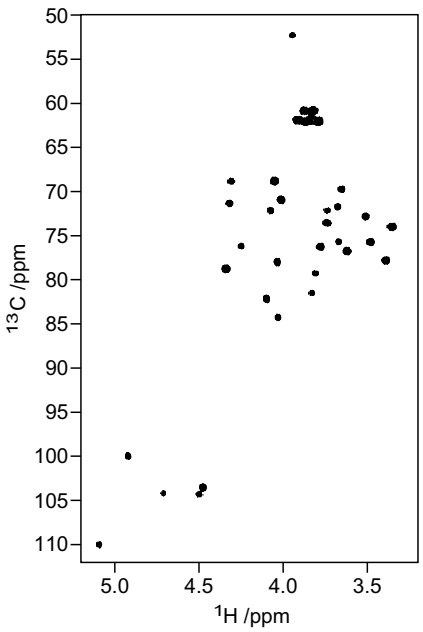
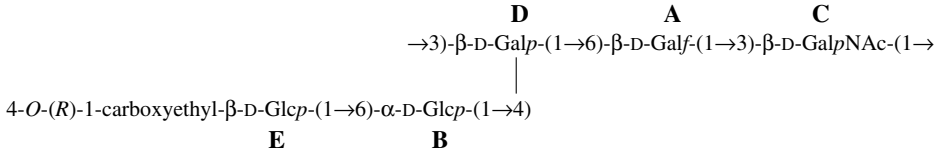


Figure 1. Part of the ^{1}H,^{13}C HSQC NMR spectrum of the LPS from *S. dysenteriae* type 3.

investigated by a comparison of the ^{1}H,^{13}C HSQC NMR spectra of their LPS. Since the O-polysaccharides are composed of repeating units, usually 10–25, these smooth strains show signals primarily from the O-antigen part of the polymer. This makes it possible to identify the complete set of signals from the sugar residues within the repeating unit (Fig. 1). It was evident that the spectra from *E. coli* O124 and *S. dysenteriae* type 3 were essentially identical. In addition, they were highly similar to those of *E. coli* O164, for which the ^{1}H and ^{13}C resonances have been assigned.[10] Furthermore, it is known that both the *E. coli* O124 and *S. dysenteriae* type 3 O-polysaccharides contain a 4-*O*-[(*R*)-1-carboxyethyl]-D-glucopyranosyl residue,[8,9] which is not present in the O-antigen of *E. coli* O164. Consequently, the O-antigens of *E. coli* O124 and *S. dysenteriae* type 3 should consist of pentasaccharide repeating units having D-Glc, 4-*O*-(*R*)-1-carboxyethyl-D-Glc, D-Gal and D-GalNAc as components.

The ^{1}H and ^{13}C NMR resonances of the O-antigen parts of the two LPS molecules were assigned using 2D NMR techniques and the chemical shifts are compiled in Table 1, in which the sugar residues are referred to as **A**–**E**, in the order of decreasing chemical shifts of their anomeric proton resonances. The $J_{H\text{-}1,H\text{-}2}$ and $J_{H\text{-}1,C\text{-}1}$ coupling constants together with the chemical shifts of anomeric protons and carbons indicated that both different ring forms and different anomeric configurations were present for the five sugar residues in the O-antigen repeating unit. The spin system of **A** having the anomeric resonance at δ_H 5.09 could be assigned to a →6)-β-D-Gal*f*-(1→ residue due to the high chemical shift of C-1 and low $J_{H\text{-}1,H\text{-}2}$ value as well as a large glycosylation shift of C-6, $\Delta\delta_C$ 8.5. Residue **B** with $\delta_{H\text{-}1}$ 4.92 was assigned to a →6)-α-D-Glc*p*-(1→ residue due to the low $J_{H\text{-}1,H\text{-}2}$ value of 3.7 Hz and a large glycosylation shift of C-6, $\Delta\delta_C$ 7.0. The ^{1}H, ^{1}H spin system of residue **C**, having $\delta_{H\text{-}1}$ 4.71 and $J_{H\text{-}1,H\text{-}2} = 8.0$ Hz, revealed that it had the *galacto*-configuration and from $\delta_{C\text{-}2}$ 52.3 and $\Delta\delta_{C\text{-}3}$ 7.3 it is a →3)-β-D-Gal*p*NAc-(1→ residue. Residue **D** is β-linked, $J_{H\text{-}1,H\text{-}2} = 7.7$ Hz, disubstituted and has the *galacto*-configuration with $\Delta\delta_{C\text{-}3}$ 7.7 and $\Delta\delta_{C\text{-}4}$ 6.5; thus it is a →3,4)-β-D-Gal*p*-(1→ residue. Finally, residue **E** is the 4-*O*-[(*R*)-1-carboxyethyl]-β-D-Glc*p*-(1→ residue having $J_{H\text{-}1,H\text{-}2} = 8.0$ Hz. In the ^{1}H,^{13}C HMBC spectrum a cross-peak was observed between C-1′ and H-4 of residue **E** thereby confirming the presence of the 1-carboxyethyl substituent.[11]

The sequence of the sugar residues in the O-antigen repeating units were determined from ^{1}H,^{13}C HMBC experiments (Fig. 2 and Table 2). Starting from the terminal residue the sequence 4-*O*-(*R*)-1-carboxyethyl-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→ can be deduced for the side-chain linked to the branch-point residue. For the backbone of the polymer, the sequence of residues can be determined as →3)-β-D-Gal*p*-(1→6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*NAc-(1→. Consequently, the structure of the O-antigen repeating unit of *E. coli* O124 and *S. dysenteriae* type 3 is



Since there is only one →3)-β-D-Gal*p*NAc-(1→ residue in the repeating unit, it should be found at the reducing end of the biological repeating unit.[5] The O-antigen from *E. coli* O164 is identical to that from *E. coli* O124, but for the 4-*O*-[(*R*)-1-carboxyethyl]-substituent of the terminal sugar residue of the side chain in the polymer.

Recently, oligosaccharides related to the O-antigen structures from *S. dysenteriae* type 3 have been synthesized.[12,13] However, since the oligosaccharides synthesized were based on a different structure than that presented herein, the usefulness of the materials may be doubtful. It must be emphasized that prior to the

Table 1. ^{1}H and ^{13}C NMR chemical shifts (ppm) of the signals from the O-antigen part of the LPS from *S. dysenteriae* type 3

Sugar residue		$^{1}H/^{13}C$									
		1	2	3	4	5	6	1′	2′	Me	CO
→6)-β-D-Gal*f*-(1→	**A**	5.09 [3][a]	4.10	4.03	4.05	4.01	3.74, 4.08				
		(−0.13)	(0.08)	(−0.07)	(0.00)	(0.20)					
		110.0 {175}[b]	82.1	78.0	84.2	70.9	72.1				
		(8.0)	(−0.3)	(1.1)	(1.0)	(−0.8)	(8.5)				
→6)-α-D-Glc*p*-(1→	**B**	4.92 [3.7]	3.51	3.73	3.65	4.32	4.05, 4.31				
		(−0.31)	(−0.03)	(0.01)	(0.23)	(0.48)					
		100.0 {173}[b]	72.8	73.5	69.7	71.3	68.8				
		(7.0)	(0.3)	(−0.3)	(−1.0)	(−1.1)	(7.0)				
→3)-β-D-Gal*p*NAc-(1→	**C**	4.71 [8.0]	3.94	3.81	4.05	3.67	3.79, 3.86			2.04	
		(0.03)	(0.04)	(0.04)	(0.07)	(−0.05)					
		104.2 {162}[b]	52.3	79.3	68.8	75.7	62.0			23.2	175.4
		(7.9)	(−2.5)	(7.3)	(0.0)	(−0.3)	(0.1)				
→3,4)-β-D-Gal*p*-(1→	**D**	4.50 [7.7]	3.67	3.83	4.25	3.77	3.82, 3.87				
		(−0.03)	(0.22)	(0.24)	(0.36)	(0.12)					
		104.3 {164}[b]	71.7	81.5	76.2	76.2	60.8				
		(6.9)	(−1.3)	(7.7)	(6.5)	(0.3)	(−1.0)				
4-*O*-[R]-β-D-Glc*p*-(1→[c]	**E**	4.47 [8.0]	3.35	3.62	3.39	3.48	3.84, 3.91	4.34	1.36		
		(−0.15)	(0.11)	(0.01)	(0.07)	(0.01)		(0.29)	(0.00)		
		103.5 {163}[b]	74.0	76.7	77.7	75.7	61.9	78.7	19.7		182.2
		(7.7)	(−0.5)	(1.8)	(−1.1)	(0.4)	(1.6)	(1.3)	(0.1)		

$J_{H\text{-}1,H\text{-}2}$ values are given in hertz in square brackets and $J_{H\text{-}1,C\text{-}1}$ values in braces. The chemical shift differences as compared to the corresponding monosaccharides are given in parenthesis.

[a] Width at half peak-height from the ^{1}H NMR spectrum of *E. coli* O124 LPS.

[b] From the coupled $^{1}H,^{13}C$ HSQC NMR spectrum of *E. coli* O124 LPS.

[c] R = (*R*)-1-carboxyethyl.

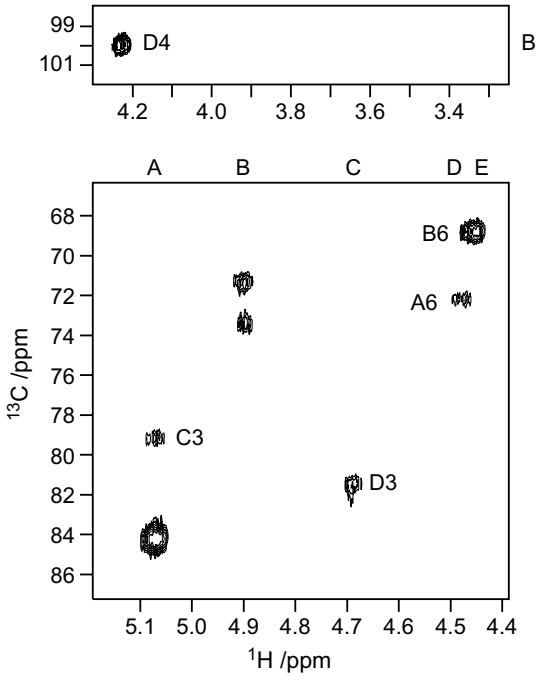


Figure 2. Part of the $^{1}H,^{13}C$ HMBC NMR spectrum of the LPS from *E. coli* O124.

undertaking of synthesis of complex oligosaccharides to be used as vaccine candidates,[14,15] one should try to ascertain whether the published structures are correct or the synthesis effort may be in vain.

Table 2. Inter-residue correlations observed in the $^{1}H,^{13}C$ HMBC NMR spectrum from the O-antigen part of the LPS from *S. dysenteriae* type 3

Residue	Anomeric atom	Residue	Atom
A	H-1	C	C-3
A	C-1	C	H-3
B[a]	H-1	D	C-4
B	C-1	D	H-4
C	H-1	D	C-3
C[a]	C-1	D	H-3
D[a]	H-1	A	C-6
E	H-1	B	C-6

[a] Additional correlations found in the $^{1}H,^{13}C$ HMBC NMR spectrum from the O-antigen part of the LPS from *E. coli* O124.

1. Experimental

1.1. Bacterial strains and conditions of growth

E. coli O124 (strain 931-78/O124:NM, Centers for Disease Control and Prevention, CDC, Atlanta, GA) and *S. dysenteriae* type 3 (strain Y797/86, a clinical isolate from the culture collection at Karolinska University Hospital) were grown in a glucose-containing

tryptone/yeast extract medium.[16] The LPS was extracted by the hot phenol/water method.[17]

1.2. NMR spectroscopy

NMR spectra of LPS materials in D_2O solutions were recorded at 45 °C using a Varian Inova 600 spectrometer equipped with a 5 mm PFG triple-resonance probe and on a Bruker Avance 400 MHz spectrometer. Data processing was performed using a vendor-supplied software. The chemical shifts are reported in parts per million using internal sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)-propanoate (TSP, δ_H 0.00) or external 1,4-dioxane in D_2O (δ_C 67.40) as references. The LPS from *S. dysenteriae* type 3 (6 mg) and *E. coli* O124 (20 mg) were each dissolved in 0.6 mL of D_2O. In addition to one-dimensional ^1H and ^{13}C NMR spectra, the following 2D NMR experiments were used to assign signals: $^1H,^1H$-TOCSY[18] with mixing times of 10 and 90 ms, $^1H,^{13}C$ HSQC[19] (also ^{13}C-coupled) and $^1H,^{13}C$ HMBC[20] with a 50 ms delay for the evolution of long-range couplings. The chemical shifts were compared to those of the corresponding monosaccharides.[11,21,22]

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