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Note

Structure of the O-polysaccharide of *Escherichia coli* O132

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ABSTRACT

Mild acid degradation of the lipopolysaccharide of *Escherichia coli* O132 released its O-polysaccharide. Analysis by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy prior and subsequent to O-deacetylation, in conjunction with sugar analysis, revealed a linear pentasaccharide repeating unit of the O-polysaccharide having the following structure:

→2)-α-D-Gal*f*-(1→3)-α-L-Rha*p*2Ac-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→

Putative functions of genes in the O-antigen gene cluster of *E. coli* O132 are consistent with the O-polysaccharide structure.

Escherichia coli is a gram-negative bacterium that colonizes the gastrointestinal tract of humans and warm-blooded animals where it usually is a harmless commensal beneficial to the host. However, there are pathogenic variants of *E. coli* clones that have acquired virulence factors thereby increasing their ability to adapt to new niches.[1,2] These pathogenic *E. coli* cause diseases such as diarrhea, septicemia and urinary tract infection. *E. coli* O132 is a serogroup that has been reported to cause septicemia in chickens,[3] diarrhea in rabbits where its pathotype was enteropathogenic *E. coli*,[4] diarrhea in calves where the pathotype was Shiga toxin-producing *E. coli*,[5] and diarrhea in children under three years of age (enterotoxigenic *E. coli*).[6] We herein elucidate the structure of the *E. coli* O132 O-antigen polysaccharide using chemical, NMR spectroscopic and bioinformatics methods.

Lipopolysaccharide (LPS) was isolated from bacterial cells of *E. coli* O132 and hydrolyzed with mild acid to give an O-polysaccharide, which was isolated by GPC. Sugar analysis by GLC of the alditol acetates derived after full acid hydrolysis of the polysaccharide revealed Rha, Glc, Gal, and GlcNAc in the ratios 1.9:1:0.8:0.6 (detector response). The L configuration of Rha and the D configuration of the other monosaccharides were not determined chemically but inferred from the ^{13}C NMR chemical shifts[7] and the content of the O-antigen gene cluster (see below).

The ^{13}C NMR spectrum of the O-polysaccharide (Fig. 1, bottom) showed signals for five anomeric carbons in the region δ 98.4–102.9, three C–CH_2OH groups (C-6 of Glc, Gal, and GlcN) at δ 61.0–64.0, two C–CH_3 groups (C-6 of Rha) at δ 17.7–17.8, one nitrogen-bearing carbon (C-2 of GlcN) at δ 56.7, 19 oxygen-bearing sugar ring carbons in the region δ 70.2–84.7, one N-acetyl group at δ 23.6 (Me) and 175.7 (CO), and one O-acetyl group at δ 21.9 (Me) and 174.5 (CO). The ^{1}H NMR spectrum showed signals for five anomeric protons at δ 4.58–5.18, two C–CH_3 groups (H-6 of Rha) at δ 1.26–1.28, other sugar protons in the region δ 3.34–4.35, one N-acetyl group at δ 2.07, and one O-acetyl group at δ 2.24. Therefore, the O-antigen has a pentasaccharide repeating unit containing one residue each of Glc, Gal, and GlcNAc; two residues of Rha; and one O-acetyl group.

The O-polysaccharide was O-deacetylated with aq ammonia, and the ^{1}H and ^{13}C NMR spectra of the native and O-deacetylated O-polysaccharides were assigned using 2D ^{1}H,^{1}H-COSY, TOCSY, ^{1}H,^{13}C-HSQC, and HSQC-TOCSY experiments (Table 1). Based on ^{13}C NMR chemical shifts, intra-residue ^{1}H,^{1}H correlations, and $^{3}J_{H,H}$ coupling constant values estimated from the 2D NMR spectra, five spin-systems were identified, including one each for α-Glc*p* (unit **C**), α-Gal*f* (unit **E**), and β-Glc*p*NAc (unit **A**), and two for α-Rha*p* (units **B** and **D**). The spin system for GlcNAc was recognized by correlations between the proton at the nitrogen-bearing carbon (H-2) at δ 3.74 and the corresponding carbon (C-2) at δ 56.7 in the ^{1}H,^{13}C-HSQC spectrum. A relatively large $J_{1,2}$ coupling constant ~8 Hz confirmed that unit **A** is β-linked. The C-1 chemical shift δ 102.3 of unit **E** confirmed the α-linkage of the Gal*f* residue, and the C-5 chemical shifts δ 72.3 of unit **C** and δ 70.2–70.5 of units **B** and **D** corroborated the

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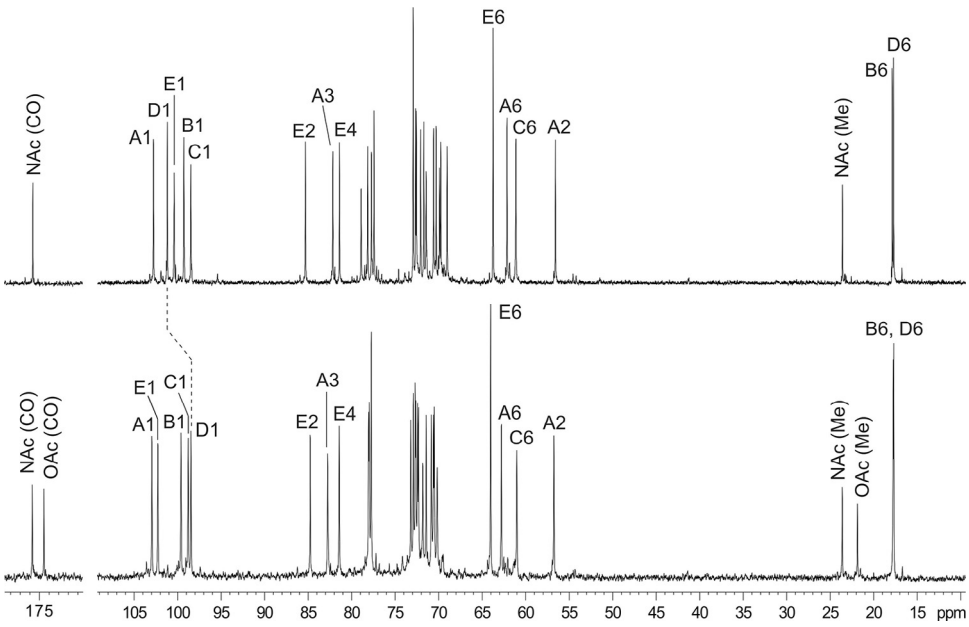

Fig. 1. ^{13}C NMR spectra of the O-polysaccharide (bottom) and the O-deacetylated polysaccharide (top). Arabic numerals refer to carbons in sugar residues denoted by letters as shown in Table 1.

α-linkages of the three other monosaccharides[8] in the native O-polysaccharide.

The 2D ^{1}H,^{1}H-ROESY spectrum of the O-deacetylated polysaccharide (Fig. 2) showed **A** H-1/**E** H-2, **B** H-1/**A** H-3, **C** H-1/**B** H-2, **D** H-1/**C** H-4, and **E** H-1/**D** H-3 cross-peaks between the transglycosidic protons. The 2D ^{1}H,^{13}C-HMBC experiment (Fig. 3) revealed the following correlations between the anomeric protons and the linkage carbons: **A** H-1/**E** C-2, **B** H-1/**A** C-3, **D** H-1/**C** C-4, and **E** H-1/**D** C-3. These data were in agreement with downfield displacements, due to glycosylation,[9] of the signals for the linkage carbons of units **A** – **E** (Table 1) as compared with their positions in the corresponding non-substituted monosaccharides.[8] Therefore, the O-deacetylated polysaccharide has the following structure:

→2)-α-D-Gal*f*-(1→3)-α-L-Rha*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→

E **D** **C** **B** **A**

Table 1
^{1}H and ^{13}C NMR chemical shifts of the polysaccharides from *E. coli* O132 (δ, ppm)

Sugar residue	Monosaccharides						Acetyl groups	
	C-1 *H-1*	C-2 *H-2*	C-3 *H-3*	C-4 *H-4*	C-5 *H-5*	C-6 *H-6 (a,b)*	C-1	C-2 *H-2*
O-Polysaccharide								
→3)-β-D-Glc*p*NAc-(1→	102.9	56.7	82.7	70.6	77.7	62.8	175.7	23.6
A	*4.58*	*3.74*	*3.63*	*3.34*	*3.45*	*3.70,3.98*		*2.07*
→2)-α-L-Rha*p*-(1→	99.6	78.0	70.8	73.2	70.5	17.7		
B	*4.98*	*3.83*	*3.86*	*3.49*	*3.98*	*1.26*		
→4)-α-D-Glc*p*-(1→	98.8	72.9	72.6	78.0	72.3	61.0		
C	*4.86*	*3.55*	*3.83*	*3.60*	*4.09*	*3.80,3.86*		
→3)-α-L-Rha*p*2Ac-(1→	98.4	72.4	77.7	71.8	70.2	17.8	174.5	21.9
D	*4.95*	*5.08*	*3.94*	*3.59*	*4.11*	*1.28*		*2.24*
→2)-α-D-Gal*f*-(1→	102.3	84.7	72.7	81.4	71.4	64.0		
E	*5.18*	*4.07*	*4.35*	*3.86*	*3.76*	*3.61,3.64*		
O-Deacetylated polysaccharide								
→3)-β-D-Glc*p*NAc-(1→	103.1	56.7	82.4	70.0	77.7	62.3	175.8	23.6
A	*4.72*	*3.88*	*3.70*	*3.50*	*3.51*	*3.76,3.98*		*2.09*
→2)-α-L-Rha*p*-(1→	99.6	78.0	70.8	73.2	70.5	17.7		
B	*5.01*	*3.86*	*3.87*	*3.50*	*4.00*	*1.27*		
→4)-α-D-Glc*p*-(1→	98.8	72.9	72.8	78.4	72.3	61.3		
C	*4.88*	*3.58*	*3.84*	*3.62*	*4.07*	*3.75,3.83*		
→3)-α-L-Rha*p*-(1→	101.6	69.2	79.2	71.7	70.1	17.9		
D	*4.93*	*4.15*	*3.79*	*3.55*	*4.08*	*1.28*		
→2)-α-D-Gal*f*-(1→	100.8	85.6	73.2	81.7	71.9	63.9		
E	*5.23*	*4.16*	*4.38*	*3.88*	*3.79*	*3.62,3.66*		