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Structure and genetics of the O-antigen of *Escherichia coli* O169 related to the O-antigen of *Shigella boydii* type 6



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ABSTRACT

The O-polysaccharide (O-antigen) of *Escherichia coli* 0169 was studied by sugar analysis along with 1D and 2D 1 H and 13 C NMR spectroscopy. The following structure of the branched hexasaccharide repeating unit was established:

 $\beta\text{-D-Glc}p$ \downarrow 6 $\rightarrow 3)-\alpha\text{-D-Gal}p-(1\rightarrow 6)-\alpha\text{-D-Man}p-(1\rightarrow 2)-\alpha\text{-D-Man}p-(1\rightarrow 3)-\beta\text{-D-Gal}p\text{NAc-}(1\rightarrow 4)$ \uparrow 1 $\beta\text{-D-Glc}pA$

The O-polysaccharide of *E. coli* O169 differs from that of *Shigella boydii* type 6 only in the presence of a side-chain glucose residue. A comparison of the O-antigen biosynthesis gene clusters between the *galF* to *gnd* genes in the genomes of the two bacteria revealed their close relationship. The glycosyltransferase gene responsible for the formation of the β -D-Glcp-(1 \rightarrow 6)- α -D-Galp linkage in the O-antigen was identified in the gene cluster.

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Escherichia coli is a clonal species, including both commensals and pathogens. The bacteria are normally identified by a combination of their O- and H- (and sometimes K-) antigens. Variations in types of sugars present, their arrangement and linkages make the O-antigen or O-polysaccharide (OPS) the most variable constituent on the cell surface and provide the basis for serotyping of the bacteria.¹ By now about 180 O-serotypes of *E. coli* have been recognized. Genes for O-antigen synthesis are normally located on the chromosome as an O-antigen gene cluster, and genetic variations in the cluster are the major basis for the diversity of the Oantigen forms. OPS structures for most serogroups have been elucidated (see *E. coli* O-antigen database at http://www.casper. organ.su.se/ECODAB/)² but several still remain to be established.

Serogroup O169 belongs to enterotoxigenic *E. coli* (ETEC) group, which has been recognized as a common cause of foodborne outbreaks.³ *E. coli* O169 has notably become one of the most prevalent ETEC pathogens associated with foodborne outbreaks in many countries including the Republic of Korea,⁴ Japan,⁵ and the USA.⁶ In

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this work, we established the OPS structure of *E. coli* O169 and compared the closely related O-antigen gene clusters of *E. coli* O169 and *Shigella boydii* type 6.

The OPS was obtained by mild acid degradation of the lipopolysaccharide of *E. coli* O169 and separated from lower molecular mass substances by GPC. Sugar analysis by GLC of the alditol acetates derived after full acid hydrolysis of the OPS revealed Man, Glc, Gal, and GalN in the ratio ~2:1:0.5:0.5 (detector response). GLC analysis of the acetylated (*S*)-2-octyl glycosides demonstrated the p configuration of all constituent monosaccharides. Further NMR studies (see below) showed that the OPS also includes p-glucuronic acid (GlcA).

The ¹³C NMR spectrum of the OPS (Fig. 1) contained signals for 6 anomeric carbons in the region δ 96.7–105.3, 5 C–CH₂OH groups (C-6 of hexoses and GalN) at δ 62.8, 63.0, 63.2, 67.4, and 71.8 (data of attached-proton test), 1 nitrogen-bearing carbon (C-2 of GalN) at δ 53.2, 1 C–CO₂H group (C-6 of GlcA) at δ 176.6, and 23 oxygenbearing sugar ring carbons in the region δ 65.7–81.3 as well as 1 N-acetyl group at δ 24.6 (CH₃) and 176.6 (CO). In the low-field region of the ¹H NMR spectrum, there were seven signals, including those for six anomeric protons at δ 4.46–5.13 and H-4 of a Gal residue at δ 4.47. The spectrum also contained signals for other sugar protons in the region δ 3.27–4.17 and one N-acetyl group at δ 2.02. These data indicated that the OPS has a hexasaccharide repeating unit (O-unit) containing one residue each of D-Glc, D-Gal, D-GalNAc, D-GlcA, and two residues of D-Man.

The ¹H and ¹³C NMR spectra of the OPS were assigned using 2D COSY, TOCSY, ¹H,¹³C HSQC, HMBC, and HSQC-TOCSY experiments (Table 1). Based on intra-residue ¹H,¹H and ¹H,¹³C correlations and coupling constant values estimated from the 2D NMR spectra, six spin systems, including two systems each for *gluco*- (Glc and GlcA, denoted as units **A** and **B**), *galacto*- (Gal and GalNAc, units **C** and **F**), and *manno*-configurated sugars (two Man residues, units **D** and **E**)

were recognized, all being in the pyranose form. The spin system for GalNAc was distinguished by a correlation between proton at the nitrogen-bearing carbon (H-2) and the corresponding carbon (C-2) at δ 4.04/53.2 in the HSQC spectrum. Unit **B** was identified as β -GlcA by a correlation of H-5 with C-6 (CO₂H) at δ 3.76/176.6 in the HMBC spectrum. A large $J_{1,2}$ coupling constant of ~7 Hz confirmed that units **A**, **B**, and **F** are β -linked, whereas a significantly smaller value of ~3 Hz indicated the α -linkage of unit **C**. The position of signals for C-5 of units **D** and **E** at δ 72.7 and 75.6, respectively, indicated that both Man residues are α -linked (compare published data δ 73.34 and 77.00 for α - and β -Manp, respectively⁷).

The OPS was subjected to a Smith degradation and the products were fractionated by GPC. The main oligosaccharide product was studied by 1D and 2D NMR spectroscopy, including the full assignment of the ¹H and ¹³C NMR signals (Fig. 1, Table 1). As a result, the following structure was elucidated:

$$\beta$$
-D-Gal*p*NAc-(1 \rightarrow 3)- α -D-Gal*p*-(1 \rightarrow 1)-Gro
F C D'

where Gro (unit \mathbf{D}') indicates glycerol derived from 6-substituted Man.

Subsequently, analysis of the OPS revealed relatively low-field positions of the signals for C-2 of unit **E**, C-3 of unit **F**, C-6 of unit **D**, C-3, 4, and 6 of unit **C** δ 80.7, 77.6, 67.4, 81.3, 78.3, and 71.8, as compared with their positions in the corresponding non-substituted monosaccharides at δ 71.69, 72.01, 61.99, 70.13, 70.28, and 62.04,⁷ respectively, showed that the OPS is branched with unit **C** at the double-branching point and demonstrated the modes of glycosylation of the monosaccharide residues. In accordance with the side-chain position of Glc and GlcA, their C-2–C-6 chemical



Fig. 1. ¹³C NMR spectra of the OPS (top) and oligosaccharide derived by Smith degradation of the OPS (bottom) from *E. coli* O169. Arabic numerals refer to carbons in sugar residues denoted by letters as shown in Table 1 and Chart 1.

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