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Structures of the O-antigens of *Escherichia coli* O13, O129, and O135 related to the O-antigens of *Shigella flexneri*

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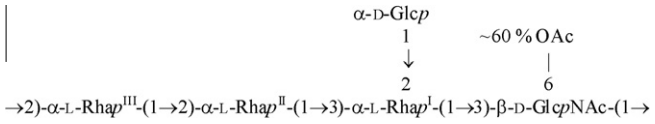
ABSTRACT

O-Polysaccharides (O-antigens) were isolated from *Escherichia coli* O13, O129, and O135 and studied by chemical analyses along with 2D ^{1}H and ^{13}C NMR spectroscopy. They were found to possess a common →2)-L-Rha-(α1→2)-L-Rha-(α1→3)-L-Rha-(α1→3)-D-GlcNAc-(β1→ backbone, which is a characteristic structural motif of the O-polysaccharides of *Shigella flexneri* types 1–5. In both the bacterial species, the backbone is decorated with lateral glucose residues or/and *O*-acetyl groups. In *E. coli* O13, a new site of glycosylation on 3-substituted Rha was revealed and the following O-polysaccharide structure was established:



The structure of the *E. coli* O129 antigen was found to be identical to the O-antigen structure of *S. flexneri* type 5a specified in this work and that of *E. coli* O135 to *S. flexneri* type 4b reported earlier.

1. Introduction

O-Antigen (O-polysaccharide, OPS) is a part of the lipopolysaccharide located on the outer membrane of Gram-negative bacteria. It contributes to the major antigenic variability and is the basis for bacterial serotyping. O-Antigen is essential for the complete function of bacteria and is related to bacterial virulence.

Escherichia coli is a clonal species, including both commensal and pathogenic strains. Currently, 166 distinct O-antigen forms have been recognized in the *E. coli* classification scheme.[1] *Shigella*, a well-known human pathogen, has long been known to be closely related to *E. coli*. Based on structure, serology, and genetic data, it was found that O-antigens of 23 *E. coli* O-serogroups have counterparts among *Shigella* O-antigens.[2] In this paper, we present new structures of the OPSs of *E. coli* O13, O129, and O135, which are identical or closely related to those of *Shigella flexneri* types 1–5, and determined the O-acetylation pattern of the OPS of *S. flexneri* type 5a.

2. Results and discussion

The OPSs were obtained by mild acid degradation of the lipopolysaccharides, which were isolated from dried bacterial cells by the phenol–water procedure. They were separated from lower-molecular mass substances by GPC on Sephadex G-50. The OPSs were studied by chemical analyses along with 2D ^{1}H and ^{13}C NMR spectroscopy.

2.1. OPS structure of *E. coli* O13

Sugar analysis by GLC of the alditol acetates derived after full acid hydrolysis of the OPS revealed Rha, Glc, and GlcN (from GlcNAc). GLC analysis of the acetylated (*S*)-2-octyl glycosides demonstrated the D configuration of Glc and GlcN and L configuration of Rha.

The ^{13}C NMR spectrum of the OPS (Fig. 1, top) contained signals having different intensities, most likely, owing to non-stoichiometric O-acetylation (there was a signal for CH_3 of an *O*-acetyl group at δ 21.8). The ^{1}H NMR spectrum of the OPS showed signals for one *N*-acetyl group at δ 2.06 and one *O*-acetyl group at δ 2.15 in the ratio ~1:0.6.

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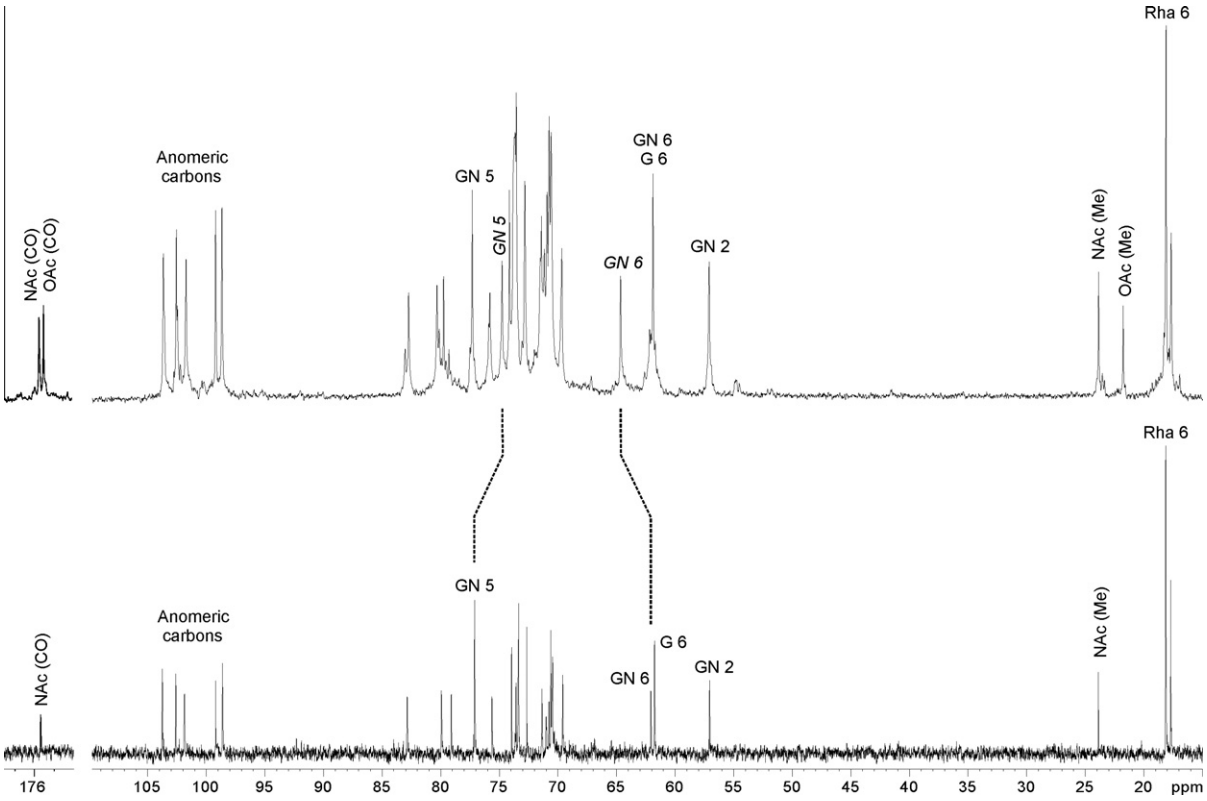

Figure 1. ^{13}C NMR spectra of the OPS (top) and DPS (bottom) of *E. coli* O13. Numbers refer to carbons in Glc (G), GlcNAc (GN) and Rha; peak annotations for 6-O-acetylated GlcNAc are shown in italics.

Therefore, the OPS was subjected to O-deacetylation with aq ammonia, and the resultant O-deacetylated polysaccharide (DPS) was found to be regular. Its ^{13}C NMR spectrum (Fig. 1, bottom) showed signals for five anomeric carbons in the region δ 98.7–103.6, two $HOCH_2$–C groups (C-6 of Glc and GlcNAc) at δ 61.9 and 62.2, one nitrogen-bearing carbon (C-2 of GlcNAc) at δ 57.1, 19 oxygen-bearing non-anomeric sugar ring carbons in the region δ 69.7–83.0, three CH_3–C groups (C-6 of three Rha residues, Rha^I–Rha^{III}) at δ 17.6–18.1, and one *N*-acetyl group at δ 23.9 (CH_3) and 175.8 (CO). Accordingly, the ^1H NMR spectrum of the DPS showed signals for five anomeric protons at δ 4.73–5.27, other sugar protons in the region δ 3.32–4.15, three methyl groups (H-6 of Rha^I–Rha^{III}) at δ 1.25–1.33, and one *N*-acetyl group at δ 2.08.

The ^1H and ^{13}C NMR spectra of the DPS were assigned using 2D $^1H/^1H$ COSY, TOCSY, $^1H/^{13}C$ HSQC, and HMBC experiments (Table 1). Based on intra-residue H/H correlations and coupling constant values estimated from the COSY and TOCSY spectra, the spin systems were assigned to Glc, GlcNAc, and Rha^I–Rha^{III}, all being in the pyranose from. A $J_{1,2}$ coupling constant of ~3 Hz showed that Glc*p* is α-linked, whereas a relatively large $J_{1,2}$ value of ~7 Hz indicated that Glc*p*NAc is β-linked. The position of the signals for C-5 at δ 70.6–70.9 demonstrated the α-linkage of all Rha*p* residues (compare published data δ 70.0 for α-Rha*p* and δ 73.2 for β-Rha*p*).[3] A heteronuclear $^1H/^{13}C$ HMBC experiment showed correlations between protons and carbons separated by two and three bonds. Taking into account the ^1H and ^{13}C NMR chemical shift data (Table 1), cross-peaks at δ 5.14/79.3; 5.27/75.8; 5.05/83.0; 4.73/80.1; and 4.92/77.3 were assigned to Rha^{III} H-1,Rha^{II} C-2; Rha^{II} H-1,Rha^I C-3; Rha^I H-1,GlcNAc C-3; GlcNAc H-1,Rha^{III} C-2; and Glc H-1,Rha^I C-2 correlations between anomeric protons and linkage carbons, respectively. These data are in agreement with the glycosylation pattern of the OPS, which was inferred from significant downfield displacements by 4.5–8.5 ppm of the signals for C-2 of Rha^{III} and Rha^{II}, C-3 of GlcNAc, C-2 and C-3 of Rha^I, as compared with their positions in the corresponding non-substituted monosaccharides.[3] These data defined the glycosylation pattern and monosaccharide sequence in the repeating unit.

Position of the *O*-acetyl group was determined by a $^1H/^{13}C$ HSQC experiment on the OPS. As compared with the HSQC spectrum of the DPS, ~60% of the GlcNAc H-6a,6b,C-6 cross-peaks shifted from δ 3.76, 3.91/62.2 to 4.32, 4.41/64.7 (Fig. 2). This displacement was evidently due to a deshielding effect of the *O*-acetyl group and indicated partial O-acetylation of GlcNAc at position 6. The O-acetylation pattern was confirmed by an upfield shift by 2.7 ppm of a part of the C-5 signal of GlcNAc (β-effect of O-acetylation).[4] Therefore, the OPS of *E. coli* O13 has the structure shown in Chart 1. It shares the backbone with the OPSs of *S. flexneri* types 1–5 but none of the latter is glucosylated at position 2 of Rha^I. The OPS of *E. coli* O13 has thus a unique structure.

2.2. OPS structure of *E. coli* O129 and *S. flexneri* type 5a

The ^{13}C NMR spectrum of the OPS of *E. coli* O129 (Fig. 3, top) contained signals of different intensities, most likely, owing to non-stoichiometric O-acetylation (there were signals for CH_3 of *O*-acetyl groups at δ 21.7 and 21.9). The ^1H NMR spectrum of the OPS showed signals for one *N*-acetyl group at δ 2.06 and two *O*-acetyl groups at δ 2.15 and 2.20 in the ratio ~1:0.33:0.25, respectively.

The ^1H and ^{13}C NMR (Fig. 3, bottom) spectra of the DPS from *E. coli* O129 were typical of a regular polymer. They were assigned using 2D $^1H/^1H$ COSY, TOCSY, ROESY, $^1H/^{13}C$ HSQC, and HMBC experiments (Table 1) and the DPS structure was established as described above for the DPS from *E. coli* O13. A comparison of the structures of the DPS from *E. coli* O129 and the OPS of *S. flexneri* type 5a[5,6] showed that they are identical.