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Note

Structure determination of the O-antigenic polysaccharide from the enterotoxigenic *Escherichia coli* (ETEC) O101

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Abstract

The O-antigenic polysaccharide of the lipopolysaccharide from the enterotoxigenic *Escherichia coli* O101 has been investigated. The composition and sequence of the repeating units was established by sugar and methylation analysis together with ^{1}H and ^{13}C NMR spectroscopy. The sequence was corroborated using the computer program CASPER. The structure of the repeating unit of the polysaccharide from *E. coli* O101 is as follows:

→ 6)-α-D-Glc*p*NAc-1 → 4-α-D-Gal*p*NAc-(1 →

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One of the major classes of *Escherichia coli* associated with diarrhoea is enterotoxigenic *E. coli* (ETEC). Members of this group are often isolated from infants and are the major cause of dehydrating infant diarrhoea in developing countries [1]. In addition, ETEC strains are often isolated from cows and piglets with diarrhoea. The diarrhoea caused by ETEC is due to the ability of these strains to produce plasmid-mediated enterotoxins (heat-labile or heat-stable). Most ETEC strains produce different adhesions that determine the host specificity. Many different serotypes within the ETEC group have been identified [2].

One of the major ETEC serotypes of porcine and bovine origin is *E. coli* O101 [3]. This serotype has also been isolated from humans [4]. The structures of the O-antigens isolated from the most common ETEC serotypes of human origin have been published previously [5–8]. In the present communication, we have elucidated the structure of the repeating unit of the lipopolysaccharide isolated from *E. coli* O101.

The lipopolysaccharide from *E. coli* O101 was delipidated under mild acidic conditions and purified by gel-permeation chromatography to give a poly-

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saccharide. Component analysis of the polysaccharide revealed a 4-substituted D-galactosamine and a 6-substituted D-glucosamine, identified on the basis of GC retention times using authentic standards. Sugar components attributed to derive from the core of the LPS were also found. From the ^{1}H NMR spectrum it was possible to identify two protons in the anomeric region at δ 5.00 and δ 4.96. The $J_{\text{H-1, H-2}}$ coupling constants, 3.4 Hz and 3.5 Hz, showed that the residues had α-configuration. The spectrum also contained, *inter alia*, two signals for *N*-acetyl groups at δ 2.10 and δ 2.07 which indicate that the 2-amino-2-deoxy sugars are *N*-acetylated. The ^{13}C NMR spectrum (Fig. 1) contained two signals in the anomeric region at δ 99.2 and δ 98.0, two signals for methyl groups of *N*-acetyl groups at δ 22.9 and δ 22.7, two signals for carbons carrying nitrogen at δ 54.9 and δ 50.9, one signal for a hydroxymethyl group at δ 61.1 and two signals for carbonyl groups at δ 175.4 and δ 175.2. A distortionless enhancement by a polarization transfer experiment showed another hydroxymethyl group at δ 66.2, indicating a 6-substituted sugar residue. From the methylation analysis and NMR spectra, it is evident that the sugars are pyranosides. The above component analysis together with ^{1}H and ^{13}C NMR spectra show that the polysaccharide is composed of a disaccharide repeating unit with the following structure:

→ 6)-α-D-Glc*p*NAc-(1 → 4)-α-D-Gal*p*NAc-(1 →

The computer program CASPER [9] was used to corroborate further the substitution pattern of the two monosaccharides. ^{13}C chemical shifts were used as input data. Two different combinations were investigated, one with a 4-substituted GalNAc residue and a 6-substituted GlcNAc residue (the substitution we have suggested) and the other with a 6-substituted GalNAc residue and a 4-substituted GlcNAc residue. In both cases α-linkages led to the best fit. The first combination had a Δδ-sum of 4.7 and the other a Δδ-sum of 7.3. The Δδ-sum is the sum of the chemical shift differences between the experimental and the simulated spectra, which is a measure of the quality of a simulated spectrum versus the experimental spectrum. The lower the Δδ-sum, the better the agreement between the experimental and the simulated spectrum. This result shows that the ^{13}C NMR data has the best fit for a repeating unit with a 4-substituted GalNAc and a 6-substituted GlcNAc, both residues being α-linked.

1. Experimental

Bacterial strain.—*E. coli* O101:K$^-$:H(33), (CCUG 11402) was obtained from the Culture Collection, University of Göteborg, Sweden.

Isolation and purification.—*E. coli* O101 bacteria were grown in 30 L TY medium in a fermentor (Belach, Sweden) at 37 °C and constant pH (7.1). Bacteria were killed by the addition of formaldehyde (1% final concentration) and harvested by centrifugation. Lipopolysaccharide (LPS) was extracted by the hot phenol–water method [10]. The lipid-free polysaccharide was prepared by treatment of the lipopolysaccharide with 1% HOAc at 100 °C for 1 h. Lipid A was removed by centrifugation and repeated washing with H_2O and the polysaccharide was purified by

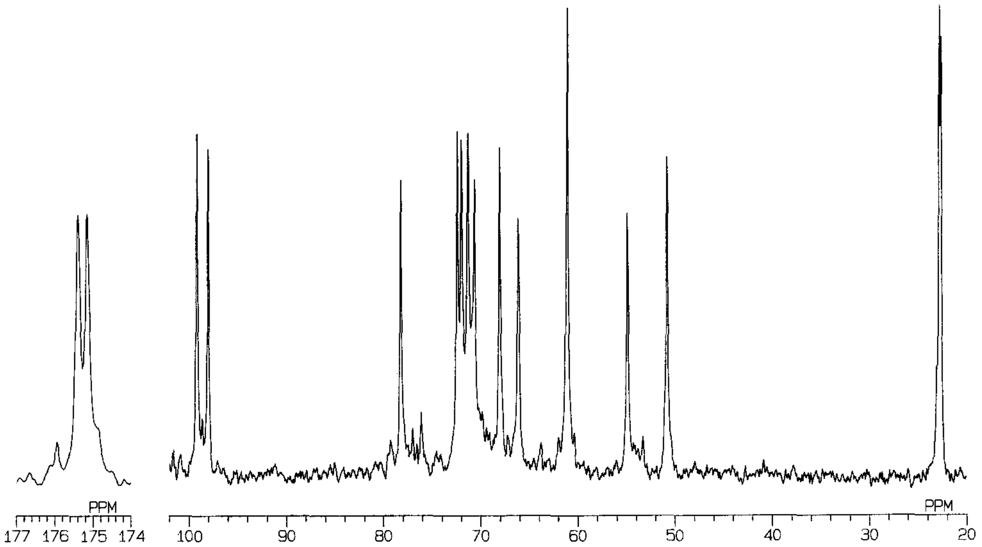

Fig. 1. ^{13}C NMR spectrum at 67 MHz of the *E. coli* O101 O-antigen polysaccharide.

gel-permeation chromatography and finally lyophilized.

General methods.—For GC a Hewlett–Packard 5890 instrument fitted with a HP-5 capillary column and a flame-ionisation detector was used. GC–MS was performed on a Hewlett–Packard 5890–5970 instrument equipped with an HP-5-MS capillary column.

Component analysis.—Hydrolysis of polysaccharide material was performed with 4 M HCl for 2 h at 100 °C. The sugars were converted into alditol acetates and analysed by GC. For linkage determination the polysaccharide was methylated [11] prior to hydrolysis, and the partially methylated alditol acetates were analyzed by GC–MS. The absolute configuration of the sugars present was determined essentially as devised by Leontein et al. [12], but with (+)-2-butanol [13].

NMR spectroscopy.—NMR spectra of solutions in D_2O were recorded at 40 °C using a JEOL GSX-270 instrument. Chemical shifts are reported in ppm relative to the signal for sodium 3-trimethylsilylpropanoate-d_4 (TSP, δ_H 0.00) as internal reference or for dioxane (δ_C, 67.4) as external reference. Data processing was performed using standard Jeol software.

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