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Structural determination of the O-antigenic polysaccharide from the Shiga toxin-producing *Escherichia coli* O171

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Abstract—The structure of the O-antigenic part of the lipopolysaccharide (LPS) obtained from the verotoxin-producing *Escherichia coli* O171 has been determined. ^{1}H and ^{13}C NMR spectroscopy techniques in combination with component analysis were used to elucidate the O-antigen structure of O-deacylated LPS. Subsequent NMR analysis of the native LPS revealed acetylation at O-7/O-9 of the sialic acid residue. The sequence of sugars was determined by inter-residue correlations in ^{1}H,^{1}H-NOESY and ^{1}H,^{13}C-heteronuclear multiple-bond correlation spectra. The O-antigen is composed of pentasaccharide repeating units with one equivalent of *O*-acetyl groups distributed over two positions:

→4)-α-Neu5Ac7,9Ac-(2→6)-β-D-Gal*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→

Based on biosynthetic considerations, this should also be the biological repeating unit.

Keywords: *Escherichia coli*; Verotoxin; Lipopolysaccharide; Sialic acid; NMR

1. Introduction

Among *Escherichia coli* strains causing diarrhoeal diseases, there are six well-described categories: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC); enterohemorrhagic *E. coli* (EHEC); enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC).[1] These categories have virulence attributes that help bacteria to cause diseases by different mechanisms.

EHEC is an etiological agent of diarrhoea with life-threatening complications like haemorrhagic colitis (HC) and haemolytic–uraemic syndrome (HUS). EHEC belongs to a group of *E. coli* called VTEC ('verotoxigenic *E. coli*') or STEC ('Shiga toxin-producing *E. coli*'). The pathological lesions associated with HC and HUS are due to the action of Shiga toxin (Stx) on endothelial cells. Whereas not all STEC strains are believed to be pathogens, all EHEC strains are considered to be pathogens. The most notorious *E. coli* serotype associated with EHEC is O157:H7, which has been the cause of several large outbreaks of disease in North America, Europe, and Japan.[2–6] The principle reservoir of STEC strains is the bovine intestinal tract, and most outbreaks of EHEC are associated with the consumption of undercooked meat, sausages, unpasteurized milk, lettuce, and apple juice. Besides *E. coli* O157:H7, several other serotypes have been associated with the STEC category. *E. coli* O171 has recently been shown in several studies to be present in cattle and in foods in Argentina and Spain.[7–13] Most of the O171 strains were of the O171:H2 serotype, and all of them possessed the *stx* gene encoding the shiga toxin. In this paper, we present the structure of the O-antigen of the LPS from *E. coli* O171.

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2. Results and discussion

The LPS from *E. coli* O171, grown in a glucose-containing tryptone/yeast extract medium,[14] was isolated from the bacterial membrane by hot phenol/water extraction.[15] Delipidation under mild acidic conditions led to a heterogeneous material not suitable for further structural studies. Our previous investigation of the O-antigen from *E. coli* O164 indicated that it should be possible to determine the O-antigen structure without resorting to acidic treatment of the material.[16] The ^{1}H NMR spectrum of the LPS indicated that *O*-acetyl groups were present in the polymer since resonances at ~2.12 ppm were observed. Subsequent O-deacylation using dilute aqueous ammonia resulted in a material of good quality. The ^{1}H NMR spectrum of the O-deacylated LPS showed the presence of four signals in the anomeric region at δ_H 4.65, 4.57, 4.48, and 4.40 (Fig. 1). Additional resonances were found, inter alia, at δ_H 2.95 and 1.76 as well as at δ_H 2.03 (3H) and 2.00 (3H) indicating a methylene group and two *N*-acetyl groups, respectively, as part of one or more residues in the polymer.

A hydrolysate of the LPS contained glucose, galactose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, and heptose in the ratio 36:43:5:14:5. Sialic acid was shown by NMR spectroscopy (vide infra) to be a component of the polymer. Determination of the absolute configuration of the hexoses in the O-antigen revealed that they had the D-configuration. However, although it is possible to identify sialic acid as its methyl glycoside methyl ester by GLC–MS analysis,[17,18] this was not possible in the present case. The absolute configuration of the sialic acid is assumed to be that previously found in nature, viz., the D-*glycero*-D-*galacto* configuration.[19]

The ^{1}H,^{13}C-HSQC spectrum of the O-deacylated LPS showed in the region for anomeric resonances four peaks corresponding to hexopyranosyl residues

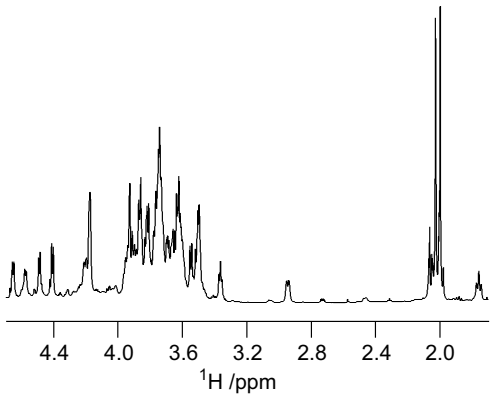

Figure 1. ^{1}H NMR spectrum of the O-deacylated LPS from *E. coli* O171.

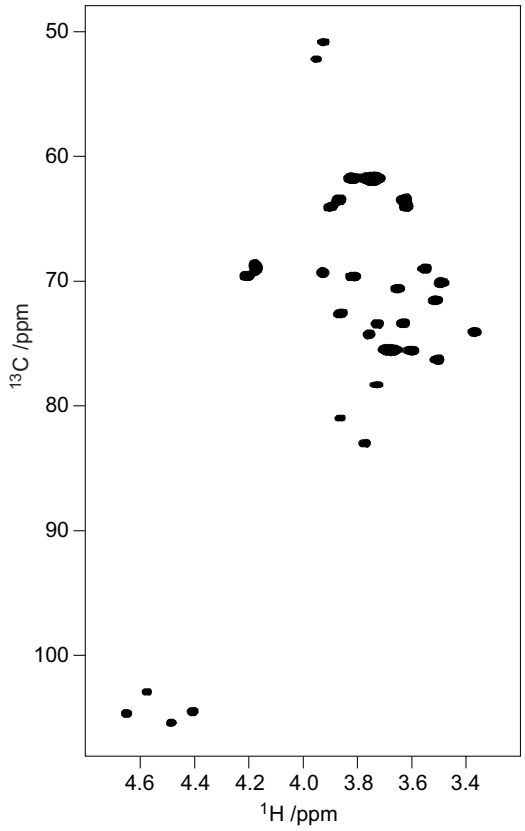

Figure 2. Part of the ^{1}H,^{13}C-HSQC spectrum of the O-deacylated LPS from *E. coli* O171.

(Fig. 2), and since $J_{H\text{-}1,H\text{-}2}$ and $J_{H\text{-}1,C\text{-}1}$ coupling constants of ~8 Hz and ~165 Hz, respectively, were observed for the anomeric protons these residues are β-linked. The ^{1}H and ^{13}C NMR resonances 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**–**D**, in decreasing chemical shift of their anomeric proton. The sialic acid residue, with a chemical shift of 101.2 ppm for its anomeric carbon, was denoted residue **E**. The presence of five anomeric carbons indicated that the O-antigen consists of pentasaccharide repeating units. From the chemical shifts and coupling constants given in Table 1 it can be concluded that all sugar residues have the pyranoid ring form. Residues **B** and **E** carried *N*-acetyl groups and these were assigned from a ^{1}H,^{13}C-HMBC spectrum.

The spin system of **A** having the anomeric resonance at δ_H 4.65 could be assigned to a →6)-β-D-Glc*p*-(1→ residue due to the low chemical shift of H-2 and a large glycosylation shift[20] of C-6, $\Delta\delta_C$ 7.9. Residue **B** with $\delta_{H\text{-}1}$ 4.57 was assigned to a →3)-β-D-Gal*p*NAc-(1→ residue due to the chemical shift of C-2 at 52.2 ppm and a large glycosylation shift of C-3, $\Delta\delta_C$ 9.1. The ^{1}H,^{1}H spin system of **C** with $\delta_{H\text{-}1}$ 4.48 revealed that it had the *galacto*-configuration and from $\Delta\delta_{C\text{-}3}$ 9.2 that it is a →3)-β-D-Gal*p*-(1→ residue. Residue **D** also has