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Note

Characterization of the polysaccharide antigen of *Klebsiella pneumoniae* O : 9 lipopolysaccharide

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The lipopolysaccharides (LPS) of *Klebsiella pneumoniae* have been implicated as virulence determinants. Although some 72 K-serospecific capsular antigens have been described for *Klebsiella* sp., it has been suggested that there exist only 8 associated unique LPS O-polysaccharide antigens in this gram-negative bacterial species[1].

In recent re-investigations of *Klebsiella* LPS O-chains[2,3], originally proposed structures have required revision, and the production of multiple unique O-chain structures by a given designated *Klebsiella* serotype has been demonstrated[2,3]. The present investigation of the *Klebsiella* O : 9 LPS polysaccharide has shown that it is a polymer of a branched pentasaccharide unit composed of only D-galactose residues. The newly deduced structure of the O-antigen differs from the originally proposed structure[4] in the mode of linkage at its branch point and, as a consequence, also in the structure of the backbone D-galactan polymer.

The O-polysaccharide, extracted by the method of Johnson and Perry[5] as described in the Experimental section, had $[\alpha]_D$ +93° (*c* 1.5, H_2O) and, by quantitative GLC[6] and capillary GLC of the derived (*R*)-2-butyl glycosides[7], was shown to be composed of D-galactose (90%). Anal. Found: C, 39.77; H, 5.71; N, 0.20; and ash 0%. The ^{1}H NMR spectrum of the native O-chain showed signals at δ 2.12 and 2.15 indicative of *O*-acetyl methyl protons while its ^{13}C NMR spectrum (Fig. 1B) also showed *O*-acetyl signals at δ 21.20 and 21.18 ($\mathit{C}H_3CO$) and 173.5 and 174.7 ($CH_3\mathit{C}O$). The ^{1}H NMR spectrum of the *O*-deacetylated O-polysaccharide (dil NH_4OH) showed *inter alia* four H-1 signals, at δ 5.31 (1 H, $J_{1,2}$ 3 Hz), 5.25 (2 H, $J_{1,2}$ 0.5 Hz), 5.08 (1 H, $J_{1,2}$ 3 Hz), and 5.05 (1 H, $J_{1,2}$ 3 Hz). The ^{13}C NMR spectrum of the same *O*-deacetylated O-polysaccharide (Fig. 1A) showed

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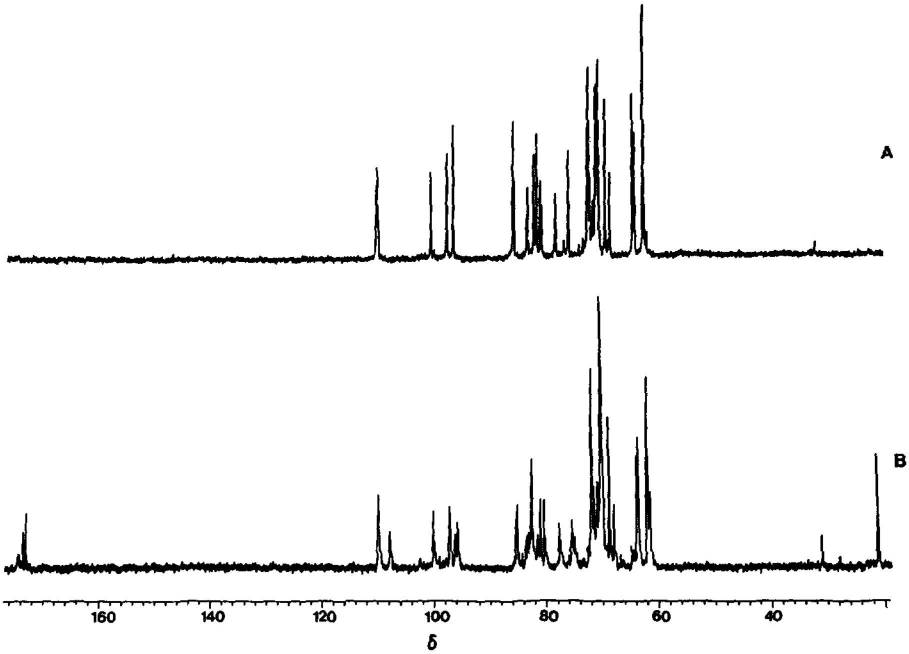

Fig. 1. ^{13}C NMR spectra (125 MHz, 47°C) of: A, *O*-Deacetylated *K. pneumoniae* O:9 LPS O-polysaccharide and B, native *K. pneumoniae* O:9 LPS O-polysaccharide.

inter alia five C-1 signals, at δ 110.2 ($J_{C,H}$ 176 Hz), 110.1 ($J_{C,H}$ 176 Hz), 100.3 ($J_{C,H}$ 170 Hz), 97.6 ($J_{C,H}$ 172 Hz), and 96.3 ($J_{C,H}$ 171 Hz). These chemical shift and $J_{C,H}$ coupling data were indicative of two β-D-Gal*f* and three α-D-Gal*p* residues in a repeating pentasaccharide unit of the D-galactan polymer. The *O*-acetyl content of the native *O*-chain corresponded to ~1.7 mol per repeating unit.

The *O*-deacetylated O-chain had $[\alpha]_D$ +86° (*c* 5.4, H_2O). Anal. Found: C, 40.90; H, 5.64; N, 0.66; and ash 0%. On methylation analysis[8] with the aid of GLC–MS the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-galactose (t_R 1.08), 2,5,6-tri-*O*-methyl-D-galactose (t_R 1.36), 2,4,6-tri-*O*-methyl-D-galactose (t_R 1.47) and 4,6-di-*O*-methyl-D-galactose (t_R 1.82) were identified in the molar ratio 1:2:1:1. This analysis is consistent with the proposed pentasaccharide structure and the presence in the repeating unit of a nonreducing D-Gal*p* end-group, two → 3)-D-Gal*f* residues, a → 3)-D-Gal*p* residue, and a branched, di-*O*-substituted residue, → 2,3)-D-Gal*p*.

As expected from the methylation evidence, the periodate oxidation of the *O*-deacetylated O-chain resulted in the oxidation of the D-Gal*p* nonreducing end-group and the exocyclic C-6–C-5 diol systems of the D-Gal*f* residues. The product of the Smith type[9] mild hydrolysis of the reduced ($NaBH_4$), periodate-oxidized O-chain afforded, on Sephadex G-50 chromatography, a polysaccharide, eluting at the void volume of the system, which had $[\alpha]_D$ +62° (*c* 1.1, H_2O) and was composed of D-Gal and L-Ara (1 : 1). This latter polymer, which resisted further periodate oxidation, on methylation analysis gave the acetylated alditol derivatives of 2,5-di-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose (1 : 1), as expected for an unbranched linear polymer composed of 1,3-linked L-Ara*f* and D-Gal*p* residues. The L-arabino-D-galactan gave ^{1}H and ^{13}C NMR spectra indistinguishable from those of previously fully characterized polymers composed of alternating 1,3-linked L-Ara*f* and D-Gal*p* residues[2,9]. The above evidence leads to the unambiguous conclusion that the backbone of the native O-polysaccharide is a linear polymer of a repeating disaccharide having the structure → 3)-β-D-Gal*f*-(1 → 3)-α-D-Gal*p*-(1 →, previously characterized as *Klebsiella* galactan I and found in *K. pneumoniae* O1 (ref. 2) and O2a (ref. 3) antigens.

The identification of 4,6-di-*O*-methyl-D-galactose in the methylation analysis of the O-chain, considered in conjunction with the characterization of the O-chain linear backbone and the α-D configurations of the D-Gal*p* residues, indicates that the nonreducing α-D-Gal*p* end-group is (1 → 2)-linked to α-D-Gal*p* units in the backbone structure. From the NMR spectra of the O-chain it can be deduced that this substitution follows a regular pattern consistent with the O-chain being composed of a repeating pentasaccharide unit in which the D-Gal*f* and D-Gal*p* residues have the β and α configurations respectively. Thus, the structure is as shown here.

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→ 3)-β-D-Galf-(1 → 3)-α-D-Galp-(1 → 3)-β-D-Galf-(1 → 3)-α-D-Galp-(1 →
                                                              2
                                                              ↑
                                                              1
             [1.7 mol Ac not located]                     α-D-Galp
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In the previously proposed structure the anomeric configurations at the glycosidic linkages were not determined[4], and the linkage sequence at the 2,3-di-*O*-substituted α-D-Gal*p* branched point was reversed.

The location and proportion of the *O*-acetyl substituents appears to be variable and dependent upon bacterial growth conditions, but may however play a role in the serology of the O:9 antigen. In the ^{13}C NMR spectra of native O-chain preparations the presence of extra C-1 signals from the D-Gal*f* residues at δ ~ 108 (Fig. 1B) suggests that a significant proportion of O-acetylation was always present at the 2 position of these units.

EXPERIMENTAL

Cells of *Klebsiella pneumoniae* O9:K^{-} (NRCC 4378, CWK 48, from strain 121205), grown in 3.7% (w/v) brain–heart infusion (Difco) at 37°C in a Microfirm fermenter, were extracted by the hot aqueous phenol method[5], and subsequent isolation procedures were performed as previously described[2]. Ultracentrifugation afforded LPS from the aqueous phase (6% yield) and the phenol phase (0.9% yield). Fission of the aqueous phase LPS with hot 2% acetic acid (2 h, 100°C) gave an insoluble lipid A (~ 10%) and Sephadex G-50 chromatography of the water soluble products gave the O-polysaccharide (69% yield) eluting at the void volume of the system. Glycan hydrolyses, aldose identifications, periodate oxidations, methylation analyses, and ^{1}H and ^{13}C NMR spectroscopy were also done under the same conditions as previously described[2].

GLC–MS analysis of acetylated methyl alditols was done with an OV-17 fused silica capillary column using a temperature program from 200°C (2 min) to 240°C at 1°C/min. Retention times are quoted relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (t_R = 1.00). ^{1}H and ^{13}C NMR chemical shifts (δ), quoted relative to tetramethylsilane, were measured from internal acetone (δ_H 2.225, δ_C 31.07); coupling constants are given in Hertz.

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