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Short Communications

Structural Studies on the *Klebsiella* O Group 4 Lipopolysaccharide

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In their studies on the chemical compositions of lipopolysaccharides (LPS) from different *Klebsiella* O groups, Nimmich and Korten [1] found that the O-specific side chains in the O group 4 LPS contained D-galactose and ribose. They further found small amounts of D-glucose, 2-acetamido-2-deoxy-glucose, 3-deoxy-octulosonic acid, and heptose. In the present communication structural studies on the LPS from *Klebsiella* O4:K36 are reported.

The LPS was isolated as previously described,[1] and showed $[\alpha]_{578}$ +77°. Analysis of the sugars in a hydrolysate by GLC-MS,[2,3] as their alditol acetates, showed that the LPS contained ribose and D-galactose residues in the proportion 1.1 : 1.0, and in addition traces of D-glucose. It seems reasonable to assume that the ribose also has the D-configuration. L-Rhamnose was used as an internal standard and the above sugars accounted for about 62 % of the LPS material.

A value of 120° can be calculated for $[\alpha]_{578}$ of the carbohydrate portion of the LPS if it is assumed that the contribution of the lipid moiety is unimportant. The LPS did not contain a significant percentage of *O*-acetyl or other *O*-acyl groups since it had no significant IR absorption around 1735 cm^{-1}.

Analysis of the methylated sugars in a hydrolysate of the methylated LPS, as their alditol acetates by GLC–MS,[4] gave equimolecular amounts of 3,5-di-*O*-methyl-D-ribose and 2,3,6-tri-*O*-methyl-D-galactose. The *T*-values (retention times relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on an ECNSS-M column at 170°) were 0.77 and 2.42, respectively. A trace of 2,3,4,6-tetra-*O*-methyl-D-glucose was detected but no significant amounts of other sugars were found.

From these results it is apparent that the D-ribose residues in the LPS are furanosidic and linked in the 2-position, while the D-galactose residues are either pyranosidic and linked in the 4-position or furanosidic and linked in the 5-position. Bacterial polysaccharides are often composed of oligosaccharide repeating units. A simple repeating unit consistent with these results is D-Rib*f*-D-Gal-(1→2) in which the D-galactose residue is either pyranosidic or furanosidic.

A mild acid hydrolysis, which should have cleaved mainly furanosidic linkages, was followed by borohydride reduction and methylation analysis. The observed ethers were 3,5-di-*O*-methyl-D-ribose, 2,3,4,6-tetra-*O*-methyl-D-galactose ($T = 1.25$) and 2,3,6-tri-*O*-methyl-D-galactose in the relative proportions 1.00:0.70:0.83. A trace of 2,3,5-tri-*O*-methyl-D-ribose ($T = 0.40$) was also obtained. The 1,3,4,5-tetra-*O*-methyl-D-ribitol derivative, derived from a D-ribose reducing terminal, which should have a high volatility and a low *T*-value, was either lost during concentrations or was hidden in the solvent peak. These results are nevertheless only consistent with the repeating unit D-Rib*f*-(1→4)-D-Gal*p*-(1→2) in which the D-galactose is in the pyranose form.

Mild acid hydrolysis of the LPS gave a number of oligosaccharides. On paper chromatography with the *p*-anisidine reagent for detection alternate spots were a brownish-red colour, indicating a terminal reducing D-ribose residue. The other spots were a faint brownish-yellow colour, indi-

cating a terminal D-galactose residue. The three most mobile materials, presumably a di-, a tri-, and a tetra-saccharide (R_{Gal} = 0.83, 0.35, and 0.25, respectively), were isolated by preparative paper chromatography. Hydrolysate of these contained D-ribose and D-galactose in the proportions 1:1, 1:2 and 1:1, respectively. The material with R_{Gal} = 0.83 was reduced with sodium borodeuteride, hydrolysed, reduced with sodium borohydride and the alditols, as their acetates were analysed by GLC–MS.[2,3] Deuterium labelling was observed in the ribitol but not in the galactitol derivative, demonstrating that D-ribose was the reducing sugar residue in a disaccharide. These results also support the assumption of the disaccharide repeating unit in the O-specific side chains.

The disaccharide alditol was acetylated and treated with chromium trioxide in acetic acid to determine the anomeric nature of the D-galactose residue.[5] *myo*-Inositol hexaacetate was present as an internal standard. Sugar analysis revealed that the disaccharide derivative was essentially intact under conditions such that a β-D-galactopyranose derivative would have been completely oxidized. The D-galactose residues are, therefore, α-linked.

The optical rotation of the LPS indicates that the D-ribose residues are β-linked. On mild acid hydrolysis of the LPS there was an initial increase in the optical rotation, supporting this assumption. This determination was not, however, very accurate, as lipid material precipitated during the hydrolysis and rendered the solution turbid.

The combined results presented above indicate that the O-specific side chains in the *Klebsiella* O group 4 LPS are composed of the disaccharide repeating unit β-D-Rib*f*-(1→4)-α-D-Gal*p*-(1→2). It cannot be decided if the "biological" repeating unit has this structure or the alternative structure, α-D-Gal*p*-(1→2)-β-D-Rib*f*-(1→4), as no methylated sugar deriving from the non-reducing terminal was observed in the methylation analysis. This negative evidence and the low percentage 2,3,4,6-tetra-*O*-methyl-D-glucose probably derived from a basal core of the LPS shows that the *O*-specific side chains are long.

Experimental. General methods. Concentrations were carried out under reduced pressure at bath temperatures not exceeding 40°. A Perkin-Elmer 990 instrument, equipped with flame ionisation detectors, was used for GLC. Glass columns (180 × 0.15 cm) containing 3 % ECNSS-M on Gas Chrom Q (100/120 mesh) were used for separation of alditol acetates (190°) and partially methylated alditol acetates (170°). For GLC-MS a Perkin-Elmer 270 instrument fitted with an OV-225 SCOT-column (50′ × 0.020″) was used. Mass spectra were recorded at an ionisation potential of 70 eV, an ionisation current of 80 μA and ion source temperature of 80°. Paper chromatography was performed on Whatman No. 1 paper and for preparative purposes Whatman No. 3 MM paper was used. The solvent system ethyl acetate : acetic acid : water = 3:1:1 was used and compounds were detected with 3 % *p*-anisidine hydrochloride in ethanol at 120°. Optical rotations, in a 10 cm micro-cell, were recorded using a Perkin-Elmer 141 photoelectric polarimeter. IR spectra were recorded using a Perkin-Elmer 257 instrument.

Isolation of the LPS, from *Klebsiella* O4:K36 (8306), was performed as previously described.[1] The LPS showed $[\alpha]_{5,7,8}^{20}$ + 77° (*c* 0.2. water). In the IR spectrum (KBr) no significant absorptions around 1735 cm^{-1} (*O*-acyl region) were observed.

Sugar and methylation analyses. For sugar analyses the LPS (5.0 mg) and L-rhamnose (2.5 mg) were treated with 0.25 M sulphuric acid at 100° for 14 h. The solution was neutralized with barium carbonate and the sugars were converted into alditol acetates as previously described.[6] The mixture was analysed by GLC-MS.[2,3] For methylation analysis a 4 mg sample of LPS was methylated by the method of Hakomori[7] and worked up as previously described.[8]

Analysis of degraded LPS. A sample (4 mg) of LPS was treated with 0.01 M sulphuric acid for 1.25 h on a boiling water bath. The solution was neutralised with Dowex 3 (free base) and reduced with sodium borohydride (25 mg) for 3 h at room temperature. The reaction mixture was treated with Dowex 50 (H^+) and boric acid was removed by repeated distillations with methanol. The product was dissolved in water and lyophilized. This partially degraded and reduced LPS was then subject to methylation analysis as in Ref. 8, except that the methylated products were recovered by partition between chloroform and water and not by dialysis.

Isolation and identification of oligosaccharides. A sample (30 mg) of LPS was hydrolysed with 0.01 M sulphuric acid for 2 h at 100°. The solution was neutralized with barium carbonate and concentrated. Paper chromatography revealed a series of oligosaccharides, of which the three most mobile (R_{Gal} = 0.83, 0.35, 0.25, respectively) were isolated by preparative paper chromatography. Sugar analyses of

hydrolysates of these, performed as described above, revealed D-ribose and D-galactose in the proportions 1.0:1.0, 1.0:2.1, and 1.0:1.1, respectively. A sample (2 mg) of the oligosaccharide with the highest mobility was reduced (sodium borodeuteride) and acetylated (acetic anhydride, pyridine) and *myo*-inositol hexaacetate (1 mg) was added as internal standard. A portion (1/3) of this preparation was hydrolysed, transformed into alditol acetates, and investigated by GLC-MS.[2,3] Deuterium labelling was observed in essentially all the ribitol but not in the galactitol.

The other part of the preparation was dissolved in acetic acid (0.06 ml), chromium trioxide (6 mg) was added and the resulting suspension was agitated at 50°C in an ultrasonic bath. The reaction mixture was diluted with water (5 ml) and extracted with chloroform (3 × 5 ml). The chloroform extracts were combined and dried (magnesium sulphate) and the material was hydrolysed, transformed into alditol acetates, and analysed by GLC-MS.[2,3] The proportions of ribitol pentaacetate, galactitol hexaacetate and *myo*-inositol hexaacetate were essentially the same as in the non-oxidized sample.

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Regulation of Branched Chain Amino Acid Transaminase Formation During the Growth of *Pseudomonas fluorescens* UK-1

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The enzyme which catalyses the transfer of amino groups from branched chain amino acids (valine, leucine, and isoleucine) to 2-oxoglutarate is classified by the Enzyme Commission as EC 2.6.1.6, L-leucine:2-oxoglutarate aminotransferase, and given the trivial name leucine aminotransferase but the name branched chain amino acid transaminase seems more correct.[1]

Branched chain amino acid transaminase catalyses the terminal reaction in the biosynthesis of branched chain amino acids in several bacteria.[2-6] In the case of an organism such as *Pseudomonas fluorescens*, which can both synthesize and metabolize the branched chain amino acids, such a transaminase is required in biosynthetic as well as catabolic pathways.[7]

Previous studies indicate that *Pseudomonas fluorescens* P-2 (previously named *Pseudomonas* P-2 by Goodhue and Snell [8]) is able to utilize pantothenate as sole source of carbon and nitrogen.[8] This substance is metabolized by way of an inducible pathway to 2-oxoisovalerate,[9-13] which is the first catabolite of valine. In the present study attention was focused on the formation and control of branched chain amino acid transaminase during the growth of *Pseudomonas fluorescens* UK-1.

Organism and culture conditions. Pseudomonas fluorescens strain UK-1 was grown in a basal mineral medium containing (per litre) KH_2PO_4, 0.82 g; $MgSO_4.7H_2O$, 0.25 g; $FeSO_4.7H_2O$, 2.8 mg. The pH was adjusted to pH 6.8 with KOH. Unless otherwise stated, the concentration of carbon source was 10 mM. $(NH_4)_2SO_4$ (10 mM) was used as nitrogen source, if necessary. The cells were precultured in the glutamate (20 mM) and pantothenate media. The culture was aerated with an aquarium pump and incubated at 30°.

Cell extract and enzyme assay. Samples (about 4 mg dry weight) were withdrawn from