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STRUCTURAL STUDIES OF THE ANTIGENIC POLYSACCHARIDE OF *Eubacterium saburreum*, STRAIN S29

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ABSTRACT

The antigenic polysaccharide produced by *Eubacterium saburreum*, strain S29, is composed of (1→6)-linked β-D-*glycero*-D-*galacto*-heptopyranosyl residues, all of which are substituted with 6-deoxy-α-D-*altro*-heptofuranosyl groups at O-2.

INTRODUCTION

The antigenic polysaccharide produced by *Eubacterium saburreum*, strain L49 (ref. 1) has been reported to be composed of D-*glycero*-D-*galacto*-heptose and 6-deoxy-D-*altro*-heptose. It contains a chain of alternating (1→3)- and (1→6)-linked β-D-*glycero*-D-*galacto*-heptopyranosyl residues, the latter being substituted with 6-deoxy-α-D-*altro*-heptofuranosyl groups at O-3. The polysaccharide further contains *O*-acetyl groups, linked to O-7 of part of the heptosyl residues, and to O-2 of part of the 6-deoxyheptosyl groups.

The antigenic polysaccharide produced by another strain, O2 (ref. 2), of the same organism has also been investigated. The antigen is composed of (1→6)-linked β-D-*glycero*-D-*galacto*-heptopyranosyl residues, all of which are substituted with 6-deoxy-α-D-*altro*-heptofuranosyl groups at O-3. Additional studies[3] of the same antigen have demonstrated that some 40% of the β-D-*glycero*-D-*galacto*-heptosyl residues carry an *O*-acetyl group at O-7.

Recently, we have isolated a new strain, S29, of the same organism. The antigenic polysaccharide produced by the new strain has the same sugar composition as the L49 and O2 antigens, but differs from these in its serological specificity. We now report a study of the structure of the new antigenic polysaccharide.

RESULTS AND DISCUSSION

Acid hydrolysis of the antigenic polysaccharide ($[\alpha]_D^{27}$ +20°) yielded two components in the approximate ratio of 1:1. The mass spectra of their per-*O*-

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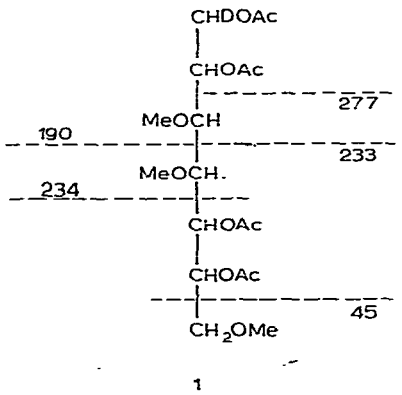
trimethylsilylalditol derivatives showed that one derived from a 6-deoxyheptose and the other from a heptose. The sugars, isolated by p.c., were identified as 6-deoxy-D-*altro*-heptose and as D-*glycero*-D-*galacto*-heptose, respectively. The ^{1}H-n.m.r. spectra of the sugars were indistinguishable from those of authentic samples[2], and their optical rotations, $[\alpha]_D^{27}$ +39° (*c* 0.7, water) for the 6-deoxyheptose, and $[\alpha]_D^{26}$ +59° (*c* 0.6, water) for the heptose, were in good agreement with the published values[2]. Bacterial polysaccharides are often composed of oligosaccharide repeating-units, and, on this basis, these results indicate that the antigenic polysaccharide contains a disaccharide repeating-structure. In agreement with this, the ^{1}H-n.m.r. spectrum showed a signal for the methylene group of the 6-deoxyheptose residue at δ 1.62 (m, 2 H) and signals for two anomeric protons at δ 4.56 ($J_{1,2}$ 7 Hz, 1 H) and 5.20 ($J_{1,2}$ low, 1 H). The polysaccharide did not contain *O*-acetyl groups, as indicated by the ^{1}H-n.m.r. and i.r. spectra.

Methylation analysis of the polysaccharide, with analysis of the partially methylated sugars as their alditol acetates, yielded 6-deoxy-2,3,5,7-tetra-*O*-methyl-D-*altro*-heptose and 3,4,7-tri-*O*-methyl-D-*glycero*-D-*galacto*-heptose (**1**) in the proportion



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of 0.83 : 1.00. This ratio differs from the expected 1 : 1 ratio, probably owing to the volatility of the former compound. The results demonstrate that 6-deoxy-D-*altro*-heptose occurs as furanosyl end-groups, and D-*glycero*-D-*galacto*-heptose as branching residues, linked through O-2 and O-6.

Acid hydrolysis of the polysaccharide under mild conditions, followed by dialysis, gave a polymeric product that was mainly composed of D-*glycero*-D-*galacto*-heptose. Methylation analysis of this product gave only low percentages of 6-deoxy-2,3,5,7-tetra-*O*-methyl-D-*altro*-heptose and 3,4,7-tri-*O*-methyl-D-*glycero*-D-*galacto*-heptose, and the preponderant sugar was 2,3,4,7-tetra-*O*-methyl-D-*glycero*-D-*galacto*-heptose. Consequently, the 6-deoxyheptofuranosyl groups are linked, in the antigen, to O-2 of the branching heptopyranosyl residues.

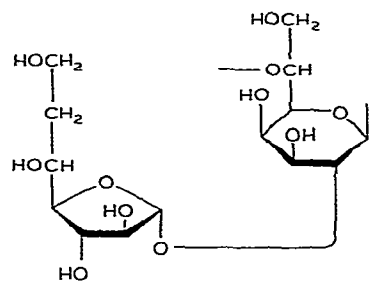
On periodate oxidation, borodeuteride reduction, and acid hydrolysis of the original polysaccharide, all sugar residues were degraded, and two degradation

products, erythritol and 2-deoxypentitol, were formed in the approximate ratio of 1:1, the former obviously deriving from the branching heptopyranosyl groups, and the latter from the terminal 6-deoxyheptofuranosyl residues. These data are in agreement with the postulated structure.

The optical rotation of the original polysaccharide ($[\alpha]_D^{27}$ +20°) decreased on mild acid hydrolysis, and the product, from which most of the 6-deoxy-heptofuranosyl groups had been removed, showed $[\alpha]_D^{27}$ −44°. These results indicate that the D-*glycero*-D-*galacto*-heptopyranosyl residues are β-linked, and the 6-deoxy-D-*altro*-heptofuranosyl residues α-linked.

The ^{1}H-n.m.r. spectrum of the polymer obtained on mild acid hydrolysis showed a signal for the anomeric proton at δ 4.56 ($J_{1,2}$ 7 Hz). Thus, the signals for two anomeric protons at δ 4.56 and 5.20 given by the original polysaccharide could be assigned to H-1 of the β-D-*glycero*-D-*galacto*-heptopyranosyl residues and to H-1 of the 6-deoxy-α-D-*altro*-heptofuranosyl residues, respectively.

From the evidence just presented, it is concluded that the antigenic polysaccharide of *E. saburreum* strain S29 is composed of (1→6)-linked β-D-*glycero*-D-*galacto*-heptopyranosyl residues, all of which are substituted at O-2 with 6-deoxy-α-D-*altro*-heptofuranosyl groups (**2**).



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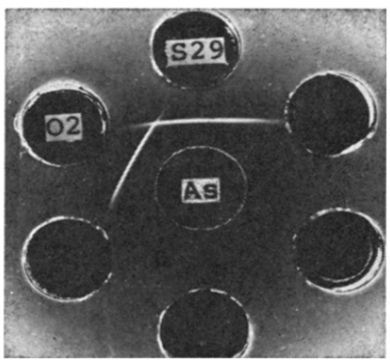

Fig. 1. Precipitation bands produced in agar by S29 and O2 antigens (peripheral wells) against the mixture of anti-S29 and anti-O2 sera (central well).

As was expected from structure **2**, the precipitation reaction between S29 antigen and anti-S29 serum was inhibited by 6-deoxy-D-*altro*-heptose, but not by D-*glycero*-D-*galacto*-heptose, *i.e.*, the 6-deoxy-α-D-*altro*-heptofuranosyl residue is immunodominant in S29 antigen. The precipitation inhibition was observed at a comparatively high concentration of 6-deoxy-D-*altro*-heptose (5, 16, and 47% inhibition, at a concentration of 0.025, 0.25, and 2.5 mg/mL, respectively). This is probably related to the fact that, at equilibrium in deuterium oxide solution[2], only 10% of the sugar exists in the α-D-furanose form.

Fig. 1 shows the precipitation reaction in gel with the two antigens S29 and O2, and the mixture of their homologous immune sera. Although both antigens contain the same sugars in equimolecular proportions, the two precipitate lines produced by them showed distinct crossing. This indicated that both antigens carry different specificities, *i.e.*, belong to different serotypes. As the *O*-deacetylation of O2 antigen by treatment with alkali did not change its serological properties[2,3], it is concluded that the difference in antigenic specificity between S29 and O2 antigens is due to the location of the 6-deoxyheptofuranosyl groups on the heptopyranosyl residues, namely, at O-2 in the former and at O-3 in the latter.

EXPERIMENTAL

Culture conditions. — The same conditions were used as in the investigation[2] of *E. saburreum* strain O2 antigen.

Antigen extraction and purification methods. — The antigenic polysaccharide, isolated as previously described[2], showed $[\alpha]_D^{27}$ +20° (*c* 0.8, water), and its elution volume on a column (1.4 × 114 cm) of Sephadex G-100 was found to be 81 mL (V_e/V_o 1.47). The lyophilized cells (1 g) yielded 10 mg of antigenic polysaccharide.

General analytical methods. — The same methods were used as these previously described[2]. G.l.c.–m.s. was performed with a Hitachi M-60 instrument. I.r. spectra were recorded with a Hitachi Model 215 apparatus for Nujol mulls. Paper chromatography was performed on Whatman No. 1 paper with 6:4:3 (v/v) 1-butanol–pyridine–water as irrigant.

Methylation analyses. — The original polysaccharide was treated by the procedure previously described[2]. G.l.c. of the product showed two components having mobilities (*t* value) relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol of 1.14 and 6.19 on an OV-225 column at 210°. One of the components (*t* 1.14) was identified as 1,4-di-*O*-acetyl-6-deoxy-2,3,5,7-tetra-*O*-methylheptitol; m.s.: *m/z* 219(2), 187(4), 162(2), 159(3), 145(1), 143(1), 129(5), 127(3), 118(27), 113(3), 103(49), 101(19), 97(2), 87(2), 75(3), 73(12), 71(24), 45(100), and 43(90). The other component (*t* 6.19) was identified as 1,2,5,6-tetra-*O*-acetyl-3,4,7-tri-*O*-methylheptitol; m.s.: *m/z* 277(1), 234(3), 233(8), 203(1), 190(8), 174(2), 173(3), 170(1), 160(3), 157(3), 143(8), 130(35), 113(30), 100(28), 88(20), 45(28), and 43(100).

The degraded polysaccharide obtained after mild acid hydrolysis (50mM sulfuric acid, 210 min, 80°) showed $[\alpha]_D^{27}$ −44° (*c* 0.7, water). The polymer was

subjected to methylation analysis. G.l.c. of the product showed one main component (*t* 3.65), which was identified as 1,5,6-tri-*O*-acetyl-2,3,4,7-tetra-*O*-methylheptitol; m.s.: *m/z* 277(1), 233(3), 203(1), 173(1), 162(4), 157(3), 143(10), 131(3), 129(3), 118(21), 113(12), 102(26), 101(7), 99(8), 87(8), 75(8), 71(2), 45(25), and 43(100).

Periodate oxidation studies. — The original polysaccharide was treated by the procedure previously described[2], except that the oxidized material was purified by dialysis against distilled water and reduced with sodium borodeuteride. G.l.c. analysis of the product showed that all sugar components had disappeared, and two degradation products were formed, having mobilities (*t* value) relative to per-*O*-trimethylsilyl-inositol of 0.35 and 0.45. One of the products (*t* 0.35) was identified as erythritol; m.s.: *m/z* 396(1, $M^+ - 15$), 321(11), 308(38), 307(21), 294(10), 218(32), 217(32), 206(38), 205(41), 191(28), 190(23), 189(27), 147(55), 118(45), 117(40), 104(38), 103(58), and 73(100). The other product (*t* 0.45) was identified as a 2-deoxypentitol; m.s.: *m/z* 410(0.1, $M^+ - 15$), 321(10), 307(11), 231(19), 219(39), 206(27), 191(18), 156(22), 147(45), 129(30), 118(21), 117(23), 104(38), 103(80), and 73(100).

Serological methods. — Preparation of antisera and gel-diffusion tests were performed as previously described[2]. Quantitative precipitation inhibition tests by sugars were undertaken as follows: four-fold diluted antiserum (0.1 mL) was mixed with an equal volume of sugar solution. Following incubation for 1 h at 37°, 0.2 mL of antigen solution (10 μg/mL) was added. After incubation for 1 h at 37° and refrigeration for 4 days, the precipitate was centrifuged off in the cold and washed three times with cold saline. The washed precipitate was dissolved in 0.1M sodium hydroxide and assayed for precipitated antibody-protein by the Lowry-Folin method[4]. Percent inhibition was calculated by the following equation: [1.0 — (amount of precipitate in presence of inhibitor / amount of precipitate in absence of inhibitor)] × 100.

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