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STRUCTURAL STUDIES OF THE CAPSULAR POLYSACCHARIDE FROM *Streptococcus pneumoniae* TYPE 37

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ABSTRACT

The structure of the capsular polysaccharide elaborated by *Streptococcus pneumonia* type 37 has been investigated; methylation analysis, Smith degradation, and n.m.r. spectroscopy were the principal methods used. It is concluded that the polysaccharide is composed of disaccharide repeating-units having the following structure.

$$\begin{array}{l} \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow \\ \qquad\quad 2 \\ \qquad\quad \uparrow \\ \qquad\quad 1 \\ \qquad \beta\text{-D-Glc}p \end{array}$$

This comb-like structure is very crowded, which influences the n.m.r. spectra of the polysaccharide.

INTRODUCTION

The capsular polysaccharide from *Streptococcus pneumoniae* type 37 (S-37) is composed of D-glucosyl residues and is the only homoglycan among the approximately 80 type-specific capsular polysaccharides elaborated by the different types of this organism. Preliminary studies[1] indicated that S-37 has a branched structure, with a linear backbone of (1→3)-linked D-glucopyranosyl residues. We now report structural studies of S-37.

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RESULTS AND DISCUSSION

The crude S-37, which was contaminated by protein, was purified by partition between water and phenol. In this procedure, used for the purification of bacterial lipopolysaccharides[2], the polysaccharide accumulates in the aqueous phase and the protein in the phenol phase. Solutions of S-37 in water or dimethyl sulfoxide were probably not monodisperse, as indicated by the poor quality of the n.m.r. spectra and the failure to achieve complete methylation by the Hakomori procedure. The ^{1}H- and ^{13}C-n.m.r. spectra showed, however, that S-37 did not contain non-carbohydrate substituents, such as acetyl or pyruvyl groups. Partial depolymerisation of S-37 by heating a solution in aqueous 80% formic acid at 85° for 20 min yielded a more soluble product. Comparison of the ^{1}H- and ^{13}C-n.m.r. spectra of this material and the original S-37 showed that structural changes introduced by the partial depolymerisation were negligible. Partially depolymerised S-37 had $[\alpha]_{578}^{20}$ −2° (water). Methylation analysis of this material yielded comparable amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose and 4,6-di-*O*-methyl-D-glucose, but no other products, indicating a highly branched structure. In agreement with previous results, Smith degradation of depolymerised S-37 gave a linear, (1→3)-linked glucan, as indicated by methylation analysis, which gave 2,4,6-tri-*O*-methyl-D-glucose as the sole product. The ^{13}C-n.m.r. spectrum of this material was indistinguishable from that of an authentic (1→3)-β-D-glucan[3].

TABLE I

CHEMICAL SHIFTS IN THE ^{1}H-N.M.R. SPECTRUM OF S-37

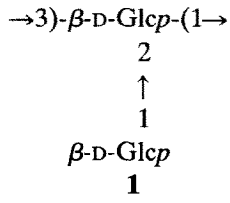
Sugar residue	*Chemical shift (δ)*						
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*
→3)-β-D-Glc*p*-(1→ 2 ↑	5.12	3.92	4.09	3.59	3.51	3.75	3.93
β-D-Glc*p*-(1→	4.97	3.40	3.54	3.40	3.45	3.74	3.93

TABLE II

CHEMICAL SHIFTS IN THE ^{13}C-N.M.R. SPECTRA OF S-37 AND (1→3)-β-D-GLUCAN

Sugar residue	*Chemical shift (δ)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
→3)-β-D-Glc*p*-(1→ 2 ↑	100.0	80.8	82.5	68.9	76.5	61.8
β-D-Glc*p*-(1→	102.9	74.9	76.9	71.1	77.1	62.3
→3)-β-D-Glc*p*-(1→	103.4	74.0	85.7	69.2	76.6	61.7

All signals in the ^{1}H- and ^{13}C-n.m.r. spectra of partially depolymerised S-37 could be identified (Tables I and II) by means of COSY and C–H correlation spectroscopy, and the results confirm that S-37 is composed of disaccharide repeating-units. The high values for the $J_{1,2}$ coupling constants (7.8 and 7.7 Hz) indicate that both D-glucopyranosyl residues in S-37 are β-linked. The values for $J_{C\text{-}1,H\text{-}1}$ (166.5 and 166.5 Hz), however, fall between those generally observed for α- and β-glycopyranosides[4]. From the combined evidence, it is concluded that S-37 is composed of disaccharide repeating-units with the structure **1**.



1

The chemical shift of the anomeric proton of a chain residue of S-37 should, to a first approximation, be equal to that of the H-1 resonance in a (1→3)-β-D-glucan (δ 4.71), plus the shift of the H-1 resonance when going from methyl β-D-glucopyranoside to methyl β-sophoroside, namely, 0.11 p.p.m.[3]. The calculated value, δ 4.82, is considerably lower than that observed, δ 5.12. A possible explanation may be that S-37, because of its crowded, comb-like structure, assumes a conformation in which O-2 of a neighbouring chain residue is in close contact with this anomeric proton.

The resonance of the anomeric proton in the terminal β-D-glucopyranosyl group, at δ 4.97, also occurs at much lower field than that of the corresponding proton in methyl β-sophoroside, at δ 4.76. In an energy-minimised model containing six sugar residues, *i.e.*, three repeating units, obtained using the GESA program[5], the middle β-D-glucopyranosyl residue assumes a conformation in which the ring oxygen in one β-D-glucopyranosyl group comes close to the anomeric proton of the adjacent group, which may account for the downfield shift. It is evident from the modelling that the molecule is severely crowded and thus other ways of escaping repulsion (for example, ring deformation) may occur which are not accounted for in the GESA program. This effect would probably not, however, change the position of the ring oxygen in terminal sugar residues very much.

The chemical shifts for the carbon atom resonances in S-37 and (1→3)-β-D-glucan are given in Table II. The shifts caused by substitution of the latter with β-D-glucopyranosyl groups in 2-positions are −3.4, 6.8, and −3.2 p.p.m. for the C-1, C-2, and C-3 resonances, respectively. The corresponding values for methyl β-sophoroside are −1.4, 7.3, and 0.0 p.p.m., respectively[3]. The signal for the C-1 resonance of the β-D-glucopyranosyl group in S-37 (δ 102.9) also differs slightly from the corresponding signal in methyl β-sophoroside (δ 103.4). These deviations also indicate that there are inter-residue atomic contacts in S-37 which are caused by the steric crowding and which are not present in the disaccharide models.

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at <40° (bath) or at room temperature by flushing with air. For g.l.c., a Hewlett–Packard 5830A instrument fitted with a flame-ionisation detector was used. Separations of alditol acetates and of partially methylated alditol acetates were performed on an SE-54 fused-silica capillary column, using a temperature program 210° (3 min)→250° at 2°/min and 150° (2 min)→220° at 2°/min, respectively. G.l.c.–m.s. was performed on a Hewlett–Packard 5970 instrument. Hydrolyses were performed with 0.5M trifluoroacetic acid for 16 h at 100°. Methylation analyses were performed as previously described[6], and methylated products were recovered by reverse-phase chromatography on Sep-Pak C_{18}-cartridges[7].

N.m.r. spectroscopy. — N.m.r. spectra of solutions in deuterium oxide were recorded at 70° (^{13}C) and 85° (^{1}H) with a JEOL GX-400 instrument. Chemical shifts are reported in p.p.m. using internal 1,4-dioxane (δ 67.4) for ^{13}C and internal sodium 3-trimethylsilylpropanoate-d_4 for ^{1}H. COSY and C–H correlation spectroscopy experiments were performed according to JEOL standard pulse sequences.

Preparation of crude S-37. — *Streptococcus pneumoniae* type 37, strain 234/77 (from the Collection of the WHO Collaborating Centre for Reference and Research on Pneumococci, Statens Seruminstitut, Copenhagen) was grown overnight in serum broth (Statens Seruminstitut). The cells were then lysed with sodium deoxycholate. Proteins were removed by treatment with chloroform–butanol, and the polysaccharide was precipitated with 4 volumes of ethanol.

Purification of S-37. — The crude polysaccharide sample (200 mg) in water (100 mL) was extracted with aqueous 90% phenol (100 mL) and worked-up as previously described[2]. The yield of polysaccharide was 105 mg.

Partial depolymerisation of S-37. — A solution of S-37 (100 mg) in aqueous 80% formic acid was kept for 20 min at 85°, then dialysed, and freeze-dried. The product was fractionated on a column (3 × 90 cm) of Bio-Gel P-10, irrigated with mM aqueous formic acid. The polysaccharide (82 mg) was eluted in the void volume and recovered as above.

Smith degradation. — A solution of partially depolymerised S-37 (60 mg) and sodium metaperiodate (200 mg) in 0.1M acetate buffer of pH 6 (15 mL) was kept in the dark for 120 h at 4°. Excess of periodate was then reduced with ethylene glycol and the product recovered as above. Reduction with sodium borohydride (200 mg) in water (10 mL) overnight, addition of aqueous acetic acid, and work-up yielded the polyalcohol (19 mg). Part of this material (10 mg) was dissolved in 0.5M trifluoroacetic acid and kept for 16 h at room temperature. The polymeric product (3 mg) was recovered by conventional work-up, including chromatography on the Bio-Gel P-10 column.

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