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STRUCTURE OF A NEUTRAL POLYMER ISOLATED FROM THE LIPOPOLYSACCHARIDE OF THE REFERENCE STRAIN FOR *Serratia marcescens* SEROGROUP O18

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ABSTRACT

A neutral polymer (the putative O antigen) has been isolated from the lipopolysaccharide of the reference strain for *Serratia marcescens* serogroup O18. From the results of spectroscopic and degradative studies, the repeating unit of the polymer was identified as a linear tetrasaccharide having the structure shown.

→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→6)-α-D-Glc*p*NAc-(1→

INTRODUCTION

As with many other Gram-negative bacteria, the typing of clinical isolates of *Serratia marcescens* for epidemiological purposes is commonly based on their O-serological reactions[1]. Initially[2], some 15 serogroups were recognized, but further studies have raised the total[3] to 24. Serogroups O16 to O20 were described by Le Minor and Pigache[4], and their specificities have been studied further[3,5,6]. Cross-reactions between serogroups O18 and O10 were noted by Le Minor and Pigache[4], and have also been observed by other workers[3,5]. During the course of a systematic survey of the surface polysaccharides produced by *S. marcescens*, we have characterized[7] the repeating unit of a neutral polymer from the O10 reference strain as **1**. We now report the structure of the repeating unit for a similar polymer from the O18 strain.

α-D-Glc*p*
1
↓
4
→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-(1→

1

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RESULTS

Lipopolysaccharide was extracted from isolated cell walls in 34% yield. Rhamnose and 2-amino-2-deoxyglucose were the major monosaccharide components; minor components included galactose, glucose, mannose, and aldoheptoses. After mild acid hydrolysis, and chromatography of the water-soluble products on Sephadex G-50, 50% of the lipopolysaccharide was recovered as polymeric material. Further fractionation on DEAE-Sepharose CL-6B gave a neutral glycan (82%), eluted partly with water but mainly (70%) with 0.1M NaCl, and an acidic glycan (18%) eluted with M NaCl. Both fractions of neutral glycan gave identical n.m.r. spectra, and consisted mainly of L-rhamnose and 2-amino-2-deoxy-D-glucose together with traces of glucose and heptoses. Studies of the acidic glycan will be described elsewhere.

The ^{1}H-n.m.r. spectrum of the neutral glycan contained four anomeric signals (each 1 H) at δ 5.18 (unresolved), 5.07 ($J_{1,2}$ ~3.5 Hz), 5.05 (unresolved), and 4.94 (unresolved), as well as methyl signals at δ 2.12 (s, 3 H) and ~1.36 (m, 9 H). These data indicated the presence of three residues of rhamnose and one of 2-acetamido-2-deoxy-α-glucopyranose. The ^{13}C-n.m.r. spectrum (Fig. 1) contained 24 signals (2 being of double intensity), again indicating a tetrasaccharide repeating-unit. Signals of particular interest included anomeric signals at δ 100.95 ($^1J_{CH}$ 169 Hz), 99.52 ($^1J_{CH}$ 172 Hz), 99.25 ($^1J_{CH}$ 171 Hz), and 96.28 ($^1J_{CH}$ 171 Hz), signals for a 2-acetamido group at δ 174.58, 53.90, and 22.20, signals for C-6 of rhamnose residues at δ 17.01, 16.93, and 16.91, and a hydroxymethyl signal at δ 66.63 derived from a 6-substituted residue of 2-acetamido-2-deoxyglucose. Signals at δ 80.23 (2 C) and 78.56, which could be assigned to glycosyloxylated carbons of the rhamnose residues, had $^1J_{CH}$ values of 151 and 152 Hz, respectively, indicative of coupling to equatorial hydrogen, *i.e.*, 2-substitution of each of the rhamnose residues[8]. The substitution patterns and the glycosidic configurations (all α-pyranosyl) inferred from the spectral data pointed to structure **2** for the repeating unit of the neutral

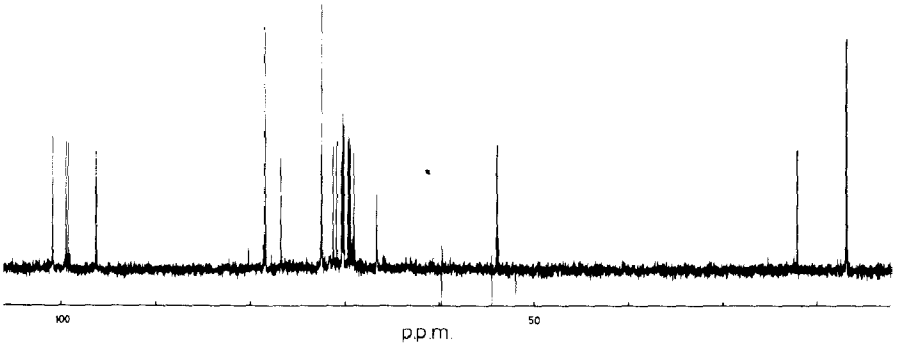

Fig. 1. ^{13}C-N.m.r. spectrum of the O18 neutral glycan. The spectrum for the sample in D_2O was obtained at 100.62 MHz and 50°, with complete proton-decoupling and tetramethylsilane as the external reference. In addition to the signals shown, the spectrum contained a carbonyl resonance at δ 174.58.

glycan. The results of methylation analysis were entirely consistent with this structure.

→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→6)-α-D-Glc*p*NAc-(1→

2

Supporting evidence for the above structure was obtained by the sequential application of *N*-deacetylation, deamination, and reduction ($NaBD_4$) to the polymer. The major product (D18), isolated by h.p.l.c., was eluted in the region expected for a tetrasaccharide-alditol. The ^{1}H-n.m.r. spectrum (270 MHz) contained three unresolved anomeric signals (each 1 H) at δ 5.10, 4.98, and 4.90, and a multiplet (9 H) at $\delta \sim 1.3$. The underivatized product was examined by laser desorption ionization, Fourier transform, ion cyclotron resonance (l.d.i.–F.t.–i.c.r.) spectroscopy. The positive-ion spectrum[9] had a base peak with *m/z* 626 (M + Na), and also contained peaks with *m/z* 642 (M + K), 480 (M + Na − Rha), and 334 (M + Na − 2Rha). Significant peaks in the negative-ion spectrum[10] included the base peak with *m/z* 638 (M + Cl) and others with *m/z* 602 (M − H) and 456 (M − H − Rha). Although the fragmentation processes in spectra of these types are not yet fully understood, the data are at least consistent with the identification of product D18 as a trirhamnosyl derivative of 2,5-anhydromannitol-*1-d*. Further structural detail was provided by c.i.-m.s. of permethylated D18, purified by reverse phase h.p.l.c. Fragment ions of the A and J series[11] (listed in the Experimental) confirmed the sequence of methylated sugar residues in the product. Conversion into methylated alditol acetates, identified by g.l.c.–m.s., gave the derivatives from 6-substituted 2,5-anhydromannitol-*1-d*, unsubstituted rhamnopyranosyl, and 2-substituted rhamnopyranosyl residues (relative peak areas in g.l.c. 0.81:1.00:2.28). These results substantiate structure **2** for the repeating unit.

DISCUSSION

Structure **2**, established for the repeating unit of the neutral O18 glycan, shows obvious similarities to the tetrasaccharide backbone in the O10 polymer (structure **1**), which, presumably, account for the serological cross-reactions that have been described. As the cross-reactivity can be removed by appropriate absorption with the heterologous cells without significant detriment to homologous reactivity, it appears that the major epitopes in the two glycans are different. The influence on serological properties of the acidic O18 glycan is not known.

Neutral glycans with repeating units constructed from rhamnose and a 2-acetamido-2-deoxyhexose are rather common in lipopolysaccharides of *S. marcescens*. Polymers in which the amino sugar has the *gluco* configuration have been isolated from strains of serogroups O1 (ref. 12) and O10 (ref. 7), and also occur in the reference strains for serogroups O17, O19, and O22 (ref. 13), as well

as the O18 strain described here. Similar polymers have been described as the O antigens in a variety of other Gram-negative bacteria, *e.g.*, *Shigella flexneri*[14], *Pseudomonas solanacearum*[15], *P. wieringae*[16], *Haemophilus pleuropneumoniae*[17], *Aeromonas hydrophila*[18], and *Pasteurella haemolytica*[19]. In the last two cases, the disaccharide repeating-unit has the same structure as the O1 polymer from *S. marcescens*[12].

EXPERIMENTAL

Growth of the bacteria, and isolation and fractionation of the lipopolysaccharide. — The methods and conditions used were identical to those described for related studies[7]. The yields of wet cells, freeze-dried cell walls, and isolated lipopolysaccharide were 149, 3.39, and 1.15 g, respectively.

General methods. — N.m.r. spectra (^1H and ^{13}C) for samples in D_2O were recorded with a Bruker WH-400 or JEOL JNM-GX270 spectrometer. ^1H-N.m.r. spectra were recorded at 60° (400 MHz) or 70° (270 MHz) with sodium 3-trimethylsilylpropanoate-d_4 (400 MHz) or sodium 4,4-dimethyl-4-silapentane-1-sulphonate (270 MHz) as the external reference. ^{13}C-N.m.r. spectra were recorded at 50° with tetramethylsilane as the external reference, and with complete or gated decoupling. Chromatographic and electrophoretic methods used to identify, determine, and assign configuration to monosaccharides have been described[7,20]. Methylation analyses were carried out by standard procedures[21], and the products were identified by g.l.c.–m.s.

Preparation and characterization of a deamination product (D18). — A sample (30 mg) of the O18 neutral glycan was *N*-deacetylated[22] for 17 h at 95°, deaminated[21], and reduced ($NaBD_4$)[7]. The products were fractionated by h.p.l.c. with a TSKgel G-Oligo-PW column (Anachem) eluted with water (1 mL.min^{-1}) at room temperature. The major product (D18) was examined by ^1H-n.m.r. spectroscopy, and by l.d.i.–F.t.–i.c.r. spectroscopy[9,10] using a Nicolet FTMS-2000 instrument. The oligosaccharide-alditol was also permethylated, and the product was purified by reverse phase h.p.l.c. with a Spherisorb S5 ODS column[20] and studied by methylation analysis and by direct e.i.-m.s. on the probe. Significant fragment ions for methylated D18 included the following (relative intensities in brackets and some assignments[11] in square brackets): *m/z* 88(100), 101(35), 125(12) [aA_3], 145(12), 157(19) [aA_2], 171(9), 189(60) [aA_1], 190(11) [dA_1], 203(11), 250(11) [cdJ_1], 299(6) [baA_3], 331(7) [baA_2], 332(10) [cdA_2], 363(5) [baA_1], 364(1) [cdA_1], 424(14) [$bcdJ_1$], 474(1) [$bcdA_3$], 506(2) [$bcdA_2$], 538(1) [$bcdA_1$], and 598(8) [$abcdJ_1$].

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