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Structure of a neutral glycan isolated from the lipopolysaccharide of the reference strain for *Serratia marcescens* serogroup O22

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ABSTRACT

Both a neutral and an acidic polymer have been isolated from a lipopolysaccharide extract of the reference strain for *Serratia marcescens* serogroup O22. The neutral polymer has a linear structure with the repeating unit shown. The same tetrasaccharide unit also forms the backbone of the branched neutral polymer isolated from the reference strain for serogroup O10, which cross-reacts strongly with O22.

→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→

INTRODUCTION

Neutral glycans with a glucorhamnan structure are rather common as the polymeric side chains of lipopolysaccharides from *Serratia marcescens*. Such polymers were originally described[1,2] for strain N.R.C. S-29, and one with a disaccharide repeating-unit was identified[3] as the O antigen in strain A.T.C.C. 264. Our structural studies of the O antigens of *S. marcescens* have shown that glucorhamnans with a disaccharide repeating-unit (partially acetylated at position 2 of the rhamnosyl residue) are present in the lipopolysaccharides from the reference strains for serogroups O4 (ref. 4), O6 (ref. 5), and O7 (ref. 4) as well as those from two pigmented O14 strains[4,6]. Similar polymers without *O*-acetyl substituents but containing 2-acetamido-2-deoxy-D-glucose in place of D-glucose are present in the lipopolysaccharides from the reference strains for serogroups O1 (ref. 7), O17, and O19 (ref. 8). A polymer with a tetrasaccharide repeating-unit of rhamnose (three residues) and 2-acetamido-2-deoxyglucose is produced by the O18 reference strain[9], and an isomeric tetrasaccharide with a lateral glucosyl substituent is the repeating unit for the O10 antigen[10]. We now report the structure of the neutral polymer isolated from the lipopolysaccharide of the O22 reference strain.

RESULTS AND DISCUSSION

Lipopolysaccharide was obtained by extraction of isolated cell walls with hot, aqueous phenol (yield, 25%). The neutral sugar components were glucose, galactose,

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rhamnose, and aldoheptoses (probably the L-*glycero*-D-*manno* and D-*glycero*-D-*manno* isomers), while 2-amino-2-deoxyglucose was the only amino sugar detected. After mild acid hydrolysis, 65% of the lipopolysaccharide was recovered as polymeric, water-soluble products (Sephadex G-50). Further chromatography of the polymeric products on DEAE-Sepharose CL-6B gave a neutral polymer (29%), most of which (66%) was eluted with water and the remainder with 0.1M NaCl, and an acidic polymer (71%) that was eluted as two peaks with 0.3M NaCl. The results of structural studies of the acidic polymer will be reported elsewhere.

Both fractions of the neutral polymer had the same monosaccharide composition (mainly L-rhamnose and 2-amino-2-deoxy-D-glucose, with small amounts of glucose and aldoheptose) and gave essentially the same n.m.r. spectra. The fraction eluted with water was used for structural studies. Methylation analysis of the polymer, monitored by g.l.c. and m.s., gave derivatives from 2-substituted and 3-substituted rhamnopyranosyl residues (relative peak areas in g.l.c., 2.2:1.0), and from 3-substituted 2-acetamido-2-deoxyglucopyranosyl residues. The n.m.r. spectra of the polymer confirmed that it had an unbranched, tetrasaccharide repeating-unit. The ^{1}H-n.m.r. spectrum contained four anomeric signals (each 1 H) at δ 5.17 (unresolved), 5.02 (unresolved), 4.98 ($J_{1,2}$ 3.0 Hz), and 4.91 ($J_{1,2} \sim 1$ Hz), as well as a methyl singlet at δ 2.06 and three methyl doublets (each with $J_{5,6} \sim 6$ Hz) at δ 1.32, 1.31, and 1.26. The ^{13}C-n.m.r. spectrum (Fig. 1) contained 25 signals (one corresponding to two carbons), including four in the anomeric region at δ 101.14 ($^1J_{CH}$ 169 Hz), 100.94 ($^1J_{CH}$ 172 Hz), 99.64 ($^1J_{CH}$ 173 Hz), and 96.44 ($^1J_{CH}$ 171 Hz), showing that all of the glycosidic linkages have the *a* configuration. Other obvious signals in the spectrum were those at δ 174.05 and 22.26 (attributable to the acetamido group), one at δ 53.34 (corresponding to C-2 of the acetamido sugar), and others at δ 16.93 (2 C) and 16.70 (attributable to C-6 of the three rhamnose residues).

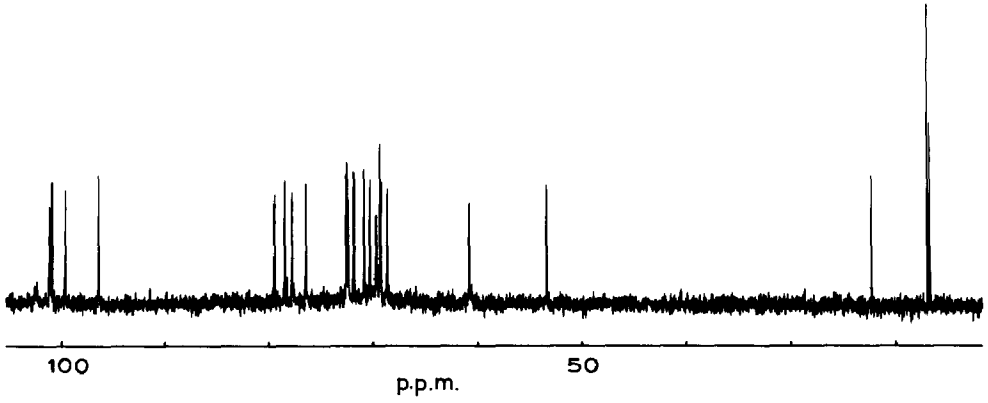

Fig 1. ^{13}C-N.m.r. spectrum of the neutral polymer.

Initial sorting of the proton resonances for the polymer (Table I) was carried out with the aid of COSY and relayed COSY spectra. The residues designated *a*, *b*, and *d* (listed in order of decreasing chemical shift for H-1) corresponded to rhamnose, and residue *c* to 2-acetamido-2-deoxyglucose. For each residue, the corresponding carbon

TABLE I

Chemical shifts (p.p.m.) of signals in the ^1H- and the ^{13}C-n.m.r. spectra for the O22 neutral polymer

	Residue			
Atom	*a*	*b*	*c*	*d*
H-1	5.17	5.02	4.98	4.91
C-1	100.94	99.64	96.44	101.14
H-2	4.07	4.10	4.09	3.87
C-2	78.48	76.42	53.34	70.84
H-3	3.93	3.92	3.84	3.80
C-3	70.26[a]	69.74[a]	79.48	77.75
H-4	3.48	3.55	3.61	3.57
C-4	72.48	72.33	68.60	71.83
H-5	3.76	3.76	4.07	4.03
C-5	69.34[a]	69.67[a]	72.55	69.24
H-6	1.31	1.32	~3.8	1.26
C-6	16.93	16.93	60.76	16.70

[a] The assignment is only tentative, as some relevant cross-peaks were not detected in the $^1H/^{13}C$ shift correlation spectrum.

resonances (Table I) were identified with the aid of a heteronuclear $^1H/^{13}C$ chemical shift correlation spectrum. The significant downfield shifts for the signals for C-2*a*, C-2*b*, C-3*c*, and C-3*d*, compared with the corresponding signals for the parent monosaccharides[11], confirmed that residue *c* was the 3-substituted 2-acetamido-2-deoxyglucose, and showed that residue *d* was the 3-substituted rhamnose. Morever, the close agreement in the ^1H-n.m.r. data for residues *a* and *d* and the corresponding residues in the structural element **1** present in the repeating unit of the type Y polysaccharide from *Shigella flexneri*[12] suggested the presence of the same disaccharide element in the polymer from *S. marcescens* O22. Further evidence for the sequence of residues was sought through 1D-n.O.e. experiments. Saturation of the signal at δ 5.02 (H-1*b*) showed an inter-residue contact with H-2*a*, indicating the overall sequence of residues in the repeating unit to be $\rightarrow b \rightarrow a \rightarrow d \rightarrow c \rightarrow$. Although other data (Table II) from the n.O.e. experiments were of limited diagnostic value, they were consistent with the sequence proposed.

$$\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow$$

1

$$\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow$$

2

In order to confirm structure **2** for the repeating unit of the neutral polymer from *S. marcescens* O22, a Smith degradation was carried out, including reduction ($NaBH_4$)

TABLE II

Observed n.O.e. contacts and assignments for the O22 neutral polymer

Proton irradiated	*Observed n.O.e. effect*
H-1*a*	H-2*a*, H-3*c* or (H-2*d* and H-3*d*)
H-1*b*	H-2*a*, H-2*b*
H-1*c*	H-2*c*, H-2*a* and /or H-2*b*
H-1*d*	H-2*d*

after the hydrolysis step. The oligomeric product gave n.m.r. spectra consistent with a trisaccharide-alditol of structure **3**. Thus, the ^{1}H-n.m.r. spectrum contained anomeric signals at δ 5.07 ($J_{1,2}$ 3.7 Hz) and 4.92 ($J_{1,2}$ 1.7 Hz), each 1 H, and methyl signals at δ 2.10 (s) and 1.29 ($J_{5,6}$ 6.3 Hz). Also, the ^{13}C-n.m.r. spectrum could be superimposed on that obtained previously[10] for the corresponding product from the O-specific polymer from *S. marcescens* O10 (allowing for a displacement of ~0.55 p.p.m. attributable to the use of a different temperature and an external reference, and the absence from the latter spectrum of the signal for C-1 of the glycerol residue as a consequence of deuterium labelling). The identification of the Smith-degradation product as **3** unambiguously confirms structure **2** as the repeating unit of the parent polymer.

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

3

This study has shown that the repeating unit of the neutral O22 polymer from *S. marcescens* has the same structure as the tetrasaccharide backbone of the branched polymer from the O10 reference strain[10]. No doubt this common feature accounts for the strong cross-reactions observed [13,14] between serogroups O10 and O22. Interestingly, no cross-reactions with serogroup O18 have been reported. The neutral polymer from the O18 reference strain[9] has a linear tetrasaccharide repeating-unit of three 2-substituted *α*-L-rhamnopyranose residues and one 6-substituted residue of 2-acetamido-2-deoxy-*α*-D-glucopyranose. Structurally related polymers have been found as the O antigens of other Gram-negative bacteria[9,12]; in fact, the polymer from a strain of *Pseudomonas solanacearum*[15] also has the repeating unit **2**.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of lipopolysaccharide. — The O22 reference strain (originally described[16] as O21) was grown and the cells were processed as in related studies[4,8–10]. The yields of products from a 20-L batch culture were as follows: wet cells, 150 g; freeze-dried cell walls, 4.08 g; lipopolysaccharide, 1.02 g. Mild acid hydrolysis of the lipopolysaccharide, followed by fractionation of the water-soluble products on Sephadex G-50 and DEAE-Sepharose CL-6B, was also carried out as described in the previous studies.

Structural methods. — N.m.r. spectra of samples in D_2O were recorded with a Bruker WH-400 or a JEOL JNM-GX270 spectrometer. The 1D ^{1}H-n.m.r. spectrum of the neutral polymer was initially recorded at 400.13 MHz and 60° with sodium 3-trimethylsilylpropanoate-d_4 as external reference: the chemical shifts reported (Table I) were adjusted using data from a second spectrum recorded at 270.05 MHz and 60° with acetone (δ_H 2.22) as internal reference. The ^{1}H-n.m.r. data for the Smith-degradation product were not so adjusted. ^{13}C-N.m.r. spectra were recorded at 100.62 MHz and 50° (neutral polymer) or 27° (Smith-degradation product) with tetramethylsilane as external reference. 2D-N.m.r. spectra were obtained using standard COSY (homo- and hetero-nuclear) and relayed COSY pulse sequences, for solutions at 55°. The n.O.e. difference spectra were recorded at 21°.

Methods used to identify and assign configuration to monosaccharides, and for methylation analysis, were those previously described[8,9]. A Smith degradation of the neutral polymer was carried out under standard conditions[10]. The products were reduced ($NaBH_4$), acidified with dilute acetic acid, passed through a column of Dowex 50 (H^+) resin, and freed from boric acid by repeated distillation of methanol. The trisaccharide-alditol produced was isolated by h.p.l.c.[9]

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