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Carbohydrate Research 267 (1995) 127–133

CARBOHYDRATE RESEARCH

Structure of the O10 antigen of *Stenotrophomonas (Xanthomonas) maltophilia*

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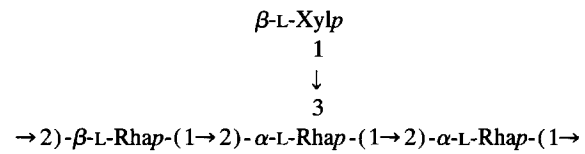
Received 16 June 1994; accepted 24 August 1994

Abstract

A polysaccharide containing L-rhamnose and L-xylose was isolated from the lipopolysaccharide extracted from the cell walls of the reference strain for *Stenotrophomonas (Xanthomonas) maltophilia* serogroup O10. By means of NMR studies and methylation analysis, the repeating unit of the polymer was identified as a branched tetrasaccharide of the structure shown.



Keywords: *Stenotrophomonas maltophilia*; Lipopolysaccharide; O Antigen

1. Introduction

Classification of the organism originally named as *Pseudomonas maltophilia* has been contentious. Although some phenotypic and genotypic similarities with *Xanthomonas* species [1] led to a proposal to transfer the bacteria to that genus [2], the suggestion did not meet with universal approval [3]. Consequently, a counter proposal to rename the organism *Stenotrophomonas maltophilia* has recently been made [4]. The bacteria are recognised as significant opportunistic pathogens of notable antibiotic resistance [5–7]. To assist in epidemiological studies, a scheme for typing clinical isolates by their heat-resistant surface antigens, expected to be the O-specific side chains of lipopolysaccharides (LPSs), has been developed [8]. Previous studies of the structures of polymers from strains representing

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SSDI 0008-6215(94)00277-0

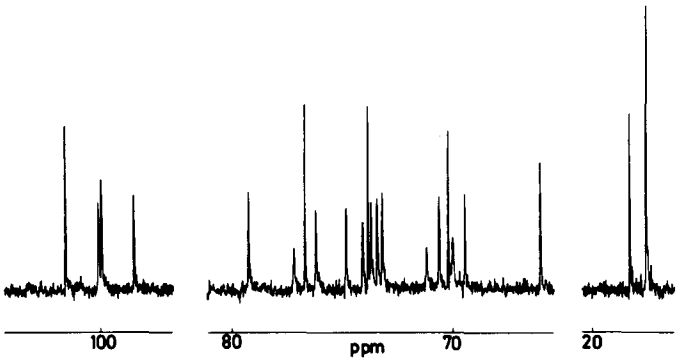

Fig. 1. ^{13}C NMR spectrum of the O10 polymer. The spectrum for the sample in D_2O was recorded at 100 MHz and 70°C with acetone (δ_C 31.07) as the internal reference. In addition to the signals shown, the spectrum contained methyl signals for Rha at δ 18.30 (1 C) and 17.58 (2 C).

serogroups O1 [9], O8 [10], O12/O27 [11], and O19 [12] have revealed the presence of various monosaccharides (e.g., 3-*O*-methyl-L-xylose, 6-deoxy-L-talose, D-arabinose, D-fucose, 4-amino-4-deoxy-D-rhamnose, 3-amino-3-deoxy-D-fucose) uncommon in lipopolysaccharides. We now report the structure of the O10 antigen, which has a rhamnan backbone substituted by β-L-xylopyranosyl groups.

2. Results and discussion

LPS of *Stenotrophomonas maltophilia* strain 558 (serogroup O10) was isolated (yield, 24%) from defatted cell walls by the aqueous phenol method; the LPS was recovered from the aqueous phase. The water-soluble products obtained (yield, 74%) after mild hydrolysis (aq 1% AcOH, 100°C, 1.5 h) of the LPS were fractionated by chromatography on Sephadex G-50. The polymeric fraction (82% of the material applied to the column) contained L-rhamnose and L-xylose (molar ratio 3:1).

A regular structure for the polymer based on a tetrasaccharide repeating-unit was apparent from the NMR data. The ^1H NMR spectrum contained four anomeric signals (each 1 H) at δ 5.39 (unresolved), 5.12 (unresolved), 4.76 (unresolved), and 4.60 ($J_{1,2}$ 7.4 Hz), and three methyl doublets (Rha H-6) at δ 1.35, 1.31, and 1.30 (each with $J_{5,6}$ ~6 Hz). The ^{13}C NMR spectrum (Fig. 1) contained 22 signals (one at δ 17.58 with double intensity), including anomeric signals at δ 101.59 ($^1J_{CH}$ 156.2 Hz), 100.09 ($^1J_{CH}$ ~170 Hz), 99.95 ($^1J_{CH}$ 176.6 Hz), and 98.47 ($^1J_{CH}$ 158.0 Hz). From these results it can be inferred that the repeating unit is constructed from β-Xyl*p* (1), β-Rha*p* (1), and α-Rha*p* (2) units. Methylation analysis, monitored by GLC and GLC-MS of the methylated alditol acetates, showed that the β-Xyl*p* residue was unsubstituted, that two Rha*p* residues were 2-substituted, and that the third was 2,3-disubstituted, thus pointing to a branched tetrasaccharide repeating-unit.

Other structural details of the repeating unit were established by further interpretation of the NMR data for the polymer, and the acquisition of additional spectroscopic results. The four monosaccharide residues were coded **A–D** (Table 1), in order of decreasing chemical

Table 1
NMR data [a] for the polymer

Atom		Residue			
		→2)-α-Rha-(1→ **A**	→2,3)-α-Rha-(1→ **B**	→2)-β-Rha-(1→ **C**	β-Xyl-(1→ **D**
1	C	99.95	100.09	98.47	101.59
	H	5.39	5.12	4.76	4.60
2	C	79.23 [b]	74.06	76.18 [b]	73.82
	H	4.08	4.41	4.09	3.39
3	C	70.59	77.18	74.81	76.69
	H	~4.04	3.97	3.70	3.48
4	C	73.40 [c]	71.15	73.17 [c]	70.17
	H	~3.44	3.53	~3.44	3.64
5	C	69.41	69.96	73.70	66.04
	H	4.06	3.76	3.42	3.32 (H*ax*)
					3.99 (H*eq*)
6	C	18.30	17.58	17.58	
	H	1.31	1.30	1.35	

[a] Values for chemical shifts relative to acetone (δ_H 2.22; δ_C 31.07).
[b,c] Pairs of signals for which assignments are tentative, and within which assignments may be interchanged.

shift for the anomeric protons. Residue **D** was clearly identified as the β-Xyl*p* residue from the values of δ and $J_{1,2}$ for the anomeric signal. The assignment was confirmed by a HETCOR experiment (Fig. 2), which showed a correlation with the anomeric carbon with δ 101.59 ($^1J_{CH}$ 156.2 Hz). Similar correlations showed that residues **A** and **B** were both α-Rha*p* and

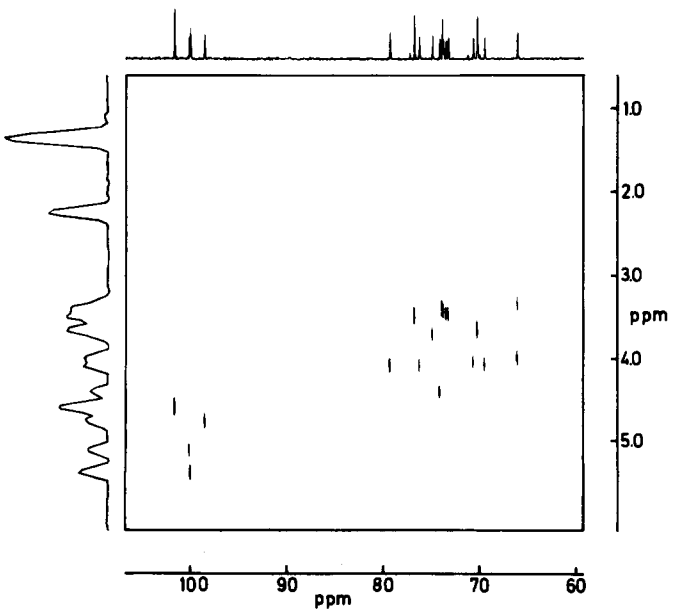

Fig. 2. HETCOR spectrum of the O10 polymer. Signals corresponding to the methyl groups are omitted.

that residue **C** was the β-Rha*p*. Complete assignments of the proton resonances for the lateral Xyl*p* residue **D** were readily made with the aid of COSY, relayed COSY, and TOCSY spectra, and correlations were made with the prominent signals (δ 76.69, 73.82, 70.17, and 66.04) in the ^{13}C NMR spectrum (Fig. 1). The spectral data for **D** are comparable with those for a similar β-L-Xyl*p* group in polysaccharides from *Burkholderia* (*Pseudomonas*) *solanacearum* [13,14].

The spin system in the 2D NMR spectra for the protons in residue **B** could also be followed readily, but the HETCOR spectrum (Fig. 2) did not show the expected correlations for H-3, H-4, and H-5. However, the more sensitive proton-detected heteronuclear multiple-quantum coherence (HMQC) technique provided the missing data. The large difference in chemical shift for C-3 (δ 77.18) compared with that in free α-Rha*p* (δ 71.00) [15] shows that this is a site of glycosylation, and hence that residue **B** is present at the branch point. By contrast, C-2 (δ 74.06), which must also be a site of substitution, shows only a modest glycosylation shift from δ 71.81 in free α-Rha*p* [15]. However, the magnitude of glycosylation effects (α and β) depends markedly on stereochemical factors, and is often influenced by vicinal substitution [16,17]. Significant deviations from additivity have been reported for some 2,3-disubstituted rhamnopyranosides [18,19].

Tracing the spin systems, and establishing the C–H correlations, for residues **A** and **C** was more difficult because of (*a*) the virtual superposition of the respective H-2 and H-4 signals and (*b*) the proximity of the signals for H-2, H-3, and H-5 in **A**, *inter alia*. However, intra-residue NOE contacts between H-1 and both H-3 and H-5 (as well as H-2) for **C** identified these signals and confirmed the β-configuration of this residue. The proton assignments (Table 1) were completed with the aid of COSY, relayed COSY, and TOCSY spectra, and most C–H correlations were then clear from the HETCOR and HMQC experiments. The exceptions were the signals for C-2 and C-4 of both **A** and **C**. The C-4 signals could not be distinguished conclusively, but the tentative assignments for C-2 (Table 1) were supported by further evaluation of the glycosylation shifts observed (see below).

The remaining structural problems were (*a*) to identify the substituents and their positions at the branch point, and (*b*) to determine the location and/or sequence of the two 2-substituted Rha*p* residues. NOE difference and NOESY spectra were used for this purpose. Although problems of spin diffusion and the difficulty in differentiating between H-2 of **A** and **C** complicated the spectra and their interpretation, there was a clear inter-residue NOE contact between H-1 of **D** and both H-2 and H-3 of **B**, showing that the Xyl*p* substituent was attached directly to the branch-point Rha*p* residue. The apparent ambiguity about the **D** → **B** linkage is consistent with the effect expected for a β-L-Xyl*p* substituent at position 3, adjacent to the equatorial H-2 of α-L-Rha*p* [18,20]. Irradiation of H-1 of **B** gave a strong NOE response at δ ~4.09 (H-2 of **A** or **C**), as well as an intra-residue response at H-2 (δ 4.41). Likewise, irradiation of H-1 of **A** failed to distinguish conclusively between the possible sequences **B** → **A** → **C** and **B** → **C** → **A**. However, irradiation of H-1 of **C** gave the expected [18,20] NOE contacts at both H-1 and H-2 of **B** in addition to the intra-residue contacts noted above, and a strong cross-peak for the respective anomeric protons was present in the NOESY spectrum. These results point to structure **1** for the repeating unit of the O10 polymer. The proposed trisaccharide sequence **C** → **B** → **A** for the backbone is also consistent with the assignments for the respective C-2 of each of the Rha*p* residues (Table 1). Thus, the chemical shifts for C-2 in free α-Rha*p* and β-Rha*p* are similar [15], and the

α-effect of glycosylation is expected [16,21] to be largest in **A** and least in **B** (which is also subjected to an upfield shift due to the presence of the β-L-Xyl*p* substituent at position 3 [18]).

D

β-L-Xyl*p*
1
↓
3
→2)-β-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→

C **B** **A**

1

3-*O*-Me-β-L-Xyl*p*
1
↓
4
→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→

2

Although rhamnans or rhamnose-rich polymers are common as bacterial O antigens, xylose is encountered much less frequently. L-Xylose also occurs (largely as the 3-*O*-methyl derivative) in the O8 antigen of *S. maltophilia* with the repeating unit of structure **2** [10], and is also present in the O18 antigen, again as lateral pyranosyl substituents of a linear rhamnan [22]. Similar polymers have also been isolated from several strains of *B. solanacearum* [13,14], while LPS containing xylose and rhamnose is present in many [23,24] but not all [25,26] pathovars of *Xanthomonas campestris.*

3. Experimental

Growth of bacteria, and isolation and fractionation of the LPS.—*S. maltophilia* strain 558, the O10 reference strain [8], was grown in Nutrient Broth No. 2 (Oxoid, 20 L) for 16 h at 37°C with aeration at 20 L min^{-1} and stirring at 300 rpm. Cell walls (yield, 6.1 g dry weight) were prepared by mechanical disintegration of the cells (148 g wet weight). LPS (yield, 1.24 g) was extracted from the defatted cell walls as in related studies [12]. The putative O-specific polymer was obtained by hydrolysis of the LPS with aq 1% AcOH for 1.5 h at 100°C, followed by chromatography of the water-soluble products on Sephadex G-50.

Chemical methods.—The monosaccharide composition of the O-specific polymer was determined by PC, HPLC, and high pH anion-exchange chromatography (Dionex) of the free sugars, and GLC of the alditol acetates as in related studies [12]. The L configuration for both rhamnose and xylose was established by GLC (BP1) of the (−)-but-2-yl glycoside

acetates [27], after separation of the monosaccharides by HPLC (HPX-87P, Bio-Rad). Methylation analysis was carried out by standard procedures [28–30].

NMR methods.—The 1D NMR spectral data (^1H and ^{13}C) reported were obtained with a Bruker WH-400 spectrometer for a solution of the polymer in D_2O at 70°C with acetone as the internal reference (δ_H 2.22, δ_C 31.07). This instrument was also used to obtain values of $^1J_{CH}$ for the anomeric signals, and for COSY, relayed COSY, and NOE difference spectra. Additional 1D and 2D NMR spectra, including TOCSY, NOESY, and HMQC, were obtained with a Varian DXR600S spectrometer.

Acknowledgements

We thank Dr. B. Schable (Centers for Disease Control, Atlanta) for the O10 reference strain of *S. maltophilia*, the University of Hull for a Scholarship (A.M.W.), and the S.E.R.C. for a grant to purchase the Dionex HPLC system and for time on the high-field NMR services at the Universities of Warwick and Edinburgh. We are also grateful to the following: Dr. O. Howarth (Warwick) and Dr. J.A. Parkinson (Edinburgh) for NMR spectra; Mr. A.D. Roberts for GLC-MS; Miss L. Galbraith for technical assistance.

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