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Carbohydrate Research 272 (1995) 225–230

CARBOHYDRATE RESEARCH

Structure of the O6 antigen of *Stenotrophomonas (Xanthomonas* or *Pseudomonas) maltophilia*

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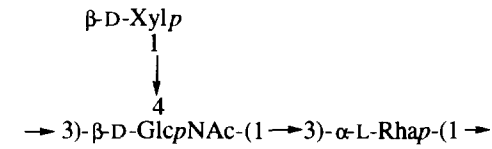
Received 18 November 1994; accepted 8 December 1994

Abstract

A polysaccharide containing D-xylose, L-rhamnose, and *N*-acetyl-D-glucosamine was released on mild acid hydrolysis of the lipopolysaccharide extracted from defatted cell walls of *Stenotrophomonas (Xanthomonas* or *Pseudomonas) maltophilia* strain 557, the reference strain for serotype O6. By means of NMR spectroscopy and chemical degradations, the repeating unit of the polymer was identified as a branched trisaccharide with the structure shown.



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Keywords: Stenotrophomonas maltophilia; Lipopolysaccharide; O Antigen

1. Introduction

The Gram-negative organism classified now as *Stenotrophomonas maltophilia* [1], and previously as a member of the genera *Pseudomonas* [2] or *Xanthomonas* [3], is of increasing importance as a cause of nosocomial infections, particularly among immunocompromised patients receiving treatment with broad-spectrum antibiotics [4–7]. Concern with this problem has stimulated the development of various systems for typing

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SSDI 0008-6215(95)00052-6

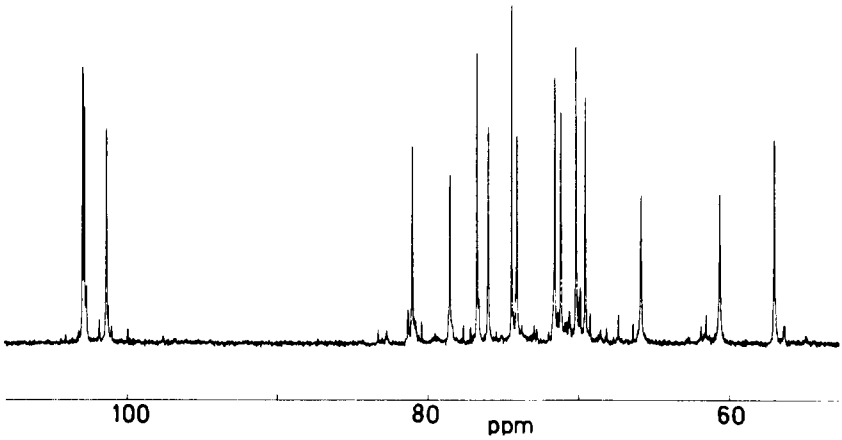

Fig. 1. ^{13}C NMR spectrum of the O6 polymer. The spectrum for the sample in D_2O was recorded at 100 MHz and 70°C with acetone (δ_c 31.07 as internal reference. In addition to the signals shown, the spectrum contained acetyl signals (δ 175.23 and 23.19) and methyl signal (Rha C-6, δ 17.62).

clinical isolates. Structural studies of their heat-stable antigens [the O-specific side chains of lipopolysaccharides (LPSs)] have revealed polymers constructed from branched-chain repeating-units, often incorporating unusual monosaccharides [8–12]. Continuing our systematic investigations [8–11] of these polymers from strains belonging to different serogroups [13], we now report the structure of the O6 antigen.

2. Results and discussion

LPS of *S. maltophilia* strain 557 (serogroup O6) was isolated as a water-soluble product (yield, 15%) from isolated cell walls. Chromatography on Sephadex G-50 of the water-soluble products from mild acid hydrolysis of the LPS gave a polymeric fraction (the putative O6 antigen; yield, approx. 48%). Monosaccharide analyses showed that the polymer was constructed from D-xylose, L-rhamnose, and 2-amino-2-deoxy-D-glucose.

The ^1H NMR spectrum of the polymer contained three signals (each 1 H) in the anomeric region, with δ 4.94 (unresolved, Rha H-1), 4.72 ($J_{1,2}$ 7.3 Hz), and 4.48 ($J_{1,2}$ 7.7 Hz), as well as methyl signals at δ 2.05 (s, *N*-acetyl) and 1.23 (d, *J* 6.0 Hz, Rha H-6). From these data it is clear that Xyl and GlcN (present as the *N*-acetyl derivative) both have the β-pyranoid structure; Rha was assigned the α-pyranoid structure from the downfield location of the signal for H-5 [14], established as δ 4.26 from the COSY spectrum. The ^{13}C NMR spectrum (Fig. 1) contained 19 discrete major signals, including signals for three anomeric carbons (δ 102.95, 102.84, and 101.37), an *N*-acetyl group (δ 175.23 and 23.19), an unsubstituted hydroxymethyl carbon (δ 60.64), C-2 of GlcNAc (δ 57.06), C-5 of Xyl (δ 65.86), and C-6 of Rha (δ 17.62).

The spectroscopic data point clearly to a regular polymer based on a trisaccharide repeating-unit of GlcNAc, Rha, and Xyl. Methylation analysis showed that Xyl occurred

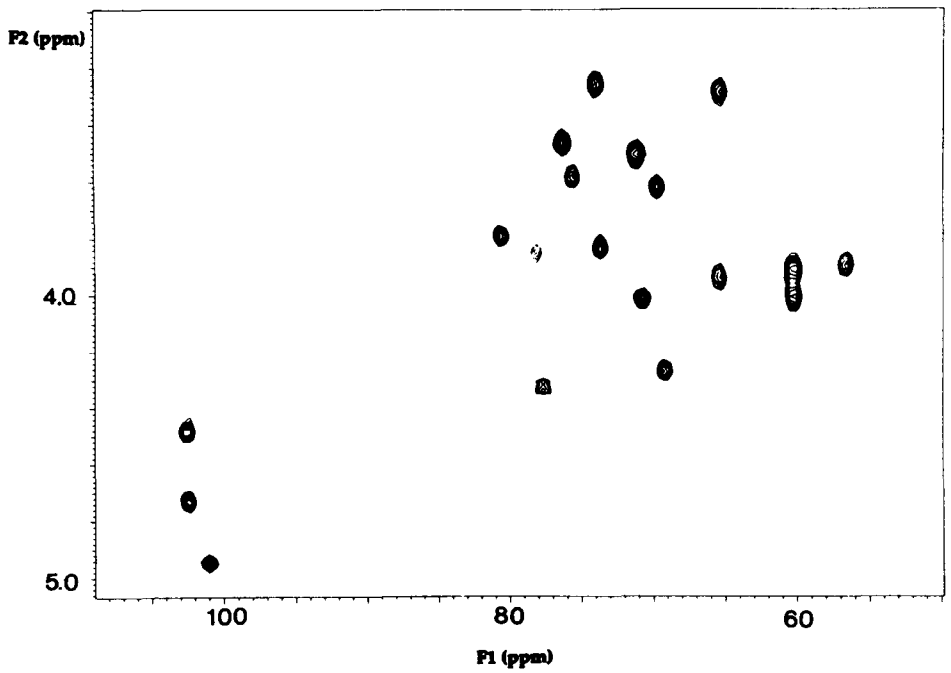

Fig. 2. HMQC spectrum of the O6 polymer.

as unsubstituted pyranosyl residues, Rha as 3-substituted pyranosyl residues, and GlcNAc as 3,4-disubstituted pyranosyl residues. Direct attachment of the Xyl substituents to GlcNAc at the branch point was demonstrated by a Smith degradation. The ^{1}H NMR spectrum of the polymeric product contained signals for two anomeric protons, δ 4.87 (unresolved) and 4.71 ($J_{1,2} \sim 8$ Hz), and methylation analysis showed that both surviving sugars (Rha and GlcNAc) were 3-substituted. Thus, the repeating unit of the parent polymer is the branched trisaccharide of structure **1**. An assignment of the ^{1}H NMR spectrum is given in Table 1. Connectivities for GlcNAc in the COSY spectrum could not be established with confidence beyond H-2. However, the remaining assignments were facilitated by an HMQC spectrum (Fig. 2), which permitted other COSY cross-peaks to be identified after the assignment of H-6a and H-6b. The glycosylation effects apparent in the ^{13}C NMR data (Table 1), where signals for C-3 of Rha, C-3 of GlcNAc, and C-4 of GlcNAc show significant downfield shifts compared with those for the free monosaccharides [15], also confirm the positions of substitution.

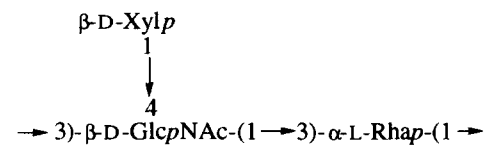

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Table 1
NMR data [a] for the O6 polymer

Atom		Residue		
		→ 3)-α-Rha-(1 →	→ 3,4)-β-GlcNAc-(1 →	β-Xyl-(1 →
1	H	4.94	4.72	4.48
	C	101.37	102.84	102.95
2	H	4.00	3.87	3.25
	C	71.20	57.06	74.46
3	H	3.79	~ 3.85	3.46
	C	81.03	78.54	76.75
4	H	3.49	~ 3.83	~ 3.60
	C	71.59	74.11	70.18
5	H	4.26	~ 3.58	~ 3.29 (H*ax*) ~ 3.92 (H*eq*)
	C	69.56	76.01	65.86
6	H	1.23	~ 3.91 ~ 4.00	
	C	17.62	60.64	

[a] Values for chemical shifts relative to acetone (δ_H 2.22; δ_C 31.07). The *N*-acetyl signal had δ_H 2.05, δ_C 175.23 and 23.19.

Like other O antigens of *S. maltophilia* so far characterised, the O6 polymer has a branched repeating unit. Several of these are unusual among LPS antigens in having an aldopentose as a lateral substituent: D-arabinofuranose in O1 [9], D-ribofuranose in O16 [16], and xylopyranose in O8 [8], O10 and O18 [11] as well as O6 (the present study). In contrast to the other antigens listed, the xylose in the O6 polymer has the D configuration. The occurrence of both isomers (D and L) of a monosaccharide in O antigens of a single species is unusual, but prior examples include rhamnose (e.g., in some pathovars of *Pseudomonas syringae* [17]), 2-amino-2-deoxyglucose in *Burkholderia cepacia* [18], and both 2-amino-2,6-dideoxygalactose (fucosamine) and 2-amino-2-deoxygalacturonic acid in *Pseudomonas aeruginosa* [19].

3. Experimental

Growth of bacteria, and isolation and fractionation of the LPS.—Strain 557 of *S. maltophilia* [13] was grown in Nutrient Broth No. 2 (Oxoid, 20 L) for 24 h at 37°C with aeration at 20 L min^{-1} and stirring at 300 rpm. The cells (wet weight 187 g) were harvested (Sharples) and disintegrated mechanically (Dyno Mill KDL). Cell walls were purified, freeze-dried (yield, 11.2 g), and extracted with 2:1 $CHCl_3$–MeOH at room temperature for 3 h. The defatted cell walls were treated with hot aqueous phenol as in related studies [8–11], and the LPS (1.68 g) was recoverd from the aqueous phase. Samples of LPS were subjected to mild acid hydrolysis (1% AcOH, 100°C, 2 h), and the polymeric material was isolated by chromatography of the water-soluble products on Sephadex G-50.

Monosaccharide analysis.—Conditions used to release monosaccharides were treatment with 2 M HCl at 105°C for 2 h (neutral sugars), 6.1 M HCl at 105°C for 4 h (amino sugars), or 2 M trifluoroacetic acid at 98°C for 16 h (both classes) [20]. When

necessary, amino sugars were separated from neutral sugars by adsorption onto Dowex 50 (H^+) resin and elution with 2 M HCl. Products were identified by high-pH anion-exchange chromatography on CarboPac PA100 (Dionex) eluted with 16 mM NaOH (neutral sugars) or 25 mM NaOH (amino sugars), and by capillary GLC of the alditol acetates on BP1 (SGE). Configurations of sugars were determined by GLC of the (-)-but-2-yl glycoside acetates.

Structural methods.—Methylation analyses, monitored by GLC and GLC-MS of the methylated alditol acetates, were carried out by standard procedures [21–23]. Smith degradation of the polysaccharide (30 mg) involved treatment with 50 mM $NaIO_4$ (8 mL) at 4°C for 5 days, addition of ethylene glycol, reduction ($NaBH_4$), dialysis, hydrolysis with 1 M trifluoroacetic acid for 16 h at room temperature, and recovery of the polymeric product (10 mg) by chromatography on Sephadex G-15. NMR spectra for samples in D_2O were recorded with a Jeol JNM-GX270, Bruker WH-400, or Varian spectrometer, with acetone (δ_H 2.22, δ_C 31.07) as an internal reference. The ^1H NMR spectra (1D and COSY), the ^{13}C NMR spectrum, and the HMQC spectrum were obtained at 70°C.

Acknowledgements

We thank Dr B. Schable (Centers for Disease Control, Atlanta) for the O6 reference strain of *S. maltophilia*, the University of Hull for a Scholarship (A.M.W.) and an allocation from the University Research Support Fund, and the S.E.R.C. for a grant to purchase the Dionex HPLC system. We are also grateful to the following: Dr O.W. Howarth (University of Warwick), Dr I.H. Sadler and Dr J.A. Parkinson (University of Edinburgh), and Mrs B. Worthington (Hull) for NMR spectra; Mr A.D. Roberts for GLC-MS; Miss L. Galbraith for help with the preparation and processing of the bacterial culture.

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