ELSEVIER

Carbohydrate Research 298 (1997) 213–217

CARBOHYDRATE RESEARCH

Structure of the O2 antigen of *Stenotrophomonas* (*Xanthomonas* or *Pseudomonas*) *maltophilia*

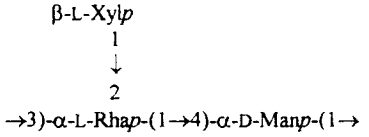
Angela M. Winn, Stephen G. Wilkinson *

School of Chemistry, University of Hull, Hull HU6 7RX, UK

Received 29 August 1996; accepted 5 November 1996

Abstract

An O antigenic polymer containing L-rhamnose, D-mannose, and L-xylose was isolated from the lipopolysaccharide present in the reference strain for *Stenotrophomonas* (*Xanthomonas* or *Pseudomonas*) *maltophilia* serogroup O2, by mild acid hydrolysis and gel-permeation chromatography. By means of NMR spectroscopy and chemical degradations, the polysaccharide was found to be based on a branched trisaccharide repeating-unit of the structure shown.



Keywords: *Stenotrophomonas maltophilia*; Lipopolysaccharide; O Antigen

1. Introduction

The organism now known as *Stenotrophomonas maltophilia* [1], but initially as *Pseudomonas maltophilia* [2] and subsequently as *Xanthomonas maltophilia* [3], is a free-living organism which is also commonly present in clinical specimens. The organism has a growing reputation as an opportunistic pathogen [4,5], particularly as an agent of nosocomial infection and a potential threat to the cystic fibrosis population [6–11], which is partly based on the multidrug resistance of many strains. Among the methods proposed for the epidemiological monitoring of clinical isolates of *S. maltophilia* is serotyping of the heat-stable O antigens [12]. As part of a systematic structural study of these antigens, we have characterised the polymer isolated from the lipopolysaccharide (LPS) of the reference strain for serogroup O2.

2. Results and discussion

The LPS from *S. maltophilia* strain 447 (serogroup O2) [12] was recovered as a water-soluble product (yield, 8%) following aqueous-phenol treatment of

* Corresponding author.

0008-6215/97/$17.00
PII S0008-6215(96)00306-0

defatted cell walls. Mild acid hydrolysis of the LPS released water-soluble products (yield, 46%) which were fractionated by chromatography on Sephadex G-50 to give a polymeric fraction (overall yield, ca. 49%). The monosaccharide components of the polymer were identified as L-rhamnose, D-mannose, and L-xylose.

Three dominant anomeric signals (each 1 H) were apparent from the ^{1}H NMR spectrum at δ 5.06 (unresolved), 5.01 (unresolved), and 4.36 ($J_{1,2}$ 7.7 Hz), and a methyl doublet (Rha H-6) at δ 1.30 ($J_{5,6}$ 6.2 Hz). These results provisionally suggest that within the repeating unit both Rha and Man have the α-pyranoid structure, and show that Xyl has the β-pyranoid structure. The ^{13}C NMR spectrum (Fig. 1) comprised 15 discrete major signals (with δ 73.47 and δ 69.95 being of double intensity), including three anomeric carbons of pyranosyl residues (δ 104.72, 99.90, and 96.70), two unsubstituted hydroxymethyl carbons (δ 66.04 and 61.28), and a methyl group (Rha C-6, δ 17.39).

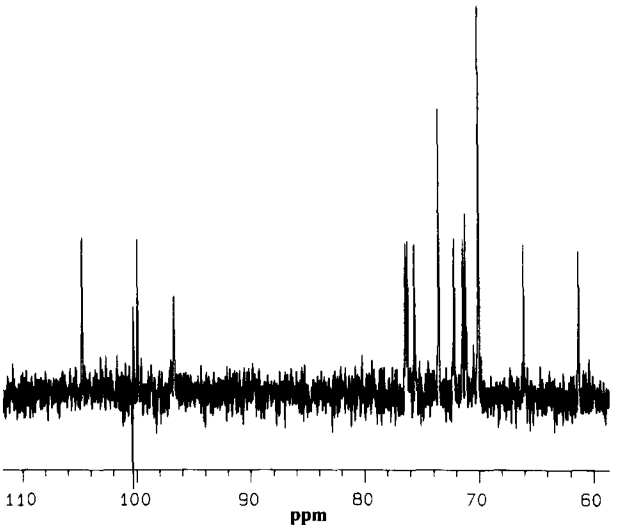


Fig. 1. ^{13}C NMR spectrum of the O2 polymer. The spectrum for the sample in D_2O was recorded at 150 MHz and 25 °C with acetone (δ_C 31.07) as the internal reference. In addition to the signals shown, the spectrum contained a methyl signal (δ 17.39).

Analysis of the NMR data for the material indicated the presence of a major polymer with a regular trisaccharide repeating-unit. GLC and MS examination of the methylated alditol acetates from methylation analysis of the polymer revealed four residues, derived from terminal Xyl*p*, 3-substituted Rha*p*, 2,3-disubstituted Rha*p*, and 4-substituted Man*p* (relative ratio of the methylated alditol acetates on GLC 0.53:0.21:0.63:1.00, respectively). Therefore a branched trisaccharide repeating-unit is indicated for the major polymer, with Xyl as the lateral substituent at the Rha branch-point. The existence of a minor proportion of 3-substituted Rha residues could indicate the absence of Xyl from certain repeating-units, the loss of this residue during methylation analysis, or the presence of minor polysaccharide. Smith degradation of the material resulted in the destruction of the Xyl and Man residues, accounting for the detection of unsubstituted Rha*p* residues from methylation analysis of the oligomeric degradation product (SD). The ^{1}H NMR spectrum of SD included two major anomeric signals equivalent to one proton at δ 5.00 and 4.92 (both unresolved) and a methyl doublet

Table 1
NMR data [a] for the native O2 polymer

Atom		Residue		
		→ 4)-α-Man-(1 →	→ 2,3)-α-Rha-(1 →	β-Xyl-(1 →
		a	**b**	**c**
1	H	5.06	5.01	4.36
	C	96.70	99.90	104.72
2	H	3.93	4.30	3.30
	C	71.34	75.59	73.47
3	H	3.97	3.88	3.42
	C	69.95	73.47	76.36
4	H	3.86	3.48	3.63
	C	76.21	71.11	~ 70.05
5	H	3.94	4.08	~ 3.96, ~ 3.29
	C	72.13	69.95	66.04
6	H	~ 3.82, ~ 3.75	1.30	
	C	61.28	17.39	

[a] Values for chemical shifts at 25 °C (^{1}H 600 MHz) relative to an internal standard of acetone (δ_H 2.22; δ_C 31.07).

(Rha H-6) at δ 1.28. The isolation of two products from the Smith degradation was presumably due to the formation of rhamnosyl derivatives following the expected degradative pathway and the other due to transacetalation during the hydrolytic step [13].

The anomeric configurations for the Rha and Man residues were verified and other structural details of the repeating unit established by further interpretation of the NMR data for the native polymer (Table 1). For this purpose, the monosaccharide residues in the O2 polymer were labelled **a**–**c** (Table 1), in order of decreasing chemical shift for the anomeric proton signals. The signals were assigned with the aid of COSY, relayed COSY and TOCSY spectra, and the C–H correlations established by an HMQC spectrum (Fig. 2).

Residue **b** was deduced to be the Rha residue following identification of the proton signals in the COSY, relayed COSY and TOCSY spectra, particularly the spin-system leading from H-4 (δ 3.48) to H-6 (δ 1.30) [14]. The α-pyranoid configuration for residue **b** was confirmed by the downfield location of the signal for H-5 [15], established as δ 4.08. The spin system in the 2D proton NMR spectra for residue **a** could not be traced with any accuracy beyond H-2 due to proximity of the signals, and hence superposition of cross-peaks. However, the remaining assignments were established by the location of C-6 (δ 61.28) and correlations made with H-6a and H-6b (δ 3.75 and 3.82) by an HMQC spectrum (Fig. 2), which allowed determination of the remaining cross-peaks in the COSY spectrum. Residue **a** was identified as the 4-substituted α-Man*p* residue from the assignments for H-4 (δ 3.86) correlated with C-4 (δ 76.21) [14]. Residue **c** was readily identified as the β-Xyl*p* residue from the values of δ and $J_{1,2}$ for the anomeric proton. Assignments for the remaining protons were readily made via the 2D proton NMR spectra, and correlations made with the corresponding carbon signals using the HMQC spectrum. However, the assignment given in Table 1 for C-4 of residue **c** is only tentative because of overlap with another signal (δ 69.95) of double intensity in the ^{13}C spectrum.

Only modest downfield glycosylation effects at C-2 (δ 75.59) and C-3 (δ 73.47) of the branch-point residue **b** are seen when the values are compared with those of corresponding carbons in free α-Rha*p* (δ 71.81 and 71.00, respectively) [14]. However, the magnitude of such effects is substantially dependent on stereochemical factors, and the effects observed were consistent with substitution by the β-L-Xyl*p* residue at position 2 and the α-D-Man*p* residue at position 3 [16,17]. Deviations from additivity of glycosylation effects have also been recorded for certain 2,3-disubstituted α-L-rhamnopyranosides [18,19].

The presence of a methoxysugar residue was indicated by the correlation of an unassigned sharp singlet at δ 3.54 in the ^{1}H NMR spectrum with a signal

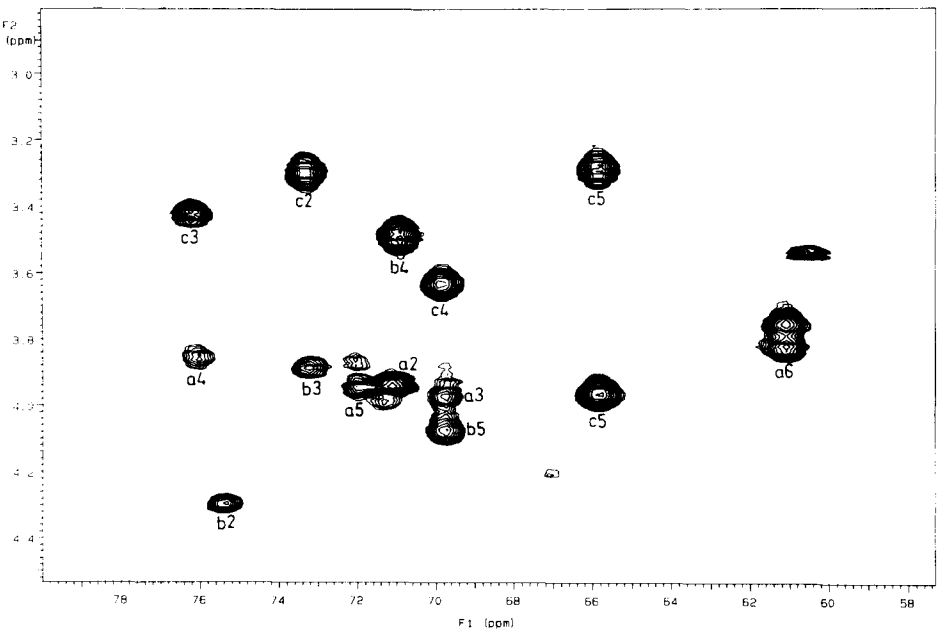


Fig. 2. HMQC spectrum of the O2 polymer. Signals corresponding to the anomeric signals and the methyl group are omitted.

for $\delta_C \sim 60.5$ in the HMQC spectrum, the latter being similar to the value for the lateral 3-*O*-methylxylose residue in the O8 antigen of *S. maltophilia* [20]. However, the signal was not clearly visible above the background in the ^{13}C NMR spectrum (Fig. 2), and analysis of the monosaccharides by PC, and the alditol acetates by GLC and MS did not detect any significant additional component. Therefore, these results indicate that the O2 polymer has the branched trisaccharide repeating-unit of structure **1**, but contains minor proportions of unidentified material.

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

1

The architecture of the repeating unit in the O2 antigen of *S. maltophilia* is comparable with the majority of other polymers reported for this species [20–26] with respect to its branched nature. The O2 polymer contains xylose with the relatively uncommon L configuration, and the incidence of both isomeric forms of a monosaccharide in O antigens of the same species is also unusual. L-Xylose has also been found as a lateral pyranosyl substituent in other O antigens of *S. maltophilia*, namely in the O8 antigen (predominantly as the 3-*O*-methyl derivative) [20,21] and in the O10 antigen [23]. L-xylose has also been isolated from the O antigen polymers in several strains of *Ralstonia* (*Burkholderia* or *Pseudomonas*) *solanacearum* [27].

3. Experimental

Growth of bacteria, and isolation and fractionation of the LPS.—*S. maltophilia* strain 447, the O2 reference strain [12], was grown in Nutrient Broth No. 2 (Oxoid, 20 L) at 37 °C for 24 h with aeration at 20 L min^{-1} and stirring at 300 rpm. Cell walls (dry weight, 7.1 g) were isolated by mechanical disintegration of the cells (wet weight, 94 g), with repeated washings and enzymatic purification [20]. The cell walls were then treated with 2:1 $CHCl_3$–MeOH at room temperature for 2 h. Treatment of the defatted cell walls with hot aqueous phenol as in related studies [20–25,28] recovered the LPS (yield, 0.67 g) from the aqueous phase. Water-soluble material was released upon hydrolysis of the LPS with aq 1% AcOH at 100 °C for 1.5 h, and fractionated on Sephadex G-50 to isolate the polymeric side chain.

General methods.—The solvent system used for PC was 13:5:4 EtOAc–pyridine–water. The monosaccharide mixtures were resolved by high-pH anion-exchange chromatography (HPAEC) using a CarboPac PA-100 column (Dionex) eluted with 16 mM NaOH. Alditol acetates, methylated alditol acetates, and (-)-but-2-yl glycoside acetates were separated by capillary GLC on a fused-silica column (BP1) fitted to a Carlo Erba Mega 5160 chromatograph. GLC–MS of the alditol acetates and methylated alditol acetates were carried out on a BP10 column situated in a Finnigan 1020B instrument.

NMR data for samples in D_2O were obtained at 25 °C with acetone (δ_H 2.22, δ_C 31.07) as an internal reference since, at 70 °C, two of the anomeric signals within the ^1H NMR spectrum of the native polymer were superimposed and the other was obscured by the HOD signal. 1 D (^1H and ^{13}C) and 2 D (COSY, relayed COSY, TOCSY, and HMQC) NMR spectra for the O2 polymer were recorded using a Varian DXR600 spectrometer at the University of Edinburgh. ^1H NMR data for the oligomeric product SD were recorded with a JEOL JNM-GX270 spectrometer.

Analysis of monosaccharide composition.—Sugar residues were released by treatment with 2 M CF_3CO_2H at 98 °C for 16 h [29]. The monosaccharide composition was deduced by PC and HPAEC of the free sugars, and by GLC and GLC–MS of the alditol acetates. The L-configuration for rhamnose and xylose, and the D-configuration for mannose, was determined by resolution of the acetylated (-)-but-2-yl glycosides [30] on GLC.

Structural methods.—Methylation analyses, monitored by GLC and GLC–MS of the methylated alditol acetates, were carried out according to standard procedures [31–33]. Smith degradation of the polymer (30 mg) required oxidation with 50 mM $NaIO_4$ (12 mL) at 4 °C for 5 days, followed by reduction ($NaBH_4$) and dialysis. The resulting product was hydrolysed with 1 M CF_3CO_2H at room temperature overnight, followed by chromatography on Sephadex G-15 which isolated an oligomeric product SD (16 mg).

Acknowledgements

We thank Dr. B. Schable (Centers for Disease Control, Atlanta) for the O2 reference strain of *S.*

maltophilia, the University of Hull for a scholarship (A.M.W.), the BBSRC for a grant to purchase the Dionex HPAEC system, and for allocations on the high-field NMR service at the University of Edinburgh. We are also grateful to the following for their contributions to this work: Dr. I.H. Sadler and Dr. J.A. Parkinson (Edinburgh), Mrs. B. Worthington (Hull), for NMR spectra; Mr. A.D. Roberts for GLC–MS; Miss L. Galbraith for assistance with the growth and processing of the bacterial cells.

References

[1] N.J. Palleroni and J.F. Bradbury, *Int. J. Syst. Bacteriol.*, 43 (1993) 606–609.
[2] R. Hugh and E. Ryschenkow, *J. Gen. Microbiol.*, 26 (1961) 123–132.
[3] J. Swings, P. De Vos, M. Van den Mooter, and J. De Ley, *Int. J. Syst. Bacteriol.*, 33 (1983) 409–413.
[4] P.E. Schoch and B.A. Cunha, *Infect. Control*, 8 (1987) 169–172.
[5] W.F. Marshall, M.R. Keating, J.P. Anhalt, and J.M. Steckelberg, *Mayo Clin. Proc.*, 64 (1989) 1097–1104.
[6] F. Karpati, A.-S. Malmborg, H. Alfredsson, L. Hjelte, and B. Strandvik, *Infection*, 22 (1994) 258–263.
[7] D.R. Burdge, M.A. Noble, M.E. Campbell, V.L. Krell, and D.P. Speert, *Clin. Infect. Dis.*, 20 (1995) 445–448.
[8] S. Ballestero, I. Vírseda, H. Escobar, L. Suárez, and F. Baquero, *Eur. J. Clin. Microbiol. Infect. Dis.*, 14 (1995) 728–729.
[9] F.P.Y. Laing, K. Ramotar, R.R. Reed, N. Alfieri, A. Kureishi, E.A. Henderson, and T.J. Louie, *J. Clin. Microbiol.*, 33 (1995) 513–518.
[10] M. Chatelut, J.L. Dournes, G. Chabanon, and N. Marty, *J. Clin. Microbiol.*, 33 (1995) 912–914.
[11] J. Wüst, R. Frei, H. Günthard, and M. Altwegg, *Scand. J. Infect. Dis.*, 27 (1995) 499–502.
[12] B. Schable, D.L. Rhoden, R. Hugh, R.E. Weaver, N. Khardori, P.B. Smith, G.P. Bodey, and R.L. Anderson, *J. Clin. Microbiol.*, 27 (1989) 1011–1014.
[13] G.O. Aspinall, in G.O. Aspinall (Ed.), *The Polysaccharides*, Vol. 1, Academic Press, New York and London, 1982, pp 81–89.
[14] P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 188 (1989) 169–191.
[15] A. Adeyeye, P.-E. Jansson, B. Lindberg, S. Abaas, and S.B. Svenson, *Carbohydr. Res.*, 176 (1988) 231–236.
[16] A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, and N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.
[17] N.K. Kochetkov, A.S. Shashkov, G.M. Lipkind, and Y.A. Knirel, *Sov. Sci. Rev., Sect. B.*, 13 (1989) 1–73.
[18] N.K. Kochetkov, G.M. Lipkind, A.S. Shashkov, and N.E. Nifant'ev, *Carbohydr. Res.*, 221 (1991) 145–168.
[19] A.S. Shashkov, N.E. Nifant'ev, and N.K. Kochetkov, *Bio-org. Khim.*, 19 (1993) 756–759.
[20] D.J. Neal and S.G. Wilkinson, *Eur. J. Biochem.*, 128 (1982) 143–149.
[21] S.G. Wilkinson, L. Galbraith, and W.J. Anderton, *Carbohydr. Res.*, 112 (1983) 241–253.
[22] A.M. Winn, L. Galbraith, G.S. Temple, and S.G. Wilkinson, *Carbohydr. Res.*, 247 (1993) 249–254.
[23] A.M. Winn, A.W. Miller, and S.G. Wilkinson, *Carbohydr. Res.*, 267 (1995) 127–133.
[24] A.M. Winn and S.G. Wilkinson, *Carbohydr. Res.*, 272 (1995) 225–230.
[25] A.M. Winn, C.T. Miles, and S.G. Wilkinson, *Carbohydr. Res.*, 282 (1996) 149–156.
[26] J.L. Di Fabio, M.B. Perry, and D.R. Bundle, *Biochem. Cell Biol.*, 65 (1987) 968–977.
[27] Y.A. Knirel and N.K. Kochetkov, *Biochemistry (Moscow)*, 59 (1994) 1325–1383.
[28] A.M. Winn and S.G. Wilkinson, *Carbohydr. Res.*, 294 (1996) 109–115.
[29] C.C. Yu Ip, V. Manam, R. Hepler, and J.P. Hennessey Jr., *Anal. Biochem.*, 201 (1992) 343–349.
[30] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349–357.
[31] B. Lindberg and J. Lönngren, *Methods Enzymol.*, 50C (1978) 3–33.
[32] L.R. Phillips and B.A. Fraser, *Carbohydr. Res.*, 90 (1981) 149–152.
[33] A.J. Mort, S. Parker, and M.-S. Kuo, *Anal. Biochem.*, 133 (1983) 380–384.