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Carbohydrate Research 319 (1999) 199–203

CARBOHYDRATE RESEARCH

Note

Structure of an acidic O-specific polysaccharide of *Proteus mirabilis* O5

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Received 15 February 1999; accepted 24 May 1999

Abstract

The following structure of the O-specific polysaccharide of *Proteus mirabilis* O5 was established by ^{1}H and ^{13}C NMR spectroscopy at 500 MHz, including two-dimensional COSY, TOCSY, NOESY, and H-detected ^{1}H, ^{13}C heteronuclear multiple-quantum coherence (HMQC) experiments:

$$\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow$$
$$\begin{array}{l} 3,6 \\ | \\ OAc_2 \end{array}$$

where O-acetylation of α-D-GlcNAc at both positions is nonstoichiometric.

Keywords: *Proteus mirabilis*; O-antigen; Acidic polysaccharide; Lipopolysaccharide; Structure

Bacteria of the genus *Proteus* are a common cause of urinary tract infections that can lead to severe complications, such as acute or chronic pyelonephritis and formation of bladder and kidney stones. Cell-surface lipopolysaccharide is considered among potential virulence factors mediating the infectious processes and serves as the main surface antigen of *Proteus* [1–3]. Strains of *Proteus* are serologically heterogeneous due to the high diversity of composition and structure of the O-specific polysaccharide chains of the lipopolysaccharides (O-antigen) [4,5]. Accordingly, strains of *Proteus mirabilis* and *Proteus vulgaris* have been classified into 60 O-serogroups [6,7], and some more serogroups proposed for strains of *Proteus penneri* [5].

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0008-6215/99/$ - see front matter
PII: S0008-6215(99)00132-9

Structures of the O-specific polysaccharides of a number of *Proteus* strains have been elucidated with the aim of creating the chemical basis for the serological classification [4,5]. Most of the polysaccharides ($\sim 80\%$) were found to be acidic, some of them containing more than one acidic function in the oligosaccharide repeating unit. Typical acidic components of the *Proteus* O-antigens are uronic acids, their amides with amino acids, phosphate groups, ether-linked lactic acid and acetal-linked pyruvic acid [4,5]. Now we report the structure of a new acidic O-specific polysaccharide of *P. mirabilis* O5, which contains two residues of D-galacturonic acid in a tetrasaccharide repeating unit.

The O-specific polysaccharide (PS-1) was obtained by mild acid degradation of the lipopolysaccharide isolated from dried bacterial cells of *P. mirabilis* O5 by the phenol–water procedure [8]. Sugar analysis after acid hydrolysis of PS-1 revealed the presence of GlcN and GalA, which were identified using amino acid and sugar analysers, respectively. GLC of acetylated (+)-2-butyl glycosides indicated the D configuration of both monosaccharides.

The ^{13}C NMR spectrum of PS-1 (Fig. 1) contained signals for four anomeric carbons at δ 97.5, 99.4 and 102.0 (2C), two *N*-acetyl groups (CH_3 at δ 23.2 and 23.3), and two *O*-acetyl groups (CH_3 at δ 21.5 and 21.7). The signals for other sugar ring carbons in the region δ 52.7–83.6 had different intensities, most probably owing to nonstoichiometric O-acetylation. In contrast, the ^{13}C NMR spectrum of the O-deacetylated polysaccharide (PS-2) was typical of a regular polymer spectrum (Fig. 1). It contained signals for four anomeric carbons at δ 97.1–102.2, two carbons bearing nitrogen (C-2 of GlcNAc) at δ 54.1 and 55.2, 14 other sugar ring carbons at δ 68.0–82.9, two $HOCH_2$–C groups (C-6 of GlcNAc) at δ 60.9 and 61.7 and two *N*-acetyl groups at δ 23.2 and 23.3 (CH_3), but no *O*-acetyl groups; four signals for CO groups (NAc and C-6 of GalA) were at δ 174.8–175.4.

The ^{1}H NMR spectrum of PS-2 contained, inter alia, signals for four anomeric protons at δ 4.66–5.32 and two *N*-acetyl groups at δ 2.01 and 2.08 (both s). Three more signals in the region close to the anomeric proton resonances at δ 4.41–4.58 were assigned to C-4,5 of GalA (see below).

Therefore, PS-1 has a tetrasaccharide repeating unit containing two residues each of

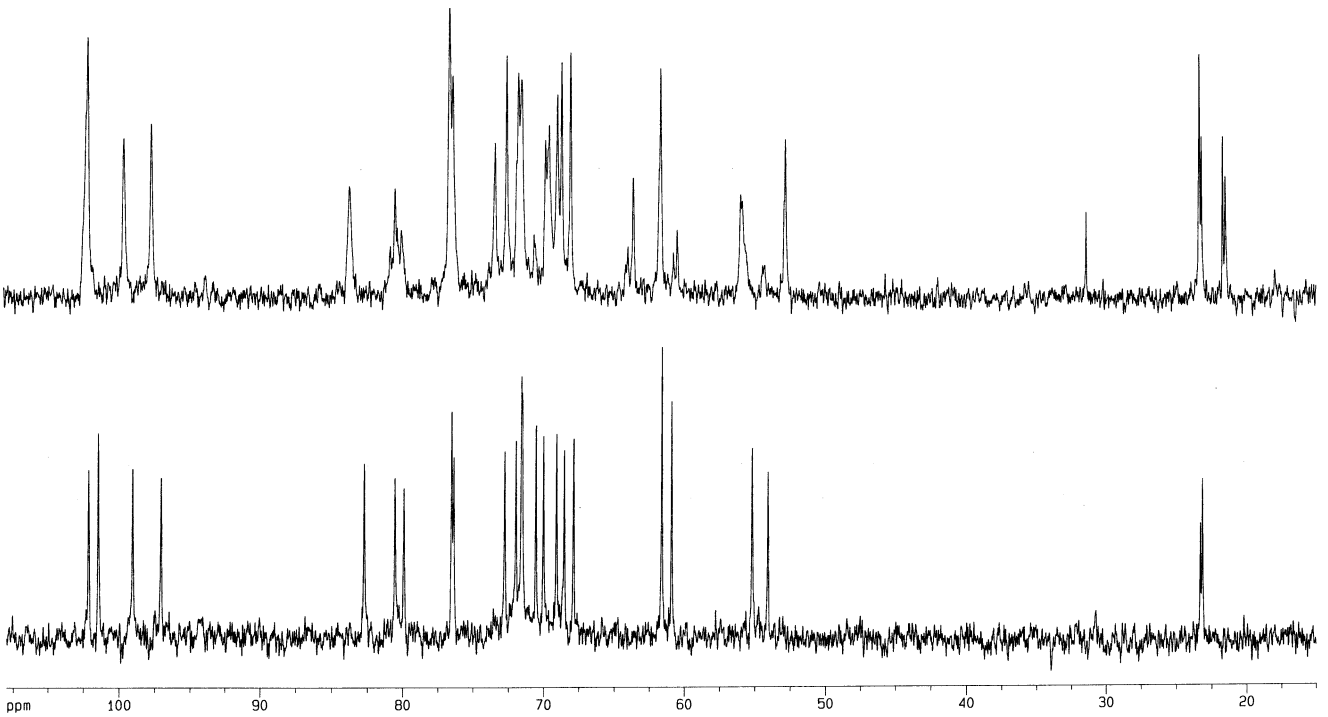

Fig. 1. ^{13}C NMR spectra of the O-specific polysaccharide (PS-1, top) and O-deacetylated polysaccharide (PS-2, bottom).

Table 1
^{1}H NMR data (δ, ppm) of the O-deacetylated polysaccharide (PS-2) [a]

Sugar residue	Proton						
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
→4)-α-D-Glc*p*NAc^{I}-(1→	4.95	3.93	3.88	3.64	4.17	3.67	3.78
→3)-β-D-Glc*p*NAc^{II}-(1→	4.66	3.80	3.79	3.69	3.52	3.74	3.90
→3)-α-D-Gal*p*A^{I}-(1→	5.32	3.95	3.98	4.49	4.23		
→4)-α-D-Gal*p*A^{II}-(1→	5.22	3.92	4.05	4.41	4.58		

[a] Additional chemical shifts: NAc at δ 2.01 and 2.08.

D-GlcNAc and D-GalA, as well as two *O*-acetyl groups in nonstoichiometric amounts. Both the monosaccharides and *O*-acetyl groups are typical components of *Proteus* O-antigens [4,5].

The ^{1}H NMR spectrum of PS-2 (Table 1) was assigned using 2D COSY and TOCSY experiments. The latter displayed cross-peaks of H-1 with H-2,3,4,5,6a,6b of both GlcNAc residues and H-2,3,4,5 of both GalA residues. This allowed the identification of the four sugar spin systems, which was confirmed by typical $^3J_{H,H}$ coupling constant values [9]. The $J_{1,2}$ coupling constant values of <4 Hz indicated that both residues of GalA (GalA^{I} and GalA^{II}) and one of the GlcNAc residues (GlcNAc^{I}) are α-linked, whereas the $J_{1,2}$ value of 8 Hz showed that GlcNAc^{II} is β-linked.

A NOESY experiment with PS-2 showed the following inter-residue cross-peaks between the transglycosidic protons: GlcNAc^{I} H-1, GalA^{II} H-4, GlcNAc^{II} H-1, GlcNAc^{I} H-4 and GalA^{I} H-1, GlcNAc^{II} H-3 at δ 4.95/4.41, 4.66/3.64 and 5.32/3.79, respectively. GalA^{II} H-1 gave two inter-residue cross-peaks with GalA^{I} H-3 and H-4 at δ 5.22/3.98 and 5.22/4.49, respectively, which is typical of α(1→3)-linked disaccharides with the *galacto* configuration of the glycosylated pyranose and the same absolute configuration of the constituent monosaccharides [10]. Therefore, these data demonstrated the linear sequence and the glycosylation pattern of the sugar residues.

The ^{13}C NMR spectrum of PS-2 (Table 2) was assigned using an ^{1}H, ^{13}C HMQC experiment. The spectrum revealed significant downfield displacements to δ 82.9, 76.5, 80.7 and 80.1 of the signals for C-3 of GlcNAc^{II} and GalA^{I} and C-4 of GlcNAc^{I} and GalA^{II}, as compared with their positions in the spectra of the corresponding unsubstituted monosaccharides at δ 74.81, 70.26, 71.26 and 71.64, respectively [11]. These data independently confirmed the modes of substitution of the monosaccharides in PS-2.

Therefore, the PS-2 has the following structure:

→4)-α-D-Gal*p*A^{II}-(1→3)-α-D-Gal*p*A^{I}-(1→3)-β-D-Glc*p*NAc^{II}-(1→4)-α-D-Glc*p*NAc^{I}-(1→

Similarly, the ^{1}H and ^{13}C NMR spectra of PS-1 were assigned, and the latter was compared with the spectrum of PS-2. Partial displacements of two characteristic signals for C-2 and C-6 of GlcNAc^{I} were clearly observed. The former shifted upfield from δ 54.1 in PS-2 to δ 52.7 in PS-1, thus indicating O-acetylation of GlcNAc^{I} at a neighbouring HO-group, i.e., at position 3 [12]. The latter shifted downfield from δ 60.9 in PS-2 to δ 63.5 (major) and 63.7 (minor) in PS-1, showing O-acetylation of GlcNAc^{I} also at position 6 [12]. Two signals for AcO*C*H$_2$–C at δ 63.5 and 63.7 in the spectrum of PS-1 corresponded to 3,6-di-O-acetylated and 6-O-acetylated GlcNAc^{I}; accordingly, the spectrum also contained two minor signals for HO*C*H$_2$–C at δ 60.7 and 60.9 which belonged to 3-O-acetylated and non-O-acetylated GlcNAc^{I}, respectively. As judged by the ratios of the integral intensities of the signals in the O-acetylated and non-O-acetylated residues, the degrees of O-acetylation of GlcNAc^{I} at positions 3 and 6 are $\sim$80 and $\sim$70%, respectively.

On the basis of the data obtained, it was concluded that the O-specific polysaccharide of *P. mirabilis* O5 has the following structure:

Table 2
^{13}C NMR data (δ, ppm) of the O-deacetylated polysaccharide (PS-2) [a]

Sugar residue	Carbon					
	C-1	C-2	C-3	C-4	C-5	C-6
→4)-α-D-Glc*p*NAc^{I}-(1→	99.1	54.1	70.7	80.7	71.7	60.9
→3)-β-D-Glc*p*NAc^{II}-(1→	102.2	55.2	82.9	71.7	76.6	61.7
→3)-α-D-Gal*p*A^{I}-(1	101.5	68.0	76.5	68.6	72.9	175.3 [b]
→4)-α-D-Gal*p*A^{II}-(1→	97.1	69.2	70.1	80.1	72.2	174.8 [b]

[a] Additional chemical shifts: NAc at δ 23.2 and 23.3 (CH_3), 175.2 [b] and 175.4 [b] (CO).
[b] Assignment could be interchanged.

→4)-α-D-Gal*p*A^{II}-(1→3)-α-D-Gal*p*A^{I}-(1→3)-
β-D-Glc*p*NAc^{II}-(1→4)-α-D-Glc*p*NAc^{I}-(1→
3,6
|
OAc_2

where O-acetylation of GlcNAc^{I} at both positions is nonstoichiometric.

1. Experimental

Preparation of lipopolysaccharide and O-specific polysaccharide; O-deacetylation.—*P. mirabilis* O5, strain PrK 12/57 from the Czech National Collection of Type Cultures (Institute of Epidemiology and Microbiology, Prague) was grown as described [13]. Lipopolysaccharide was isolated from dried bacterial cells by extraction with a hot phenol–water mixture [8] and purified by enzymatic treatment [14]. Degradation of the lipopolysaccharide with 0.1 M sodium acetate buffer (pH 4.5) at 100 °C for 1.5 h followed by GPC on a column (3 × 65 cm) of Sephadex G-50 in 0.05 M pyridinium acetate buffer (pH 5.4) gave PS-1. O-Deacetylation of PS-1 was performed with aq 12% ammonia at 60 °C for 1.5 h, the resultant PS-2 being isolated by GPC on a column (3.5 × 95 cm) of Sephadex G-25 in water.

Sugar analysis.—PS-1 was hydrolysed with 3 M CF_3CO_2H (100 °C, 4 h). Amino sugars were identified using a Biotronik LC-2000 amino acid analyser, a column (0.4 × 25 cm) of an Ostion LG AN B cation-exchange resin and the standard 0.35 M sodium citrate buffer (pH 5.28) at 80 °C. Hexuronic acids were analysed with a Biotronik LC-2000 sugar analyser at 70 °C using a column (0.4 × 15 cm) of a Dionex A × 8-11 anion-exchange resin and 0.02 M potassium phosphate buffer (pH 2.4), respectively. The absolute configurations of the monosaccharides were determined by the published method [15] modified as described [16], using GLC of acetylated (*S*)-2-butyl glycosides on a Hewlett–Packard 5890 chromatograph equipped with an Ultra 2 capillary column.

NMR spectroscopy.—^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer in D_2O at 30 and 60 °C for PS-1 and PS-2, respectively. Internal acetone (δ_H 2.225, δ_C 31.45) was used as reference. Standard Bruker software (XWINNMR 1.2) was used to acquire and maintain the NMR data. Mixing times of 200 and 100 ms were used in TOCSY and NOESY experiments, respectively.

Acknowledgements

This work was supported by grant 99-04-48279 of the Russian Foundation for Basic Research and grant 4PO5A 078 14 of the Sciences Research Committee (KBN, Poland).

References

[1] J.W. Warren, in H.L.T. Mobley, J.W. Warren (Eds.), *Urinary Tract Infections. Molecular Pathogenesis and Clinical Management*, ASM Press, Washington, 1996, pp. 3–27.
[2] J.L. Penner, in A. Ballows, H.G. Trüper, W. Harder, H. Schleifer (Eds.), *The Prokaryotes*, Springer-Verlag KG, Berlin, 1992, pp. 2849–2863.

[3] A. Rozalski, Z. Sidorczyk, K. Kotelko, *Microbiol. Mol. Biol. Rev.*, 61 (1997) 65–89.
[4] Y.A. Knirel, E.V. Vinogradov, A.S. Shashkov, Z. Sidorczyk, A. Rozalski, J. Radziejewska-Lebrecht, W. Kaca, *J. Carbohydr. Chem.*, 12 (1993) 379–414.
[5] Y.A. Knirel, W. Kaca, A. Rozalski, Z. Sidorczyk, *Pol. J. Chem.*, 73 (1999) 895–907.
[6] P. Larsson, *Methods Microbiol.*, 14 (1984) 187–214.
[7] J.L. Penner, C. Hennessy, *J. Clin. Microbiol.*, 12 (1980) 304–309.
[8] O. Westphal, K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
[9] C. Altona, C.A.G. Haasnoot, *Org. Magn. Reson.*, 13 (1980) 417–429.
[10] G.M. Lipkind, A.S. Shashkov, S.S. Mamyan, N.K. Kochetkov, *Carbohydr. Res.*, 181 (1988) 1–12.
[11] P.-E. Jansson, L. Kenne, G. Widmalm, *Carbohydr. Res.*, 188 (1989) 169–191.
[12] P.-E. Jansson, L. Kenne, E. Schweda, *J. Chem. Soc., Perkin Trans. 1*, (1987) 373–383.
[13] K. Kotelko, W. Gromska, M. Papierz, Z. Sidorczyk, D. Krajewska, K. Szer, *J. Hyg. Epidemiol. Microbiol. Immunol.*, 21 (1977) 271–284.
[14] P.R. Lambden, J.E. Heckels, *J. Immunol. Methods*, 48 (1982) 233–240.
[15] G.J. Gerwig, J.P. Kamerling, J.F.G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349–357.
[16] A.S. Shashkov, S.N. Senchenkova, E.L. Nazarenko, V.A. Zubkov, N.M. Gorshkova, Y.A. Knirel, R.P. Gorshkova, *Carbohydr. Res.*, 303 (1997) 333–338.