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Note

Structure of the O-antigen of *Yersinia pseudotuberculosis* O:4b

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ABSTRACT

The structure of the long-chain O-antigen of *Yersinia pseudotuberculosis* O:4b containing two uncommon deoxy sugars, tyvelose (3,6-dideoxy-D-*arabino*-hexose, Tyv) and 6-deoxy-D-*manno*-heptose (D-6dmanHep), was established by 1D and 2D NMR spectroscopy as

α-Tyv*p*-(1→3)-β-D-6dmanHep*p*
1
↓
4
→3)-α-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→

A zoonotic pathogen Yersinia pseudotuberculosis is the causal agent of a broad range of acute and chronic gastrointestinal disorders and the ancestor of *Yersinia pestis*[1], the cause of plague. As typical for Gram negative bacteria, the immunospecificity of *Y. pseudotuberculosis* strains is determined by the structures of their O-antigens (O-polysaccharides) and they are classified accordingly into 15 serovars, some of which are divided into subgroups.[2] Structures of the O-antigens (O-polysaccharides) of several serovars have been established.[3,4] Recently, we have determined the O-antigen structure of *Y. pseudotuberculosis* O:2a[5] and reinvestigated those of *Y. pseudotuberculosis* O:2c and O:3, the O:2c structure being revised and the O:3 structure confirmed.[6] In this work, we elucidated the O-polysaccharide structure of *Y. pseudotuberculosis* O:4b and confirmed a tentative structure proposed for this O-antigen earlier based on incomplete chemical[7] and genetic[8,9] data.

The LPS were isolated from bacterial cells by the phenol–water procedure[10] and degraded under mild acidic conditions (sodium acetate buffer, pH 4.5, 100 °C) to give an O-polysaccharide isolated by GPC on Sephadex G-50. According to published data,[7–9] the O-polysaccharide repeating unit contains one residue of D-Gal, tyvelose (3,6-dideoxy-D-arabino-hexose, Tyv), 6-deoxy-D-manno-heptose (D-6dmanHep) and D-GlcNAc.

The ^{1}H and ^{13}C NMR (Fig. 1) spectra of the O-polysaccharide show signals for anomeric atoms of four monosaccharides at δ_H 4.76, 4.93, 4.99 and 5.42 and δ_C 100.6, 101.3, 102.1 and 103.8, a methyl group of Tyv at δ_H 1.30 and δ_C 18.4, methylene groups of Tyv (H-3 and C-3) and 6dmanHep (H-6 and C-6) at δ_H 1.92, 2.05; δ_C 34.6 and δ_H 1.74, 2.14; δ_C 34.7, respectively, hydroxymethylene groups of Gal, GlcNAc (both C-6) and 6dmanHep (C-7) at δ_C 59.5–62.1, and a nitrogen-bearing carbon (C-2 of GlcNAc) at δ 55.8. The signals for other sugar atoms were located at δ_H 3.39–4.36 and δ_C 68.7–81.4, and those for an *N*-acetyl group at δ_H 2.04 and δ_C 23.7. Therefore, the O-polysaccharide consists of a tetrasaccharide repeating unit containing one residue each of the reported[7–9] monosaccharides.

The NMR spectra were fully assigned using 2D COSY, TOCSY, ROESY and ^{1}H,^{13}C HSQC experiments (Table 1). Particularly, the spin systems of Tyv and 6dmanHep were established by correlations for the methylene groups (in case of Tyv also for the methyl group) and that of GlcNAc by a correlation of H-2 to a nitrogen-bearing carbon C-2 at δ 3.85/55.8.

A relatively large $J_{1,2}$ coupling constant of 7 Hz showed that GlcNAc is β-linked, whereas relatively small $J_{1,2}$ values (<3 Hz) indicated that Gal and Tyv are α-linked. The β-linkage of 6dmanHep was inferred by comparison of its ^{1}H and ^{13}C chemical shifts with those of the corresponding free monosaccharide[11] and β-6dmanHep in the O-polysaccharide of *Y. pseudotuberculosis* O:2a.[5] The anomeric configurations were confirmed by a 2D ROESY experiment, which showed a H-1, H-2 correlation for the α-linked Gal and Tyv and H-1, H-3 and H-1, H-5 correlations for the β-linked GlcNAc and 6dmanHep.

Downfield displacements of the signals for C-3 of GlcNAc, C-3 of 6dmanHep, C-3 and C-4 of Gal to δ 77.3–81.4, as compared with their positions in the spectra of the corresponding unsubstituted monosaccharides,[11–13] revealed the glycosylation pattern in the

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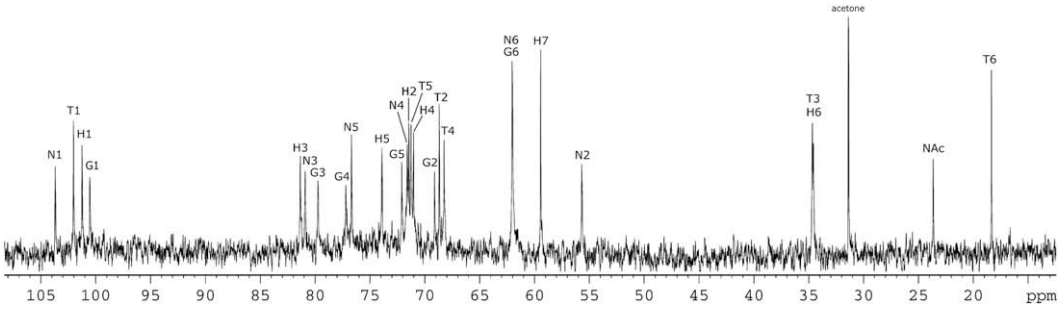

Figure 1. 125-MHz ^{13}C NMR spectrum of the O-polysaccharide of *Y. pseudotuberculosis* O:4b. Numbers refer to carbons in sugar residues denoted as follows: G, galactose; T, tyvelose; H, 6-deoxy-D-*manno*-heptose; N, 2-amino-2-deoxyglucose.

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ, ppm) of the O-polysaccharide of *Y. pseudotuberculosis* O:4b. Chemical shifts for NAc are δ_H 2.04 and δ_C 23.7

Residue		Nucleus	1	2	3	4	5	6	7
→3)-β-D-Glc*p*NAc-(1→	N	^{1}H	4.76	3.85	3.77	3.70	3.50	3.75, 3.94	
		^{13}C	103.8	55.8	81.0	72.2	76.7	62.1	
→3,4)-α-D-Gal*p*-(1→	G	^{1}H	5.42	3.96	3.93	4.36	3.92	3.75	
		^{13}C	100.6	69.2	79.8	77.3	72.5	62.1	
→3)-β-D-6dmanHep*p*-(1→	H	^{1}H	4.99	4.17	3.66	3.54	3.39	1.74, 2.14	3.77
		^{13}C	101.3	71.7	81.4	71.1	74.0	34.7	59.5
α-Tyv*p*-(1→	T	^{1}H	4.93	4.07	1.92, 2.05	3.62	3.88	1.30	
		^{13}C	102.1	68.7	34.6	68.3	71.3	18.4	

α-Tyv*p*-(1→3)-β-D-6dmanHep*p*
1
↓
4
→3)-α-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→

Chart 1. Structure of the O-polysaccharide of *Y. pseudotuberculosis* O:4b.

O-unit with Gal at the branching point and Tyv being the terminal residue of the side chain. The terminal position of Tyv was confirmed by the similarity of its ^{13}C NMR chemical shifts to those of the terminal Tyv residue in synthetic oligosaccharides[13] and a capsular polysaccharide of *Salmonella enteridis*.[14]

The monosaccharide sequence in the O-units was established by ROESY based on the following inter-residue correlations between anomeric protons and protons at the linkage carbons: Tyv H-1,6dmanHep H-3 at δ 4.93/3.66; 6dmanHep H-1,Gal H-4 at δ 4.99/4.36; Gal H-1,GlcNAc H-3 at δ 5.42/3.77 and GlcNAc H-1,Gal H-3 at δ 4.76/3.93. This pattern was confirmed by a ^{1}H, ^{13}C HMBC experiment, which revealed a correlation between the anomeric proton and the transglycosidic carbon atom for all four monosaccharides.

Therefore, the O-polysaccharide of *Y. pseudotuberculosis* O:4b has the structure shown in Chart 1. It differs from the O-polysaccharides of *Y. pseudotuberculosis* O:1a[15] and O:2a[5] only in the presence of tyvelose in place of paratose (3,6-dideoxy-D-*ribo*-hexose) or abequose (3,6-dideoxy-D-*xylo*-hexose), respectively, in the lateral position of the O-unit.

1. Experimental

1.1. Bacterial strain, isolation and degradation of the lipopolysaccharide

The wild-type strain of *Y. pseudotuberculosis* O:4b was kindly provided by Professor M. Skurnik (Helsinki, Finland). Cultivation of bacteria was performed at 22 °C as described.[16] The lipopolysaccharides were isolated by the Westphal procedure.[10] A lipopolysaccharide sample (80 mg) was heated at 100 °C for 2 h in 0.1 M NaOAc buffer, pH 4.5, the precipitate separated by centrifugation (13 000*g*, 20 min) and the supernatant fractionated on a column (56 × 2.6 cm) of Sephadex G-50 (S) in 0.05 M pyridinium acetate buffer, pH 4.5, with monitoring using a differential refractometer (Knauer, Germany) to give poly- and oligo-saccharide fractions. The yield of the O-polysaccharide was 12.5%.

1.2. NMR spectroscopy

An O-polysaccharide sample was deuterium-exchanged by freeze-drying twice from 99.9% D_2O and then examined as solution in 99.96% D_2O at 40 °C on a Bruker DRX-500 NMR spectrometer (Germany) using internal acetone (δ_H 2.225, δ_C 31.45) as reference. 2D NMR spectra were obtained using a standard Bruker software, and the Bruker XWINNMR 2.6 program was used to acquire and process the NMR data. Mixing times of 200 and 100 ms were used in TOCSY and ROESY experiments, respectively. Other NMR parameters were set essentially as described.[17]

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References

1. Achtman, M.; Zurth, K.; Morelli, G.; Torrea, G.; Guiyoule, A.; Camiel, E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14043–14048.
2. Tsubokura, M.; Aleksic, S. *Contrib. Microbiol. Immunol.* **1995**, *13*, 99–105.
3. Ovodov, Y. S.; Gorshkova, R. P. *Khim. Prirod. Soed.* **1988**, 163–171.
4. Ovodov, Y. S.; Gorshkova, R. P.; Tomshich, S. V.; Komandrova, N. A.; Zubkov, V. A.; Kalmykova, E. N.; Isakov, V. V. *J. Carbohydr. Chem.* **1992**, *11*, 21–35.
5. Kondakova, A. N.; Ho, N.; Bystrova, O. V.; Shashkov, A. S.; Lindner, B.; Creuzenet, C.; Knirel, Y. A. *Carbohydr. Res.* **2008**, *343*, 1383–1389.

6. Kondakova, A. N.; Bystrova, O. V.; Shaikhutdinova, R. Z.; Ivanov, S. A.; Dentovskaya, S. V.; Shashkov, A. S.; Knirel, Y. A.; Anisimov, A. P. *Carbohydr. Res.* **2008**, *343*, 2486–2488.
7. Samuelsson, K.; Lindberg, B.; Brubaker, R. R. *J. Bacteriol.* **1974**, *117*, 1010–1016.
8. Skurnik, M.; Zhang, L. *APMIS* **1996**, *104*, 849–872.
9. Reeves, P.; Pacinelli, E.; Wang, L. *Adv. Exp. Med. Biol.* **2003**, *529*, 199–209.
10. Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.
11. Knirel, Y. A.; Paramonov, N. A.; Shashkov, A. S.; Kochetkov, N. K.; Yarullin, R. G.; Farber, S. M.; Efremenko, V. I. *Carbohydr. Res.* **1992**, *233*, 185–193.
12. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
13. Bock, K.; Pedersen, C.; Pedersen, H. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 193–225.
14. Snyder, D. S.; Gibson, D.; Heiss, C.; Kay, W.; Azadi, P. *Carbohydr. Res.* **2006**, *341*, 2388–2397.
15. Komandrova, N. A.; Gorshkova, R. P.; Isakov, V. V.; Ovodov, Y. S. *Bioorg. Khim.* **1984**, *10*, 232–237.
16. Knirel, Y. A.; Lindner, B.; Vinogradov, E. V.; Kocharova, N. A.; Senchenkova, S. N.; Shaikhutdinova, R. Z.; Dentovskaya, S. V.; Fursova, N. K.; Bakhteeva, I. V.; Titareva, G. M.; Balakhonov, S. V.; Holst, O.; Gremyakova, T. A.; Pier, G. B.; Anisimov, A. P. *Biochemistry* **2005**, *44*, 1731–1743.
17. Hanniffy, O. M.; Shashkov, A. S.; Senchenkova, S. N.; Tomshich, S. V.; Komandrova, N. A.; Romanenko, L. A.; Knirel, Y. A.; Savage, A. V. *Carbohydr. Res.* **1998**, *307*, 291–298.