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Note

Structure of the O-antigen of *Yersinia pseudotuberculosis* O:4a revised

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ABSTRACT

The O-specific polysaccharide was isolated by mild acid degradation of the lipopolysaccharide of *Yersinia pseudotuberculosis* O:4a and studied by NMR spectroscopy, including 2D ROESY and ^{1}H, ^{13}C HMBC experiments. The following structure of the pentasaccharide repeating unit of the polysaccharide was established, which differs from the structure reported earlier [Gorshkova, R. P. et al., *Bioorg. Khim.* **1983**, *9*, 1401–1407] in the linkage modes between the monosaccharides:

α-Tyvp
1
↓
3
→6)-α-D-Manp-(1→2)-α-D-Manp-(1→2)-β-D-Manp-(1→3)-α-D-GlcpNAc-(1 →

Yersinia pseudotuberculosis O:4a O-polysaccharide repeating unit

where Tyv stands for 3,6-dideoxy-D-*arabino*-hexose (tyvelose). The structure of the *Y. pseudotuberculosis* O:4a antigen resembles that of *Y. pseudotuberculosis* O:2c, which differs in the presence of abequose (3,6-dideoxy-D-*xylo*-hexose) in place of tyvelose only.

Strains of *Yersinia pseudotuberculosis*, a zoonotic pathogen causing acute and chronic gastrointestinal disorders in humans, are classified into 15 serovars, some of which are divided into subgroups.[1] The immunospecificity of these bacteria is determined by O-antigens, and the chemical structures of the O-antigens (O-specific polysaccharides) of a number of *Y. pseudotuberculosis* serovars have been established.[2,3] In early works, old methods were used for structural analysis of carbohydrates, which were not always reliable, and it seemed that complex polysaccharides studied >25 years ago required reinvestigation to confirm or to revise their structures. Recently, using high-resolution 2D NMR spectroscopy, we have determined the O-antigen structures of *Y. pseudotuberculosis* O:2a,[4] O:2b,[5] and O:4b[6] for the first time, and reinvestigated those of *Y. pseudotuberculosis* O:2c and O:3,[7] the O:2c structure being revised and the O:3 structure being confirmed. In this work, we reinvestigated the O-specific polysaccharide of *Y. pseudotuberculosis O:4a,* and revised the structure proposed earlier.[8]

Lipopolysaccharide was isolated from dried bacterial cells by the phenol–water procedure,[9] and degraded under mild acidic conditions (sodium acetate buffer, pH 4.5, 100 °C) to give a polysaccharide isolated by GPC on Sephadex G-50.

The ^{1}H and ^{13}C NMR (Fig. 1) spectra of the polysaccharide showed signals for anomeric atoms of five monosaccharides at δ_H 4.82–5.34 (H-1) and δ_C 98.6–103.8 (C-1), a methyl group of a 3,6-dideoxyhexose at δ_H 1.28 (H-6) and δ_C 18.1 (C-6), a methylene group of a 3,6-dideoxyhexose at δ_H 1.90, 2.07 (H-3); δ_C 34.8 (C-3), hydroxymethylene groups of Man and GalNAc at δ_C 62.0–66.3 (all C-6), and a nitrogen-bearing carbon of GalNAc at δ 50.4 (C-2). The signals for other sugar atoms were located in the regions of δ_H 3.40–4.33 and δ_C 66.6–79.7, and those for an N-acetyl group at δ_H 2.05; δ_C 23.5 (Me) and 175.3 (CO). These data are in agreement with published data,[8] which showed that the *Y. pseudotuberculosis* O:4a polysaccharide has a pentasaccharide repeating unit containing three residues of D-Man and one residue each of tyvelose (3,6-dideoxy-D-arabino-hexose, Tyv) and D-GalNAc.

The NMR spectra of the polysaccharide were fully assigned using 2D COSY, TOCSY and ^{1}H, ^{13}C HSQC experiments (Table 1). The spin systems for three mannose residues, designated as Man^{I}–Man^{III} according to their sequence in the repeating unit (see below), were identified by tracing connectivities from signals for H-1 and H-2 in the TOCSY spectrum. The spin system of Tyv was distinguished by correlations between H-1, methyl and meth-

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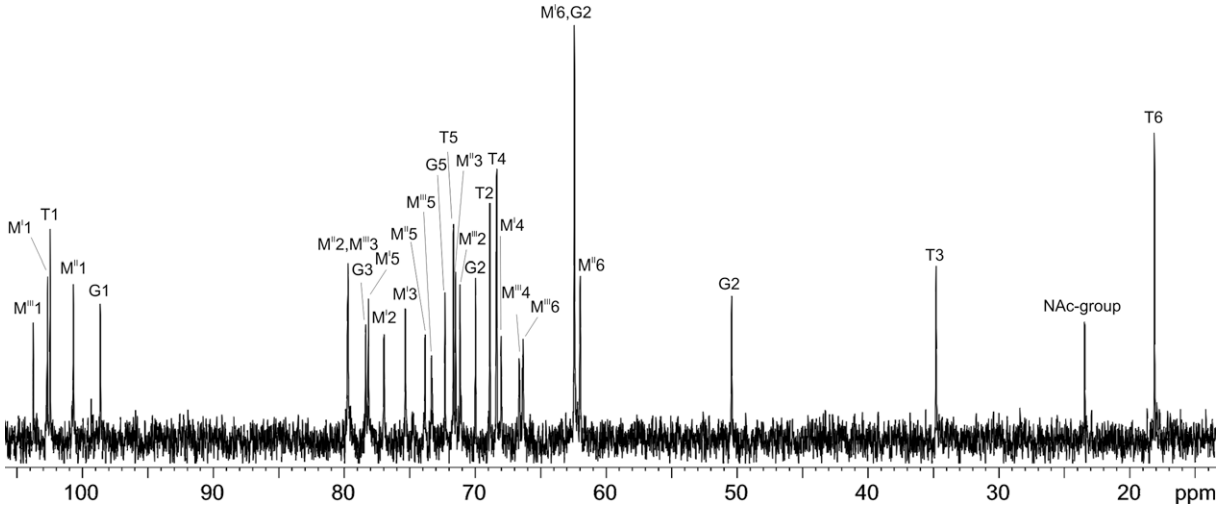

Figure 1. 125 MHz ^{13}C NMR spectrum of the O-specific polysaccharide of *Y. pseudotuberculosis* O:4a. The CO signal of NAc is not shown. M^{I}, M^{II}, and M^{III} stand for Man^{I}, Man^{II}, and Man^{III}; G and T for GalNAc and Tyv, respectively.

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ, ppm) of the O-specific polysaccharide of *Y. pseudotuberculosis* O:4a

Residue	Nucleus	**1**	2	**3** (**3a**, **b**)	**4**	**5**	**6** (**6a**, **b**)
α-Tyv*p*-(1→	^{1}H	4.90	4.06	1.90, 2.07	3.64	3.84	1.28
	^{13}C	102.4	68.9	34.8	68.3	71.6	18.1
→3)-α-D-Gal*p*NAc-(1→	^{1}H	4.93	4.33	4.05	4.19	3.99	3.76, 3.76
	^{13}C	98.6	50.4	78.3	70.0	72.3	62.4
→2)-β-D-Man*p*^{I}-(1→	^{1}H	4.82	4.00	3.72	3.64	3.40	3.74, 3.93
	^{13}C	102.7	76.9	75.3	68.3	78.1	62.4
→2)-α-D-Man*p*^{II}-(1→	^{1}H	5.34	4.08	4.04	3.81	4.01	3.81, 3.81
	^{13}C	100.7	79.7	71.5	68.0	73.8	62.0
→3,6)-α-D-Man*p*^{III}-(1→	^{1}H	5.04	4.21	3.93	4.06	3.85	3.58, 4.12
	^{13}C	103.8	71.1	79.7	66.6	73.3	66.3

Chemical shifts for NAc are δ_H 2.05; δ_C 23.5 (Me) and 175.3 (CO).

ylene groups, and the identity of the 3,6-dideoxyhexose was confirmed by characteristic $^{3}J_{H,H}$ coupling constants. GalNAc was confirmed by a correlation between proton H-2 and a nitrogen-bearing carbon C-2 at δ 3.85/55.8.

A relatively small $J_{1,2}$ value of ~3 Hz indicated that GalNAc is α-linked. The α-linkage of Tyv, Man^{II} and Man^{III} and the β-linkage of Man^{I} were inferred by comparison of their ^{1}H and ^{13}C NMR chemical shifts with those of the corresponding free monosaccharides and glycosides.[10,11] The anomeric configurations were confirmed by a 2D ROESY experiment (Fig. 2), which showed a H-1,H-2 correlation as the only intraresidue correlation for the α-linked Tyv, Man^{II}, Man^{III}, and GalNAc, whereas strong H-1,H-3 and H-1,H-5 correlations were observed for the β-linked Man^{I}.

Relatively low-field positions of the signals for C-2 of Man^{I}, C-2 of Man^{II}, C-3 of Man^{III}, C-3 of GalNAc at δ *76.9–79.7,* and C-6 of Man^{III} at δ 66.3, as compared with their positions in the spectra of the corresponding unsubstituted monosaccharides,[10] revealed the glycosylation pattern in the repeating unit with Man^{III} at the branching point and Tyv in the lateral position. The terminal position of Tyv in the side chain was confirmed by a similarity of its ^{13}C NMR chemical shifts to those of terminal α-Tyv residue in synthetic oligosaccharides[11] and the O-specific polysaccharide of O:4b.[6]

The monosaccharide sequence in the repeating unit was analyzed by the ^{1}H, ^{13}C HMBC technique (Fig. 3). The following correlations between the anomeric protons and the linkage carbons were revealed: Tyv H-1, Man^{III} H-3; Man^{III} H-1, Man^{II} H-2; Man^{II} H-1, Man^{I} H-2 and Man^{I} H-1, GalNAc H-3 at δ_H/δ_C 4.90/79.7; 5.04/79.7; 5.34/76.9; 4.82/78.3, respectively. There were no cross-peaks for GalNAc H-1 in the HMBC spectrum, but the ROESY spectrum showed a clear correlation between GalNAc H-1 and Man^{III} H-6a at δ 4.93/3.58, which is in agreement with the Man^{III} C-6 chemical shift (see above). The ROESY spectrum (Fig. 2) also confirmed the sequence of the other constituent monosaccharides.

Therefore, the O-specific polysaccharide of *Y. pseudotuberculosis* O:4a has the structure shown in Structure 1. It differs from the structure proposed earlier[8] in the linkage modes between the monosaccharides. The only basis for combining serovars O:4a and O:4b in one serogroup is evidently the occurrence of a terminal α-Tyv residue since the remainders of their O-antigen repeating units are different.[6] On the other hand, the *Y. pseudotuberculosis* O:4a antigen shares the backbone structure with the *Y. pseudotuberculosis* O:2c antigen, and the only difference between the two and, accordingly, the basis for their classification into different O-serogroups is the occurrence of either tyvelose or abequose (3,6-dideoxy-D-*xylo*-hexose) in the lateral position, respectively.

1. Experimental

1.1. Bacterial strain, isolation, and degradation of the lipopolysaccharide

Wild-type strain of *Y. pseudotuberculosis* O:4a was kindly provided by Prof. M. Skurnik (Helsinki, Finland). Cultivation of bacteria was performed at 22 °C as described.[12] The lipopolysaccharide was isolated by the Westphal procedure.[9] A lipopolysaccharide sample (80 mg) was heated at 100 °C for 2 h in 0.1 M NaOAc buffer pH 4.5,