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Note

Structure of the O-polysaccharide of *Yersinia pseudotuberculosis* O:2b

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ABSTRACT

The O-polysaccharide was isolated by hydrolysis of the lipopolysaccharide of *Yersinia pseudotuberculosis* O:2b, and studied by 1D and 2D NMR spectroscopy. The following structure of the polysaccharide was established:

α-Abe*p*
1
↓
3
→2)-α-D-Man*p*-(1→3)-α-L-Fuc*p*-(1→3)-β-D-Gal*p*NAc-(1→

where Abe stands for 3,6-dideoxy-D-*xylo*-hexose (abequose).

A zoonotic pathogen *Yersinia pseudotuberculosis* causes a broad range of acute and chronic gastrointestinal diseases in humans. Currently, strains of this bacterium are classified into 15 O-serogroups, some of which are divided into two or three subgroups (serovars).[1] Structures of the O-antigens (O-polysaccharides) of several *Y. pseudotuberculosis* serovars have been established,[2,3] but some structures require reinvestigation as old methods were used in their elucidation. Recently, we have determined the O-antigen structures of serovars O:2a[4] and O:4b,[5] and revised the structure of serovar O:2c.[6] In this work, we elucidated the O-polysaccharide structure of *Y. pseudotuberculosis* O:2b, for which only incomplete chemical data have been reported.[7]

The LPS was obtained from dried bacterial cells by the phenol–water procedure, 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 chemical[7] and genetic[8,9] data, the O-polysaccharide repeating unit contains one residue each of D-Man, L-Fuc, abequose (3,6-dideoxy-D-*xylo*-hexose, Abe) and D-GalNAc.

The ^{1}H and ^{13}C NMR spectra of the O-polysaccharide showed signals for anomeric atoms of four monosaccharides at δ_H 4.57, 5.05, 5.11 and 5.14; δ_C 100.6, 101.3, 101.7 and 102.8, methyl groups (H-6 and C-6) of Fuc and Abe at δ_H 1.22 and 1.18; δ_C 16.7 and 16.9, a methylene group (H-3 and C-3) of Abe at δ_H 1.98 and 2.07; δ_C 34.4, hydroxymethylene groups (C-6) of Man and GalNAc at δ_C 62.0 and 63.2, a nitrogen-bearing carbon (C-2) of GalNAc at δ 52.8, other sugar atoms at δ_H 3.62–4.23 and δ_C 65.0–79.8 and an *N*-acetyl group at δ_H 2.05 and δ_C 23.9 (CH_3). These data are in agreement with the suggested tetrasaccharide repeating unit of the O-polysaccharide.[6–8]

The NMR spectra of the O-polysaccharide were fully assigned using 2D ^{1}H, ^{1}H COSY, TOCSY, ROESY (Fig. 1) and ^{1}H, ^{13}C HSQC experiments (Table 1). The spin system of GalNAc was distinguished by a correlation of H-2 to a nitrogen-bearing carbon (C-2) at δ 4.08/52.8 in the ^{1}H, ^{13}C HSQC spectrum. The spin system of abequose was identified based on correlations of H-2 at δ 4.00 and of H-4 at δ 3.88 to a methylene group (H-3) at δ 1.98 and 2.07, as well as by a correlation of H-5 to a methyl group (H-6) at δ 4.23/1.18 in the COSY and TOCSY spectra. Fucose also showed a H-5, H-6 correlation at δ 4.17/1.22. The fourth spin system belonged to mannose as confirmed by a relatively small $J_{2,3}$ coupling constant (<3 Hz). The absence of ^{13}C NMR signals in a lower field than δ 80 demonstrated that all monosaccharide residues are in the pyranose form.

A relatively large $J_{1,2}$ coupling constant of 7 Hz as well as characteristic H-1, H-3 and H-1, H-5 correlations in the ROESY spectrum (Fig. 1) for GalNAc is typical of β-hexopyranosides, and indicated that this monosaccharide is β-linked. Relatively small $J_{1,2}$ values (~3 Hz) showed that all other monosaccharides are

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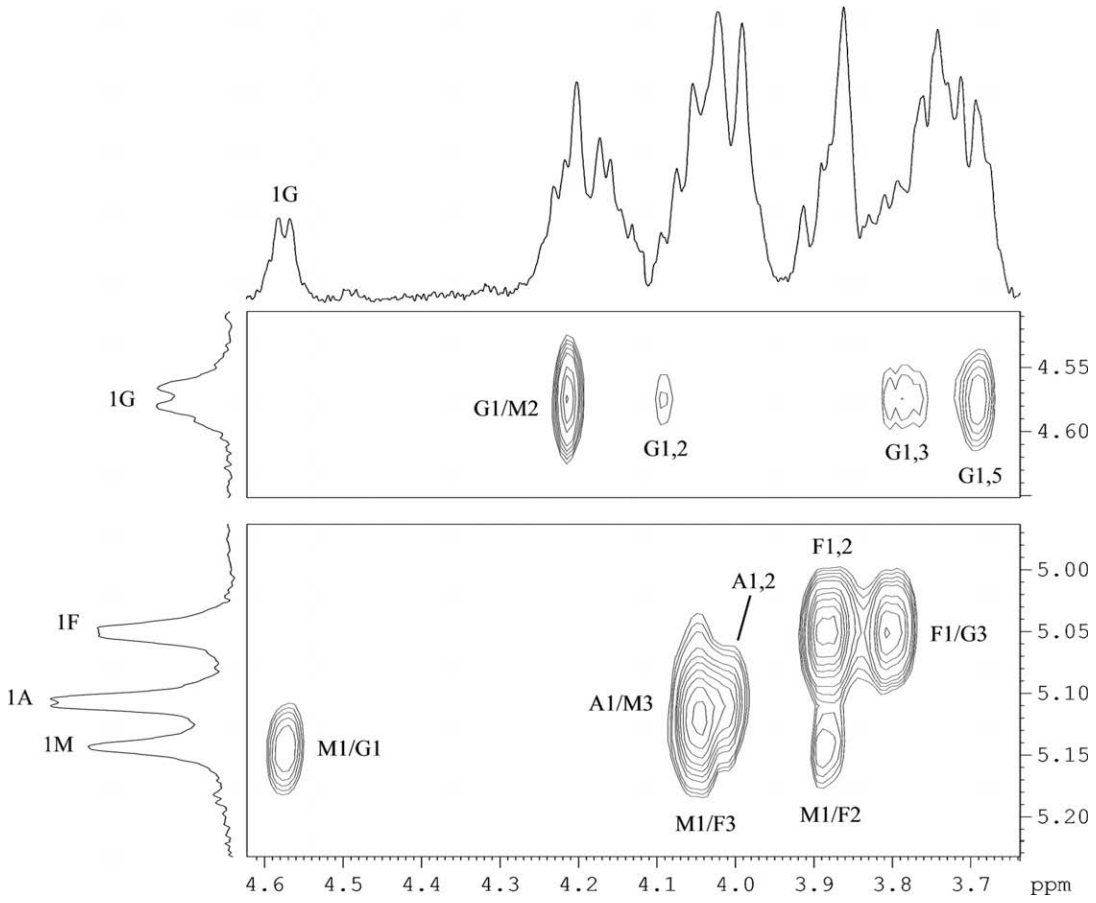

Figure 1. Parts of a 2D ^{1}H, ^{1}H ROESY spectrum of the O-polysaccharide of *Y. pseudotuberculosis* O:2b. The corresponding parts of the ^{1}H NMR spectrum are displayed along the axes. Numbers refer to protons in sugar residues denoted as follows: A, abequose; M, mannose; F, fucose; G, GalNAc.

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ, ppm) of the O-polysaccharide of *Y. pseudotuberculosis* O:2b

Sugar residue	Nucleus	Atom					
		1	2	3	4	5	6
α-Abe*p*-(1→	^{1}H	5.11	4.00	1.98, 2.07	3.88	4.23	1.18
	^{13}C	101.7	65.0	34.4	70.0	68.3	16.9
→2,3)-α-D-Man*p*-(1→	^{1}H	5.14	4.20	4.04	3.98	3.75	3.62, 3.91
	^{13}C	100.6	77.9	78.4	n/d	n/d	63.2
→3)-α-L-Fuc*p*-(1→	^{1}H	5.05	3.88	4.05	3.98	4.17	1.22
	^{13}C	102.8	69.0	77.6	73.0	68.3	16.7
→3)-β-D-Gal*p*NAc-(1→	^{1}H	4.57	4.08	3.79	4.02	3.69	3.76
	^{13}C	101.3	52.8	79.8	69.3	76.1	62.0

Chemical shifts for NAc are δ_H 2.05 and δ_C 23.9 (CH_3).

α-linked. This was confirmed by H-1, H-2 correlations in the ROESY spectrum for abequose and fucose (Fig. 1), whereas the absence of any H-1, H-2 ROESY cross-peak for mannose could be due to its coincidence with a H-1, H-2 TOCSY cross-peak having the opposite sign.

Downfield displacements of the signals for C-3 of GalNAc, C-3 of Fuc, and C-2 and C-3 of Man to δ 77.6–79.8, as compared with their positions in the spectra of the corresponding unsubstituted monosaccharides,[10] revealed the glycosylation pattern in the O-unit with Man at the branching point and Abe being the terminal residue of the side chain. The sequence of the monosaccharides in the O-units was established by ROESY (Fig. 1) based on the following inter-residue correlations between anomeric protons and protons at the linkage carbons: Abe H-1, Man H-3 at δ 5.11/4.04; Man H-1, Fuc at H-3 δ 5.14/4.05; Fuc H-1, GalNAc at H-3 δ 5.05/3.79 and GalNAc H-1, Man H-2 at δ 4.57/4.20. A 1→2-linkage between GalNAc and Man was additionally confirmed by a strong H-1, H-1 cross-peak at δ 4.57/5.14 in the ROESY spectrum (Fig. 1).

Therefore, the O-polysaccharide of *Y. pseudotuberculosis* O:2b has the structure shown in Chart 1. The O-antigens of *Y. pseudotuberculosis* serovars O:2a,[4] O:2b (this work) and O:2c[6] have the

α-Abe*p*
1
↓
3
→2)-α-D-Man*p*-(1→3)-α-L-Fuc*p*-(1→3)-β-D-Gal*p*NAc-(1→

Chart 1. Structure of the O-polysaccharide of *Y. pseudotuberculosis* O:2b.

same terminal monosaccharide of the side chain, abequose, which seems to be sufficient for combining the corresponding strains into the O:2 serogroup as the remainders of the repeating units in the three serovars are different.

1. Experimental

1.1. Bacterial strain, cultivation of bacteria, isolation and degradation of lipopolysaccharide

Wild-type *Y. pseudotuberculosis* strain L-616 (O:2) isolated in 1973 in Leningrad from a Norway Rat (*Rattus norvegicus*) came from the Leningrad Antiplague Station (St. Petersburg, Russia). The O:2b serotype was established by O-genotyping of strain L-616 as described.[11]

Cultivation of bacteria was performed at 22 °C as described.[12] The lipopolysaccharide was isolated by the Westphal procedure.[13] A portion of the lipopolysaccharide (20 mg) in 0.1 M NaOAc buffer, pH 4.5, was heated at 100 °C for 2 h, and a polysaccharide was isolated from the supernatant by GPC on Sephadex G-50 (S) as described.[4] The yield of the O-polysaccharide was 20% of the lipopolysaccharide weight.

1.2. NMR spectroscopy

An O-polysaccharide sample was deuterium-exchanged by freeze-drying twice from 99.9% D_2O, and then examined as a solution in 99.96% D_2O at 30 °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 standard Bruker software, and Bruker XWINNMR 2.6 programme 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.[14]

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