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Note

Reinvestigation of the O-specific polysaccharides of *Hafnia alvei* lipopolysaccharides isolated from strains ATCC 13337 and 1187

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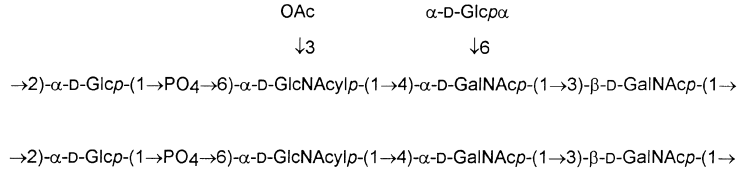
Abstract

The structure of the O-specific polysaccharides of the lipopolysaccharides produced by *Hafnia alvei* strains ATCC 13337 and 1187 was reinvestigated. The position of phosphate group in the repeating units of the polysaccharides was established with the aid of ^{1}H detected, ^{31}P edited NMR spectra. According to the results obtained, the polysaccharides are teichoic acid-like polymers with the repeating units of the following structure:

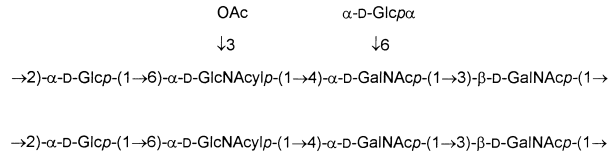


where Acyl = D-3-hydroxylbutyryl, and 3-O-acetylation was approximately 30%.

Keywords: Lipopolysaccharide; O-Specific polysaccharide; *Hafnia alvei*

1. Introduction

Basing on ^{1}H NMR spectroscopy, sugar and methylation analyses and periodate oxidation, the structure of the repeating unit of the O-specific polysaccharides of *Hafnia alvei* ATCC 13337 standard strain and 1187 strain were reported as shown [1]:



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where Acyl = D-3-hydroxylbutyryl, and 3-O-acetylation was approximately 66%.

The above studies were performed in 1989. The O-specific polysaccharides were isolated by mild acid hydrolysis of the lipopolysaccharides and purified on Bio-Gel P-4. The sugar components of the polysaccharides released after hydrolysis (0.5 M HCl or 4 M HCl, 18 h, 100 °C) were converted into alditol acetates.

The absolute configuration of sugar components was established by using enzymes: glucose oxidase, galactose oxidase and hexokinase [2]. The methylation procedure according to Hakomori [3] or to Stellner et al. [4] was used. In order to identify the sugar component acylated with 3-hydroxybutyric acid, the polysaccharides were submitted to solvolysis with anhydrous HF [5]. The alditol acetates and partially methylated alditol acetates were analyzed by gas–liquid chromatography/mass spectrometry (gas chromatograph Varian model 3700 mass spectrometer Mat-311A equipped with a fused silica OV-1-CB column (0.25 mm × 12 m)).

In recent years we have published structures of O-specific polysaccharides of *H. alvei*, in which some of these polysaccharides contain alditol phosphate showing teichoic-acid like character [6–8]. Recently the structure of the pentasaccharide repeating unit of O-specific polysaccharide of *H. alvei* 744 and PCM 1194 was established by Petersson et al. [9]. This structure is closely related to that described by us for ATCC 13337 standard strain except that it contains additionally one phosphate group per the repeating unit.

All these facts prompted us to re-examine the structure of the O-specific polysaccharides of *H. alvei* strains ATCC 13337 and 1187. First of all the determination of phosphorus [10] was carried out in the above mentioned polysaccharides. ATCC 13337 polysaccharide contains 2.5% of P and 1187 polysaccharide 3.2% of P, which means that there is one phosphate group per the repeating unit in both polysaccharides.

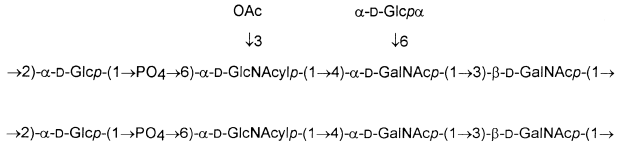
The position of phosphate group in the repeating units of the examined polysaccharides was established with the aid of NMR spectroscopy. The proton spectra of both the 1187 and ATCC 13337 polysaccharides (Fig. 1) were practically identical with those reported earlier (Fig. 3(a) and (b), and 8 in ref. [1]), except for the twice smaller content of the acetylated species of the latter in the present sample. Therefore, the assignments of all proton resonances, as well as the sequence and linkage analysis documented by NOEs, remain the same.

As for the Glc(1-6)GlcNAcyl linkage, which was deduced from indirect indications [1], it was now shown with the aid of ^{1}H detected, ^{31}P edited spectra that a phosphodiester group interpose between these two sugar residues in both polysaccharides. The spectrum in Fig. 2(a) correlates the phosphor resonance at $\delta -0.4$ with Glc H-1 and GlcNAcyl H-6 and H-6 resonances, thus establishing unambiguously the location of the phosphodiester group. A four-bond $^{31}P/^{1}H$ correlation with Glc H-2 is also visible. The expanded, resolution-enhanced Glc H-1 signal in Fig. 1(a) clearly shows a coupling with ^{31}P ($^{3}J_{H,P} = 6.7$ Hz).

Fig. 2(b) exhibits a practically identical correlation pattern for the $\delta -0.4$ phosphor resonance of the ATCC 13337 polysaccharide. However, another ^{31}P resonance, at δ 1.4, correlates with GlcNAcyl H-6 and H-6 only, thus showing that this polysaccharide is depolymerized to a considerable extent, with the phosphomonoester group being located at the end of the shorter chains. Recently we found in the same region (δ 1.2) the ^{31}P resonance for a terminal phosphomonoester group linked to C-4 of a heptose residue of the core region ATCC 13337 hexasaccharide [11].

The percentage of the 3-O-acetylated and deacetylated GlcNAcyl-III residues (30:70) was determined by integration of the well resolved HBA methyl signals (Fig. 1(b)), which differ in chemical shift due to the close proximity of the acetylation site.

The results obtained proved that ATCC 13337 and 1187 polysaccharides are teichoic acid-like polymers, of the following structure:



It is worthy of note that the dry preparation of ATCC 13337 polysaccharide showed low stability after long storage at room temperature. Its O-acetyl content decreased ×2, and the phosphodiester linkage to a substantial extent passed into phosphomonoester linked with GlcNAcyl residues only.

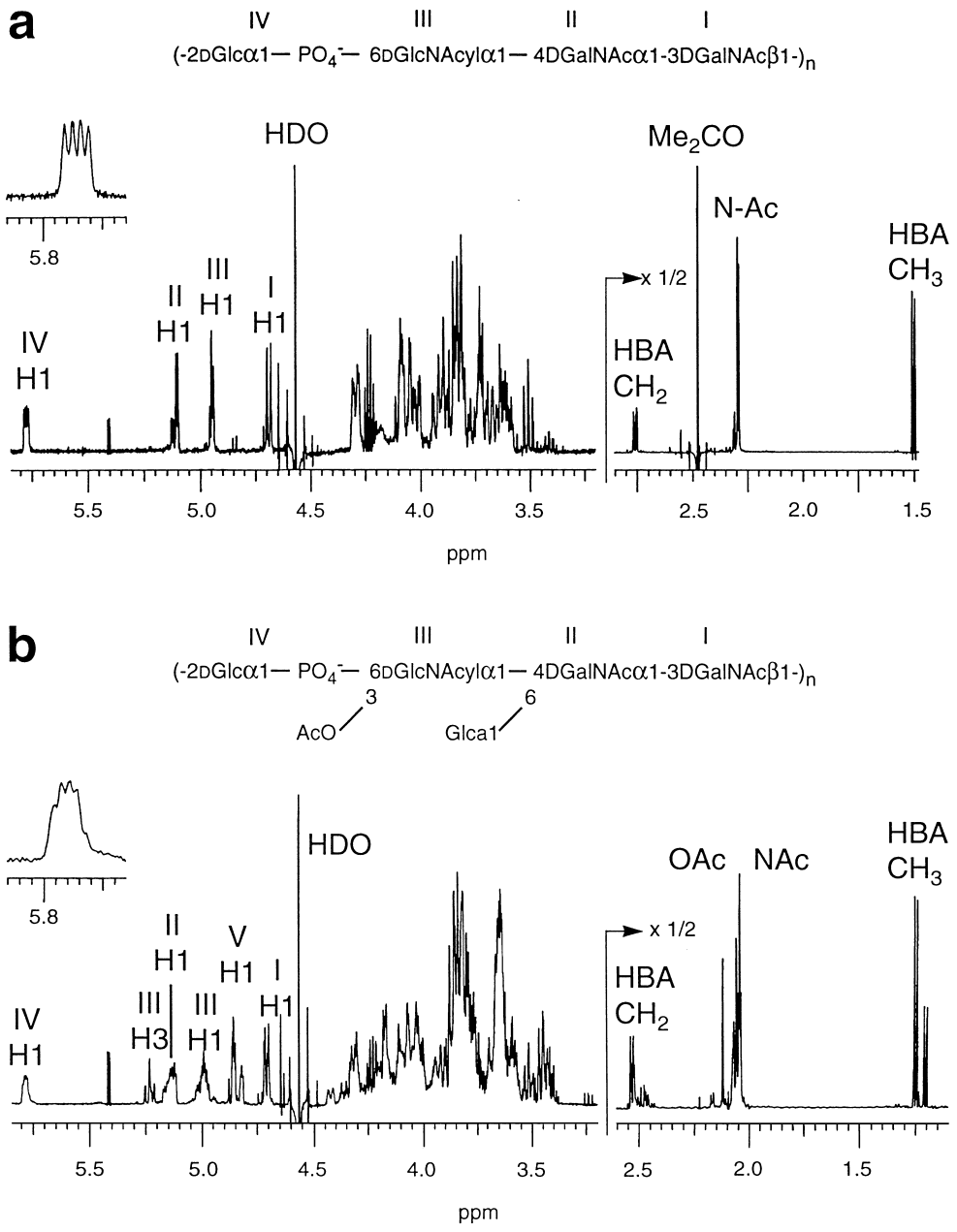


Fig. 1. The resolution-enhanced 500 MHz ^{1}H NMR spectra of the polysaccharides of (a) *H. alvei* strain 1187 and (b) *H. alvei* strain ATCC 13337. The inserts show the expansion of the Glc-IV H1 signals. Acyl = HBA = hydroxybutyric acyl residue.

2. Experimental

H. alvei standard strain ATCC 13337 and strain 1187 from the collection of Pasteur Institute (Paris) were used. Lipopolysaccharides, O-specific polysaccharides obtained after mild acid hydrolysis of lipopolysaccharides (1% acetic acid, 100 °C, 1 h) and purified on Bio-Gel P-4 column (1.6×100 cm) were prepared as described previously [12].

Phosphorus was determined according to Chen et al. [10].

NMR spectroscopy.—The samples were exchanged twice with D_2O with intermediate lyophilization, and then dissolved in 0.4 ml D_2O containing a trace of acetone, the signal of which was set at δ 2.225 as reference. The spectra were recorded at 316 K on a Bruker AM 500 spectrometer operating at 500 MHz for ^{1}H and 202 MHz for ^{31}P. Bruker standard software was applied for two-dimensional (2D)^{1}H,^{31}P inverse heterocorrelated spectra. The digital resolution in the frequency domain was 1.6 Hz and 3.9 Hz per point for ^{1}H and ^{31}P, respectively.

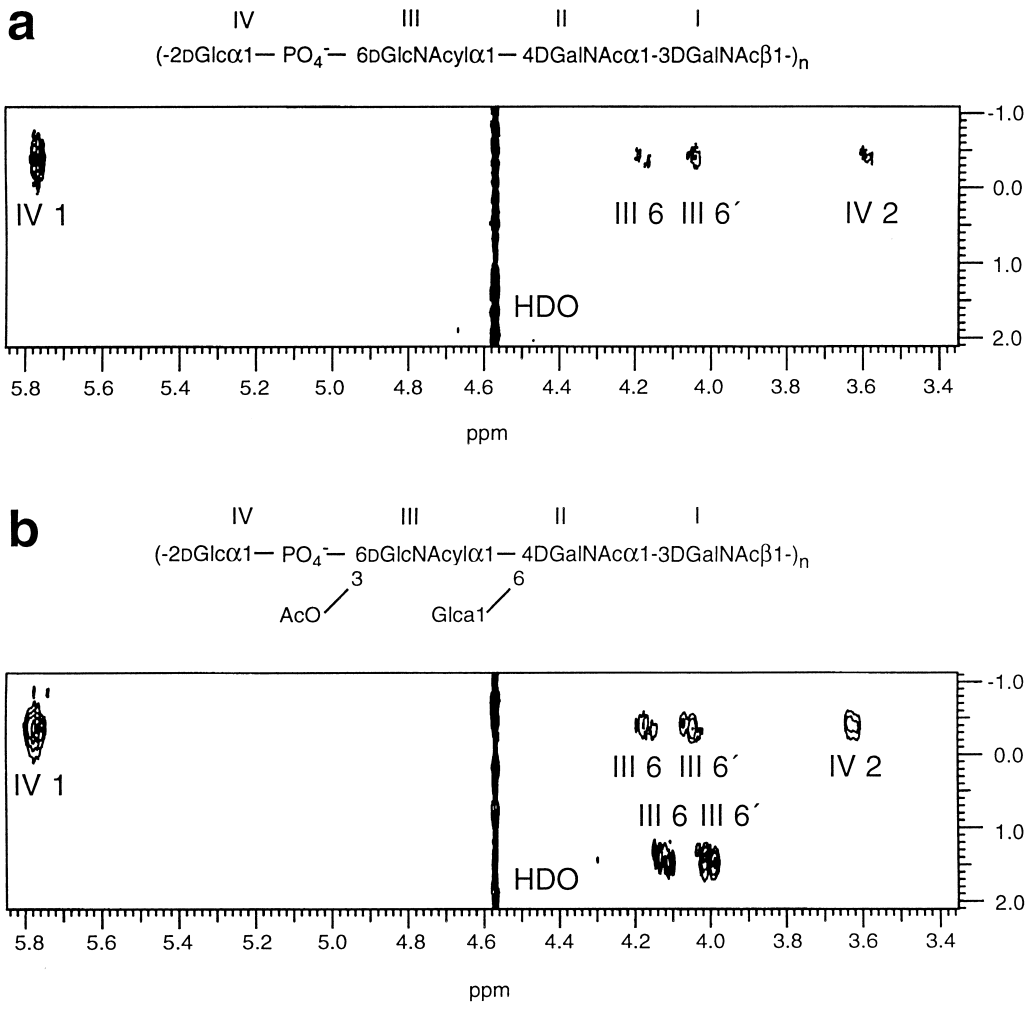


Fig. 2. The 500/202 MHz ^{1}H, ^{31}P inverse correlated spectra of the polysaccharides of (a) *H. alvei* strain 1187 and (b) *H. alvei* strain ATCC 13337. The ordinate of each correlation contour defines the chemical shift of the phosphorus atom, and the abscissa corresponds to the chemical shift of the proton coupled with this phosphorus.

References

[1] A. Gamian, E. Romanowska, H.J. Opferkuch, M. Hauck, and J. Dabrowski, *Eur. J. Biochem.*, 186 (1989) 611–620.
[2] A. Gamian, E. Romanowska, A. Romanowska, C. Ługowski, J. Dabrowski, and K. Trauner, *Eur. J. Biochem.*, 146 (1985) 641–647.
[3] S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
[4] K. Stellner, H. Saito, and S. Hakomori, *Arch. Biochem. Biophys.*, 155 (1973) 464–472.
[5] A.J. Mort, *Carbohydr. Res.*, 122 (1983) 315–321.
[6] E. Katzenellenbogen, E. Romanowska, N.A. Kocharova, Y.A. Knirel, A.S. Shashkov, and N.K. Kochetkov, *Carbohydr. Res.*, 231 (1992) 249–260.
[7] A. Gamian, E. Romanowska, U. Dabrowski, and J. Dabrowski, *Eur. J. Biochem.*, 213 (1993) 1255–1260.
[8] U. Dabrowski, J. Dabrowski, E. Katzenellenbogen, M. Bogulska, and E. Romanowska, *Carbohydr. Res.*, 287 (1996) 91–100.
[9] C. Petersson, W. Jachymek, A. Klonowska, C. Ługowski, T. Niedziela, and L. Kenne, *Eur. J. Biochem.*, 245 (1997) 668–675.
[10] P.S. Chen, T.Y. Toribara, and H. Warner, *Anal. Chem.*, 28 (1956) 1756–1758.
[11] A. Gamian, E. Katzenellenbogen, E. Romanowska, U. Dabrowski, and J. Dabrowski, *Carbohydr. Res.*, 266 (1995) 221–228.
[12] E. Romanowska, A. Romanowska, C. Ługowski, and E. Katzenellenbogen, *Eur. J. Biochem.*, 121 (1981) 119–123.