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Structural studies of the O-specific chain of *Hafnia alvei* strain 32 lipopolysaccharide

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Abstract

The structure of the O-specific side-chain of the *Hafnia alvei* strain 32 lipopolysaccharide has been investigated. Methylation analysis, partial acid hydrolysis, Smith degradations, NMR spectroscopy, MALDI-TOF and FAB mass spectrometry in combination with collision-induced decomposition MS/MS were the principal methods used. It is concluded that the polysaccharide is composed of pentasaccharide repeating units having the following structure which is partially *O*-acetylated in the 2- (20%) and 3- (50%) position of the → 4)-α-D-Gal*p*A-(1 → residue.

→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→
2,3
:
OAc

A MALDI-TOF mass spectrum of the O-specific chains indicated that they consisted of up to 16 repeating units. © 1996 Elsevier Science Ltd.

Keywords: *Hafnia alvei*; Lipopolysaccharide; O-Antigen; MALDI-TOF; MS/MS

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1. Introduction

Hafnia alvei is a typical member of Enterobacteriaceae. It is an opportunistic pathogen found in some incidences of nosocomial infections and cases of septicemia caused by these bacteria have also been reported. The serotyping scheme of *H. alvei* includes 39 O-serotypes [1]. Preliminary chemical characterization of lipopolysaccharides isolated from 33 strains of this genus has been published [2]. Recently, the structures of the O-specific polysaccharides from a number of serologically different strains of *H. alvei* have been reported [3,4].

We now report on structural studies of the O-specific polysaccharide of the *H. alvei* strain 32.

2. Results and discussion

The lipopolysaccharide (LPS) of *H. alvei*, isolated by conventional methods [5] and purified on Sepharose 2B [6], showed in SDS-PAGE analysis [7] a pattern that indicated different lengths of the O-polysaccharide chains. The O-specific polysaccharide (PS) and core oligosaccharides were liberated by mild acid hydrolysis and isolated by gel filtration on Bio-Gel P-10. The PS was eluted in four peaks which showed the same structure, according to the NMR spectra, but had different molecular mass. Three other fractions containing lower-molecular-mass components were also obtained. The first of these contained both O-antigen and core sugars. The other fractions contained a core hexasaccharide and a trisaccharide, respectively, with the same structures as those previously shown for core components in *H. alvei* lipopolysaccharides [8–10]. Sugar analysis of the PS gave L-rhamnose, D-galactose, D-galactosamine and D-glucosamine in the relative proportions 1.0:1.5:1.2:1.0, determined as their alditol acetates on GLC-MS. The absolute configurations of the sugars were determined as devised by Gerwig et al. [11,12]. The ^{13}C and the $^{1}H,^{1}H$-COSY NMR spectra indicated in addition to the four sugars the presence of a uronic acid, from a carbonyl signal and a five proton spin-system. The PS was therefore carboxyl-reduced [13] and sugar analysis of this material gave a relative proportion of 2.3 of D-galactose. The matrix-assisted laser-desorption ionisation (MALDI) mass spectrum of the O-specific PS (negative mode) (Fig. 1), using a time-of-flight (TOF) instrument, showed a series of ions up to $m/z \sim 15000$, indicating the presence of a large number of polysaccharides with different molecular weights. The difference of 919 Da between the main components indicated the size of the repeating unit. However, the molecular weight of a pentasaccharide repeating unit, consisting of 1 Rha, 1 Gal, 1 GalA, 1 GlcNAc and 1 GalNAc, is lower (890 Da). The presence of *O*-acetyl groups, on average ~ 0.7 mol per repeating unit, can explain this difference. This assumption was supported by a MALDI-TOF mass spectrum (Fig. 1) of de-*O*-acetylated PS which showed a mass difference between the main components that corresponded to 890 Da. The methylation analysis of the PS (Table 1) showed the presence of 2-substituted rhamnose, 4-substituted galactose, 3-substituted *N*-acetylgalactosamine and 4-substituted *N*-acetylglucosamine. When the PS was methylated and then reduced with "Superdeuteride" ($LiB(C_2H_5)_3D$) [14] the analysis gave also 2,3-di-*O*-methyl-galactose-*6*-d_2 (Table 1).

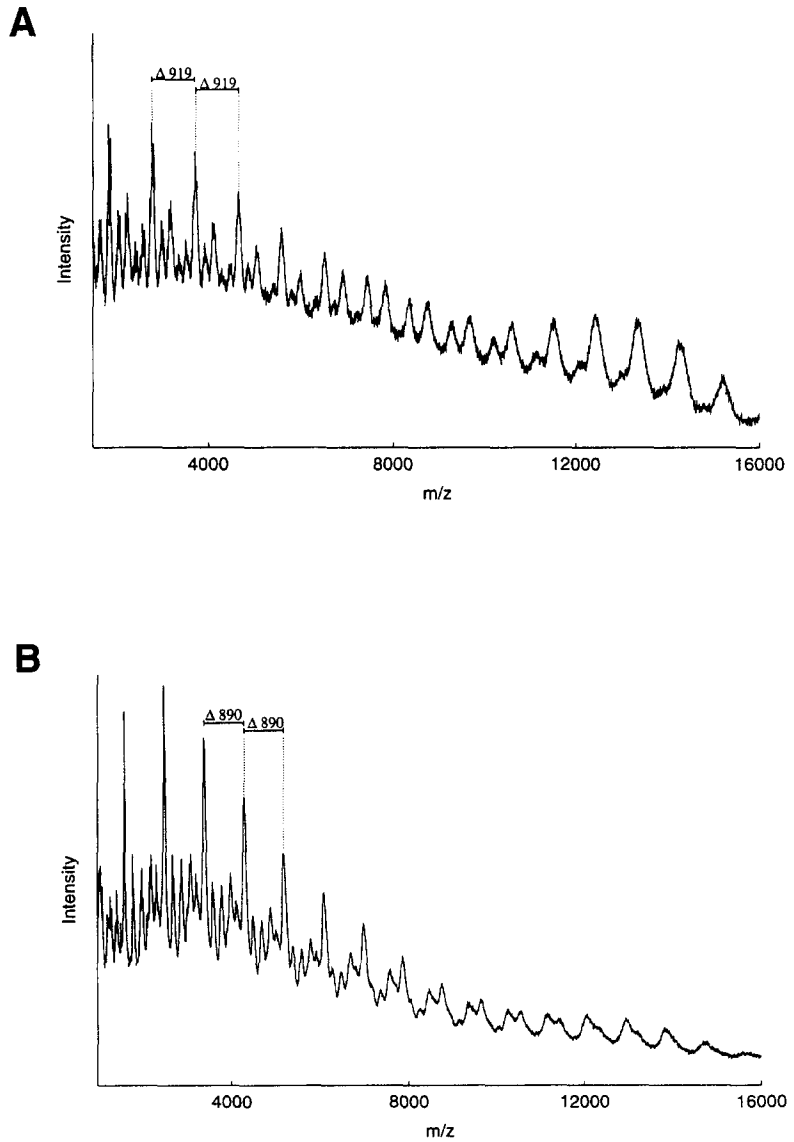


Fig. 1. MALDI-TOF mass spectra of (A) native and (B) de-*O*-acetylated polysaccharides.

The ^{1}H (Fig. 2), ^{13}C and HMQC NMR spectra of the de-*O*-acetylated PS contained signals for anomeric atoms from five sugar residues at δ_H/δ_C 5.20/100.4, $J_{H\text{-}1,H\text{-}2} < 2$ Hz (residue **I**); δ_H/δ_C 5.06/98.3, $J_{H\text{-}1,H\text{-}2}$ 3 Hz (**II**); δ_H/δ_C 4.93/99.1, $J_{H\text{-}1,H\text{-}2}$ 3 Hz (**III**); δ_H/δ_C 4.61/102.1, $J_{H\text{-}1,H\text{-}2}$ 8.2 Hz (**IV**); and δ_H/δ_C 4.48/105.8, $J_{H\text{-}1,H\text{-}2}$ 7.5 Hz (**V**), supporting a pentasaccharide repeating unit. The $^{1}J_{C,H}$-values obtained from HMQC experiments showed that three sugars had the a-configuration ($^{1}J_{C,H}$ 173, 174 and 172 Hz for residues **I**, **II** and **III**, respectively) and two the b-configuration ($^{1}J_{C,H}$ 158 and

Table 1
Methylation analysis of O-specific polysaccharide from *Hafnia alvei 32* before and after chemical modifications

Methylated sugars (as alditol acetates)	t_R [a]	Molar ratio		
		A [b]	B	C
3,4-Me_2Rha [c]	0.90	1.00	1.00	– [d]
2,3,6-Me_3Gal	1.22	1.50	0.95	–
2,3-Me_2Gal-6-d_2 [e]	1.47	–	0.80	–
3,4,6-Me_3GalNAc	1.74	–	–	1.00
3,6-Me_2GlcNAc	1.82	1.00	0.85	0.65
4,6-Me_2GalNAc	1.93	1.17	1.05	–

[a] t_R = Retention time for the corresponding alditol acetate relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (t_R = 1.00) on an HP-1 glass capillary column at 150–270 °C, 8 °C/min.
[b] **A**, original; **B**, methylated and ester reduced; **C**, Smith-degraded polysaccharide.
[c] 3,4-Me_2Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol, etc.
[d] A dash (–) indicates that the component is not present.
[e] 6,6-dideutero-2,3-di-*O*-methyl-D-galactitol.

162 Hz for residues **IV** and **V**, respectively). As the ^{1}H NMR spectrum was complex the assignments of the major signals and spin-systems were made by 2D COSY and TOCSY experiments. By these techniques five spin-systems, starting with the signals for the anomeric protons, could be determined. From the assigned proton signals and using the one-bond C-H-connectivities observed in the HMQC spectrum, the carbon signals could be assigned. By comparison of the ^{1}H and ^{13}C chemical shifts (Table 2) with earlier published NMR data for the respective monosaccharides [15] and taking the $^3J_{H,H}$-values for the coupling between the ring protons into consideration, the type of

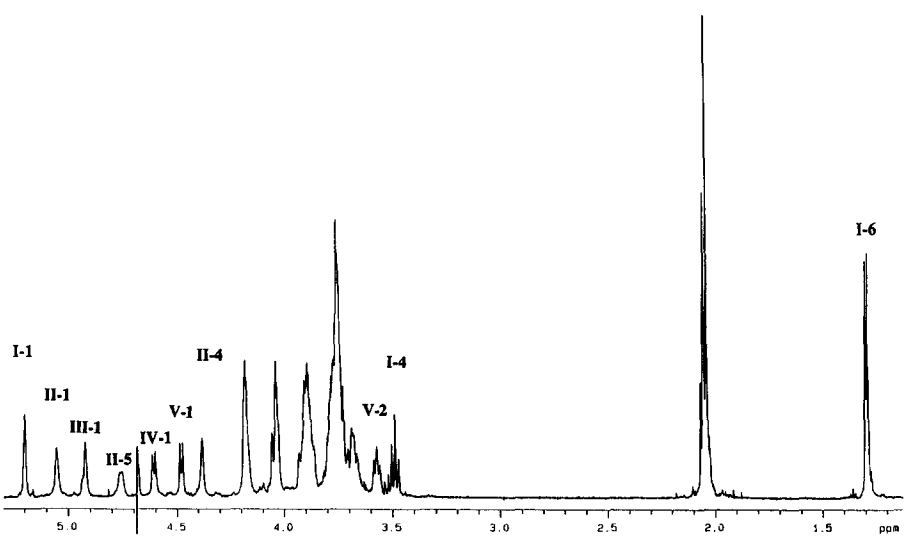


Fig. 2. The 600-MHz ^{1}H NMR spectrum of de-*O*-acetylated polysaccharide.

Table 2
^{1}H and ^{13}C NMR chemical shifts of the de-*O*-acetylated and native *H. alvei* strain 32 O-specific polysaccharide

Residue		H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-6	H-6′
I	→2)-α-L-Rha*p*-(1→	5.20	4.19	3.90	3.49	3.73	1.28	
		100.4	76.8	70.1	72.2	70.4	17.4	
II	→4)-α-D-Gal*p*A-(1→	5.06	3.88	4.05	4.38	4.76		
		98.3	68.5	69.3	79.9	71.6	175.3	
III	→4)-α-D-Glc*p*NAc-(1→	4.93	3.91	3.88	3.69	4.17	3.76	3.67
		99.1	53.8	70.22	79.7	71.2	60.7	
IV	→3)-β-D-Gal*p*NAc-(1→	4.61	4.04	3.92	4.18	3.75	≈ 3.76 [a]	≈ 3.73 [a]
		102.1	52.2	80.7	68.5	75.5	61.7 [b]	
V	→4)-β-D-Gal*p*-(1→	4.48	3.58	3.78	4.03	3.74	≈ 3.79 [a]	≈ 3.76 [a]
		105.8	71.0	73.8	77.4	75.5	62.2 [b]	
→4)-2-*O*-Ac-α-D-Gal*p*A-(1→ [c]		5.34	4.88	4.29	4.45	– [d]		
→4)-3-*O*-Ac-α-D-Gal*p*A-(1→ [c]		5.12	4.14	5.25	4.60	– [d]		

[a] These values, obtained from the HMQC spectrum, are approximative only.
[b] These assignments could be reversed.
[c] These residues are from the native PS.
[d] No cross-peaks between the signals of H-4 and H-5 were observed in the COSY and TOCSY spectra.

sugar, anomeric configuration and linkage position could be determined. Residue **I** was recognised as a 2-substituted α-L-Rha*p* residue due to a signal for a CH_3 group, the small coupling constants for the coupling between H-1, H-2 and H-3 and the high ^{13}C chemical shift of the C-2 signal (δ 76.8). Residue **II** was assigned as a →4)-α-D-Gal*p*A-(1→ residue, as this spin-system consisted of only five protons with a high chemical shift of the H-5 (δ 4.76) signal, small coupling constants for the couplings between H-3, H-4 and H-5 and a high ^{13}C chemical shift of the C-4 signal (δ 79.9). The broad signal for H-5 at δ 4.76 in the ^{1}H NMR spectrum (Fig. 2) changed to a narrow signal upon change of the pD to > 7. Residue **III**, with the anomeric proton signal at δ 4.93, was recognised as a →4)-α-D-Glc*p*NAc-(1→ residue based on a low ^{13}C chemical shift of the C-2 signal (δ 53.8), strong coupling between the ring protons and a high ^{13}C chemical shift of the C-4 signal (δ 79.7). Residue **IV** was assigned as a →3)-β-D-Gal*p*NAc-(1→ residue from a low ^{13}C chemical shift of the C-2 signal (δ 52.2), the large $J_{H\text{-}1,\ H\text{-}2}$ (8.2 Hz), small coupling constants for the coupling between H-3, H-4 and H-5 and a high ^{13}C chemical shift of the C-3 signal (δ 80.7). Residue **V** was determined as a →4)-β-D-Gal*p*-(1→ residue due to small coupling constants for the coupling between the 3-, 4- and 5-protons and a high ^{13}C chemical shift of the C-4 signal (δ 77.4).

The positions of the *O*-acetyl groups could be obtained by comparison of the ^{1}H NMR data (Table 2) for native and de-*O*-acetylated polysaccharide. This showed that the *O*-acetyl groups were located in the 2- and 3-position of the →4)-α-D-Gal*p*A-(1→ residue. The shifts of the signals, induced by the *O*-acetyl groups, were in accordance with published data for *O*-acetylated monosaccharides [16]. According to the intensities

of the three signals for the anomeric protons of the →4)-α-D-Glc*p*A-(1→ residue it was acetylated in the 2- and 3-position to approximately 20 and 50%, respectively.

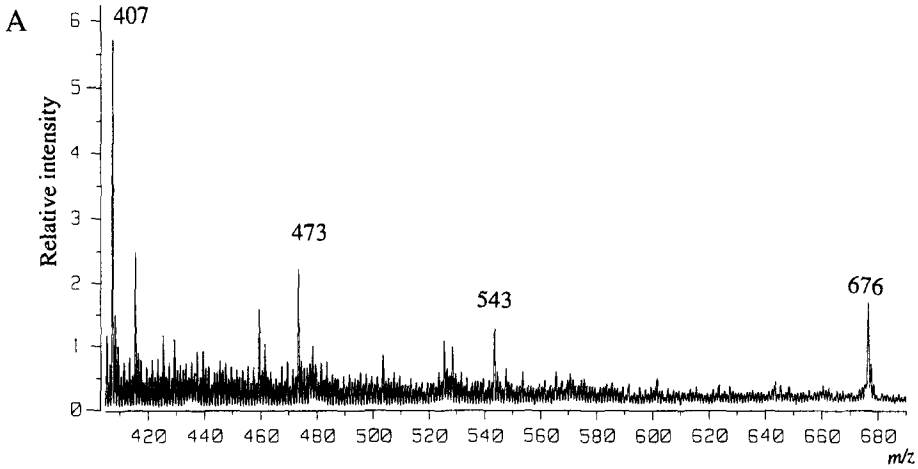
In order to obtain information on the sequence of the sugars in the repeating unit the de-*O*-acetylated PS was subjected to a Smith degradation [17] and the products were separated on Bio-Gel P-2. The main product was eluted in the disaccharide region and sugar analysis of this material showed the presence of glucosamine, galactosamine and threonic acid. Threonic acid was identified in the sugar analysis as the threitol-*1,1*-d_2 tetraacetate which was obtained by reduction with $NaBD_4$ of the threonic acid lactone formed during the hydrolysis with 2 M CF_3CO_2H at 120 °C for 2 h. Methylation analysis of the oligosaccharide, obtained by the Smith degradation, gave terminal galactosamine and 4-linked glucosamine in the molar ratio 1.0:0.6 (Table 1). FAB ionization produced an $[M + H]^+$ ion at *m/z* 543 and the high-energy collision-induced decomposition mass spectrum of this ion showed B_i ions (Domon and Costello nomenclature [18]) at *m/z* 407 and 204. This corresponds to the loss of threonic acid and of HexNAc-threonic acid, respectively. The structure of the oligosaccharide was further analysed by ^1H NMR spectroscopy which showed signals for threonic acid in addition to those from the two hexosamine units. Inter-residue NOEs, observed as increased signal intensities in NOE difference spectra, were found between H-1 of β-D-Gal*p*NAc-(1→ and H-4 of →4)-α-D-Glc*p*NAc-(1→ (δ 4.42/3.72) and between H-1 of →4)-α-D-Glc*p*NAc-(1→ and H-3 of threonic acid (δ 4.98/4.15). These results together with data from the methylation analysis and NMR spectroscopy of the de-*O*-acetylated PS suggest the following sequence as part of the repeating unit:

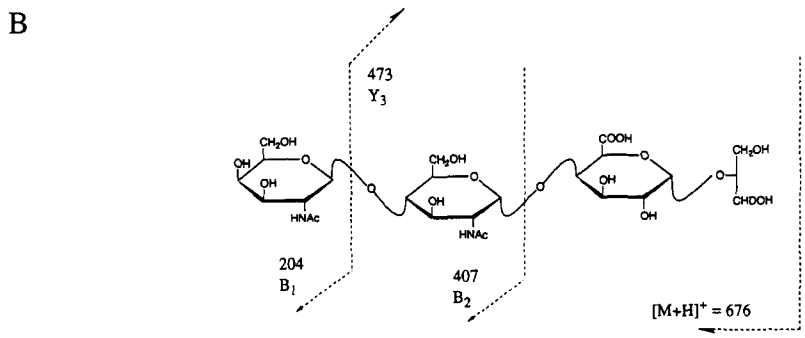
→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*A-(1→

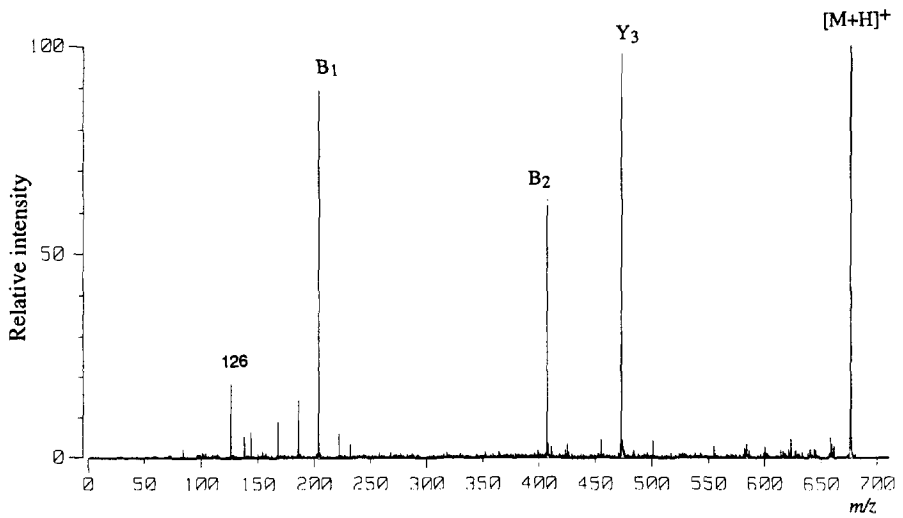
As the →4)-α-D-Gal*p*A-(1→ residue in the native PS is mainly substituted with *O*-acetyl groups this residue would resist the periodate oxidation and native PS should give the trisaccharide linked to either a glycerol or an erythritol. Thus native PS was subjected to a Smith degradation and the obtained oligosaccharides were analysed by FABMS which showed that two oligosaccharides had been formed (Fig. 3A). The high-energy collision-induced decomposition MS/MS spectrum (Fig. 3B) showed that the trisaccharide was linked to a glycerol group and consequently demonstrates the following sequence as part of the PS:

→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→
2,3
:
OAc

Fig. 3. A) FAB mass spectrum of the oligosaccharides obtained by Smith degradation of native polysaccharide. The oligosaccharide giving the $[M+H]^+$ ion at *m/z* 543 is formed when the →4)-α-D-Gal*p*A-(1→ residue is non-acetylated and that giving the ion at *m/z* 676 when it is *O*-acetylated. B) Collision-induced decomposition fragment ions observed in an MS/MS experiment using the $[M+H]^+$ ion at *m/z* 676, obtained in positive FABMS.


A
407
473
543
676
Relative intensity
m/z


B
473
Y3
204
B1
407
B2
[M+H]+ = 676


[M+H]+
Y3
B1
B2
126
Relative intensity
m/z

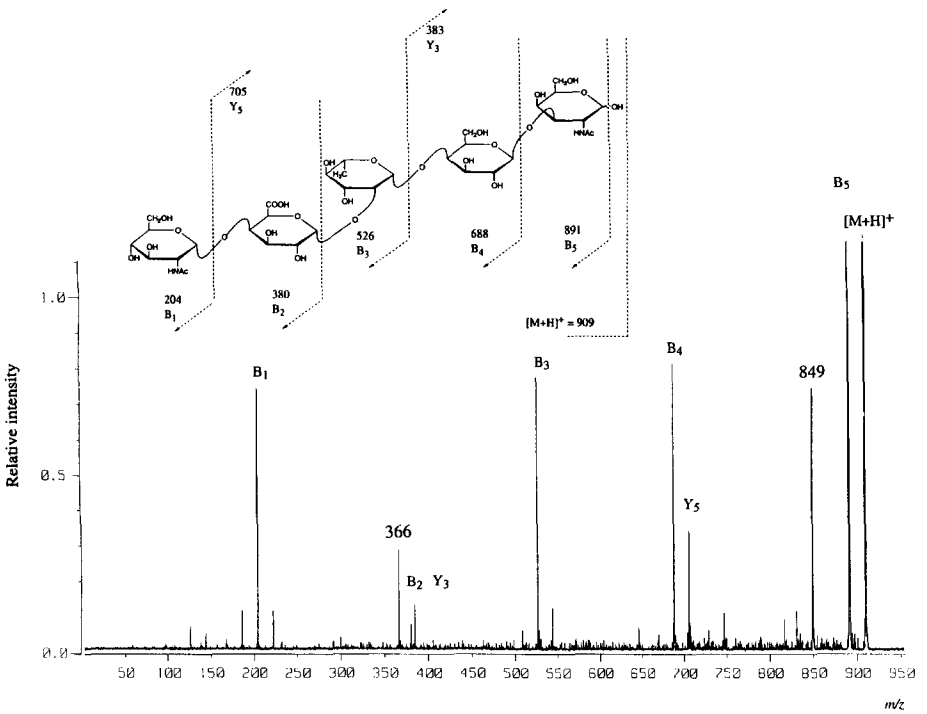


Fig. 4. Collision-induced decomposition fragment ions observed in an MS/MS experiment using the $[M+H]^+$ ion at m/z 909, obtained in positive FABMS. The pentasaccharide was obtained by partial acid hydrolysis of de-*O*-acetylated polysaccharide.

To obtain other oligosaccharides which could give additional sequence information the de-*O*-acetylated PS was subjected to partial acid hydrolysis. Several different conditions were tried on small amounts of PS and the results were checked by MALDI-TOF mass spectrometry. The hydrolysis giving the highest amount of one repeating unit ($[M + Na]^+$ m/z 931), i.e. 1 M CF_3CO_2H for 100 min at 80 °C, was repeated with a larger amount of material. FAB ionisation of the oligosaccharide, corresponding to one repeating unit, produced the $[M + H]^+$ ion at m/z 909 and the high-energy collision-induced decomposition mass spectrum of this ion (Fig. 4) showed the characteristic B type fragments giving ions at m/z 204, B_1; m/z 380, B_2; m/z 526, B_3; m/z 688, B_4; and m/z 891, B_5. To determine the sugar residue at the reducing end, the oligosaccharide was first treated with $NaBH_4$, then hydrolysed and the products were acetylated and analysed by GLC-MS. The alditol acetate of GalNAc was almost exclusively found in the analysis. These results in combination with results from the previous experiments showed that the following pentasaccharide was obtained by partial acid hydrolysis of the PS:

α-D-Glc*p*NAc-(1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-β-D-Gal*p*-(1 → 3)-D-GalNAc

Table 3
The significant NOEs for the anomeric protons of the sugar residues of the de-*O*-acetylated O-specific polysaccharide of *Hafnia alvei* strain 32 lipopolysaccharide

Anomeric proton			Observed NOE		
Residue		δ_H	δ_H	Residue, atom	
I	→ 2)-α-L-Rha*p*-(1 →	5.20	4.19	I	H-2
			4.03	V	H-4
II	→ 4)-α-D-Gal*p*A-(1 →	5.06	3.88	II	H-2
			4.19	I	H-2
III	→ 4)-α-D-Glc*p*NAc-(1 →	4.93	3.91	III	H-2
			4.38	II	H-4
IV	→ 3)-β-D-Gal*p*NAc-(1 →	4.61	3.92	IV	H-3
			3.75	IV	H-5
			3.69	III	H-4
V	→ 4)-β-D-Gal*p*-(1 →	4.48	3.78	V	H-3
			3.74	V	H-5
			3.92	IV	H-3

The combined results thus suggest the following structure for the repeating unit of the *H. alvei* 32 O-specific polysaccharide:

II **I** **V** **IV** **III**

→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→
2,3
:
OAc

This structure was confirmed by NOESY experiments on the de-*O*-acetylated PS showing the connectivities between the sugar residues. Inter-residue NOEs (Table 3), observed as cross-peaks in the NOESY spectrum, were found between H-1 of **I** and H-4 of **V** (δ 5.20/4.03), H-1 of **V** and H-3 of **IV** (δ 4.48/3.92), H-1 of **IV** and H-4 of **III** (δ 4.61/3.69), H-1 of **III** and H-4 of **II** (δ 4.93/4.38), H-1 of **II** and H-2 of **I** (δ 5.06/4.19).

From the MALDI-TOF spectra of the native and the de-*O*-acetylated PS it is evident that the O-specific chains, released by the 1% acetic acid treatment, consisted of up to 16 repeating units.

3. Experimental

General methods.—GLC-MS was carried out with a Hewlett-Packard 5971A system using an HP-1 glass capillary column (0.2 mm × 12 m) and a temperature program of 150 → 270 °C at 8 °C/min. Gel permeation chromatography was performed on columns (100 × 1.6 cm) of Bio-Gel P-10, Bio-Gel P-2 and Sephadex G-15 equilibrated with a 0.05 M pyridine-HOAc buffer (pH 5.6). Eluates were monitored with a Knauer

differential refractometer and all fractions were checked by ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry.

Mass spectrometry.—MALDI-TOF mass spectrometry was performed in both positive and negative mode on an LDI-1700XS time-of-flight instrument with 8–10 μJ energy of the laser beam and 2,5-dihydroxybenzoic acid as matrix. FABMS spectra were recorded on a JEOL JMS-SX/SX-102A four sectors tandem mass spectrometer by bombardment of samples (dissolved in a glycerol matrix) with Xe atoms of average translational energy of 6 keV. The mass spectrometer was operated at an accelerating voltage of 10 kV. Tandem mass spectrometry was conducted using the first two sectors (B_1E_1) to select the precursor ions and the second mass spectrometer (B_2E_2) to analyse the product ions. A resolution of 3000 was used to separate the ^{12}C peak of the $[M + H]^+$ precursor ion. Helium was used as the collision gas at a pressure sufficient to attenuate the precursor ion by approximately 50%. All samples were treated with a Dowex-50 (H^+) ion exchanger to remove sodium ions prior to analysis.

NMR spectroscopy.—NMR spectra were obtained for D_2O solutions at 35 °C on Varian Unity 600 and Varian VXR-400 spectrometers using sodium 3-trimethylsilylpropionate-d_4 (δ_H 0.00) and acetone (δ_C 31.00) as internal references. The signals were assigned by one- and two-dimensional experiments (dq-COSY, TOCSY, NOESY and HMQC). The mixing times used in the NOESY experiments were 200 ms and in the TOCSY experiments 30, 60 and 90 ms. The HMQC experiments were performed both with and without carbon decoupling.

Preparation of LPS, PS and core oligosaccharides.—*H. alvei* strain 32 bacteria were obtained from the collection of the Institute of Immunology and Experimental Therapy (Wroclaw). LPS was prepared by phenol-water extraction of bacterial cells and purified by column chromatography on Sepharose 2B [6]. LPS was analysed by SDS-PAGE [7] and the LPS bands were detected by silver staining [19]. LPS was degraded by mild acid hydrolysis using 1% HOAc at 100 °C for 30–45 min, then the solution was cooled and centrifuged. The supernatant was fractionated on Bio-Gel P-10. All fractions were collected separately, freeze-dried and analysed by ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry.

Sugar and methylation analyses.—A solution of the sample ($\sim$ 0.5 mg) in 2 M aq CF_3CO_2H (1.0 mL) was kept in a closed vial at 120 °C for 2 h. The sugars in the hydrolysate were then converted into alditol acetates by conventional methods. The absolute configurations of the sugars were determined as described by Gerwig et al. [11,12] using (+)-2-butanol for glycosylation.

Methylations were performed according to the method of Hakomori [20]. Products were recovered by reversed phase chromatography on Sep-Pak C_{18} cartridges [21]. The permethylated material was hydrolysed with 2 M aq CF_3CO_2H at 120 °C for 2 h. The partially methylated sugars in the hydrolysate were then converted into alditol acetates by conventional methods. Reduction of ester groups with "Superdeuteride" [$LiB(C_2H_5)_3D$] after methylation of the polysaccharide was carried out according to Bhat et al. [14].

De-O-acetylation of PS.—PS (10 mg) was treated with aq 12% NH_3 (2 mL) at room temperature for 16 h whereafter the solution was freeze-dried. The product was analysed by ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry.

Carboxyl reduction of PS.—PS (10 mg) was dissolved in water (1.5 mL) and treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (50 mg) at pH 4.75 and then with $NaBH_4$ (60 mg) at 37 °C for 16 h according to the method of Taylor et al. [13]. The excess of $NaBH_4$ was destroyed by adding aq 50% HOAc and the sample was dialysed against distilled water and freeze-dried (8 mg).

Smith degradation of native and de-O-acetylated PS.—De-*O*-acetylated PS (20 mg) was oxidized with 0.1 M $NaIO_4$ (pH 5.0, 2 mL) at 4 °C for 3 days in darkness. The oxidised sample was reduced with $NaBH_4$ (50 mg) at room temperature whereafter the reaction mixture was acidified (pH 6.0) by adding aq 50% HOAc. Reduced polymer was purified on a column of Bio-Gel P-2 and freeze-dried. The product was degraded by mild acid hydrolysis using 2% HOAc at 100 °C for 1.5 h and the products were purified on a column of Bio-Gel P-2 yielding an oligosaccharide product (8 mg) after freeze-drying. Native PS (10 mg) was treated with 0.05 M $NaIO_4$ in 0.1 M NaOAc buffer (pH 3.9) at 4 °C for 48 h. Excess of ethyleneglycol was added, the oxidised product purified on a column of Sephadex G-15, freeze-dried and then reduced with $NaBH_4$. The reaction mixture was acidified (pH 6.0) by adding aq 50% HOAc. Reduced product was degraded by treatment with 0.5 M CF_3CO_2H at room temperature for 40 h and the product purified on a column of Sephadex G-15 yielding an oligosaccharide product (3 mg) after freeze-drying. The oligosaccharide was reduced with $NaBD_4$ and the reaction mixture was deionised with Dowex-50 (H^+) and the boric acid removed by codistillation with MeOH.

Partial acid hydrolysis.—Small amounts (0.5 mg) of de-*O*-acetylated PS were used for hydrolysis using different concentrations of CF_3CO_2H (1 mL) at 80 °C. A sample (20 μL) was taken every 20 min and the progress of hydrolysis was checked by MALDI-TOF mass spectrometry. The hydrolysis conditions giving the highest proportion of one repeating unit ($[M + Na]^+$ m/z 931), i.e. 1 M aq CF_3CO_2H (1 mL) at 80 °C for 100 min, were used for a larger amount of PS (3 mg). This solution was freeze-dried after the reaction was completed. The product was analysed by FAB in combination with collision-induced decomposition MS/MS and part of the material was reduced with $NaBH_4$, hydrolysed with 2 M CF_3CO_2H and acetylated. The formed alditol acetates were analysed by GLC-MS.

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