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Note

Structure of the O-specific polysaccharide chain of the lipopolysaccharide of *Bordetella hinzii*

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Abstract

The lipopolysaccharide of *Bordetella hinzii* was analyzed after various chemical degradations by NMR spectroscopy and MALDI mass spectrometry, and the following structure of the polysaccharide chain was determined:

4-*O*-Me-α-Gal*p*NAc3NAcAN-(1→[→4)-β-Glc*p*NAc3NAcAN-(1→4)-β-Glc*p*NAc3NAcAN-(1→4)-α-Gal*p*NAc3NAcAN-(1-]$_n$-

where GlcNAc3NAcAN and GalNAc3NAcAN stand for 2,3-diacetamido-2,3-dideoxy-glucuronamide and -galacturonamide, respectively. The polysaccharide chain is terminated with a 4-O-methylated GalNAc3NAcAN residue and is rather short ($n \leq 5$).

Keywords: Lipopolysaccharide; Polysaccharide structure; *Bordetella hinzii*

A gram-negative bacterium, *Bordetella hinzii*, was found recently in patients with cystic fibrosis and bacteremia, and in poultry.[1–3] Unlike its relative *Bordetella pertussis*, *B. hinzii* produces a smooth-type lipopolysaccharide (LPS) that has an O-specific polysaccharide chain. Here we present results of structural analysis of the polysaccharide chain of the LPS of *B. hinzi*.

The LPS was isolated from bacterial cells by the phenol–water procedure.[4] Mild-acid hydrolysis of the LPS resulted in a polysaccharide connected to the LPS core.

The ^{1}H and ^{13}C NMR spectra showed that the polysaccharide contains four different *N*-acetylated amino sugar residues and an *O*-methyl group (δ_H 3.42, δ_C 62.8). The sugar residues were designated as **A**–**D** in the order of decreasing H-1 chemical shifts, and the spectra were assigned using 2D COSY, TOCSY, NOESY, HMQC, HSQC-TOCSY, and gHMBC experiments (Table 1). Correlation in the ^{1}H,^{13}C HMQC spectrum of the H-2 and H-3 protons to nitrogen-bearing carbons at δ 48.0–55.4 (Fig. 1, right) defined the positions of the amino groups. In the ^{1}H,^{13}C gHMBC spectrum, each H-5 proton showed a correlation to a CO group at δ 171.8–174.5, and, hence, all constituent sugars are 2,3-diacetamido-2,3-dideoxyhexuronic acids. Based on the vicinal proton coupling constants, it was concluded that residues **A** and **B** have the β-*gluco* configuration and residues **C** and **D** the α-*galacto* configuration.

The (1→4) linkages between the residues **A** and **C**; **C** and **D**; **D** and **B**; and **B** and **C** were confirmed by a NOESY experiment, which showed the corresponding interresidue H-1,H-4 correlations, and by a gHMBC experiment (Fig. 1, left). A correlation between the OMe group at δ 3.42 and H-4 of residue **A** in the NOESY spectrum and C-4 of residue **A** in the gHMBC spectrum (Fig. 1, left) demonstrated 4-*O*-methylation of this residue, which evidently terminates the polysaccharide chain.

NMR chemical shifts of the polysaccharide showed no pD dependence, thus indicating amidation of the carboxyl groups. This conclusion was confirmed by the failure to methylate the carboxyl groups by treatment of the polysaccharide with 1 M HCl in methanol for 24 h at 25 °C (^{1}H NMR data). Another item of evidence for the amidation is the readiness of β elimination in the uronic acids upon treatment of the LPS with aq

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Table 1
^{1}H and ^{13}C NMR data of the polysaccharide of *B. hinzii* obtained by mild-acid degradation of the LPS (δ, ppm)

Sugar residue	Nucleus	1	2	3	4	5	6	OMe
A	^{1}H	5.06	4.13	4.21	3.94	4.23		3.42
	^{13}C	98.7	48.0	49.0	78.7	71.6	174.5	62.8
B	^{1}H	5.04	4.10	4.17	4.33	4.22		
	^{13}C	98.6	48.0	48.1	75.4	71.6	173.6	
C	^{1}H	4.55	3.62	4.09	4.02	3.96		
	^{13}C	103.0	55.4	54.2	76.4	76.2	172.1	
D	^{1}H	4.48	3.81	3.95	3.89	3.91		
	^{13}C	103.2	53.9	53.6	76.1	78.1	171.8	

Signals for NAc are at $\delta_H \sim 1.9$, $\delta_C \sim 23.1$ (Me) and ~ 175.5 (CO).

12% ammonia for 24 h at 50 °C, which resulted in almost complete degradation of the polysaccharide chain to give 4,5-unsaturated acids. Interestingly, upon milder treatment at 30 °C, the polysaccharide was essentially stable but split off from the LPS core. Nitrous acid deamination also cleaved the linkage between the polysaccharide chain and the core, because of the presence in the latter of a number of amino sugars having the amino group free,[5] which may link the polysaccharide chain to the rest of the LPS. After purification by GPC on Sephadex G-50 and reversed-phase HPLC, the polysaccharides obtained by various methods showed similar ^{1}H NMR spectra.

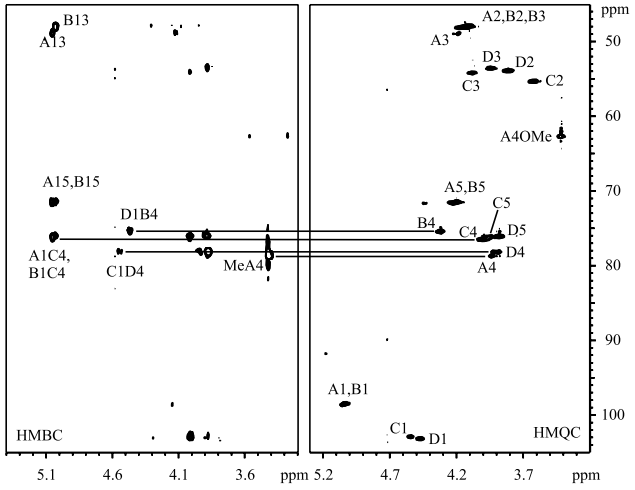

Fig. 1. Part of 2D $^{1}H,^{13}C$ gHMBC (left) and HMQC (right) spectra of the polysaccharide of *B. hinzii* obtained by mild-acid degradation of the LPS.

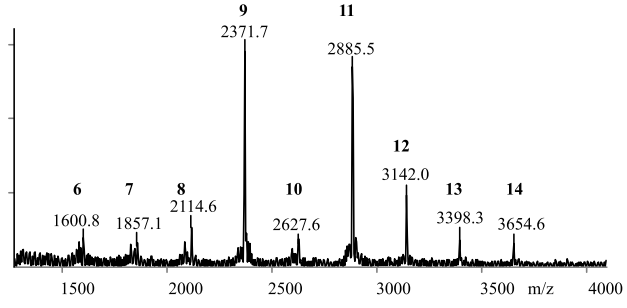

Fig. 2. Positive-ion mode MALDI mass spectrum of the polysaccharide of *B. hinzii* obtained by degradation of the LPS with aq 12% ammonia. Shown are m/z values and numbers of sugar residues.

The positive-ion mode MALDI mass spectrum of the alkaline degradation product (Fig. 2) contained a number of peaks with a mass difference of 257 Da, which corresponds to a 2,3-diacetamido-2,3-dideoxyhexuronamide residue. There were peaks for oligomers consisting of up to 15 sugar residues, including one *O*-methylated residue, with the predominance of nona- and undecasaccharides (m/z 2371.7 and 2885.5 for the $[M + Na]^{+}$ ions, respectively). However, this pattern may not reflect the actual polysaccharide chain-length distribution in the LPS, since a random cleavage of the polysaccharide by β elimination under the alkaline conditions cannot be excluded. No peaks for oligomers lacking the *O*-methyl group were observed. Similarly, in the mass spectrum of the deamination product, there were peaks for oligomers consisting of up to 15 sugar residues with the same mass difference of 257 Da. The nature of the residue at the reducing end in this product remained unknown.

Therefore, the data obtained showed that the polysaccharide of *B. hinzii* has the following structure:

4-*O*-Me-α-Gal*p*NAc3NAcAN-(1
$\underset{\mathbf{A}}{}$
$\rightarrow$[$\rightarrow$4)-β-Glc*p*NAc3NAcAN-(1$\rightarrow$4)-
$\underset{\mathbf{C}}{}$
β-Glc*p*NAc3NAcAN-(1$\rightarrow$4)-α-
$\underset{\mathbf{D}}{}$
Gal*p*NAc3NAcAN-(1$\rightarrow$]$_n$ –
$\underset{\mathbf{B}}{}$

where GlcNAc3NAcAN and GalNAc3NAcAN stand for 2,3-diacetamido-2,3-dideoxy-glucuronamide and -galacturonamide, respectively; $n \leq 5$. The absolute configurations of the monosaccharides were not deter-

mined; most likely, they all are D since previously derivatives of GlcN3NA and GalN3NA have been found exclusively in this enantiomeric form.[6] Although the total amidation of the uronic acids and *O*-methylation of the terminal sugar residue deprive the polysaccharide of free carboxy and hydroxy groups, it showed perfect solubility in water but not in organic solvents, including methanol and pyridine, and is thus less hydrophobic than one could expect.

1. Experimental

Bacterial strain and isolation of the lipopolysaccharide.—*B. hinzii* strain ATCC 51730 was originally isolated from blood of an AIDS patient. Bacterial cells were cultivated and the LPS was isolated as described previously.[7]

Mild-acid hydrolysis.—The LPS (100 mg) was hydrolyzed with aq 1% HOAc (10 mL) at 100 °C for 4 h, the precipitate was removed by centrifugation, and the supernatant was fractionated by GPC on a column (2.5 × 80 cm) of Sephadex G-50 (S) (Pharmacia) using pyridinium–AcOH buffer (4 mL pyridine and 10 mL HOAc in 1 L water) to give a polysaccharide (35 mg) and a core oligosaccharide (20 mg).

Alkaline degradation.—A solution of the LPS (100 mg) in aq 12% ammonia (4 mL) containing $NaBH_4$ (25 mg) was kept at 35 °C for 24 h, lyophilized, acidified with 2 M HCl, and the products were fractionated by GPC on a Sephadex G-50 (S) column as above. The reduced polysaccharide was further purified by reversed-phase HPLC on a Hamilton PRP1 column in a 0 → 90% gradient of MeCN in aq 0.1% CF_3CO_2H; the yield of the product was 25 mg.

Deamination.—Sodium nitrite (50 mg) and HOAc (0.5 mL) were added to a solution of the LPS (100 mg) in water (5 mL), in 4 h at 25 °C, lipid-containing material was removed by ultracentrifugation (120,000*g*, 1 h), the supernatant was fractionated by GPC on a Sephadex G-50 (S) column, and the polysaccharide was purified by reversed-phase HPLC on a Hamilton PRP1 column in a 0 → 90% gradient of MeCN in aq 0.1% CF_3CO_2H; the yield of the product was 20 mg.

NMR spectroscopy and mass spectrometry.—^1H and ^{13}C NMR spectra were recorded on a Varian Inova 500 spectrometer in D_2O solutions at 25 °C with acetone as internal standard (δ_H 2.225, δ_C 31.5), using standard pulse sequences COSY, TOCSY (mixing time 100 ms), NOESY (mixing time 200 ms), HMQC, gHMBC (optimized for a coupling constant of 5 Hz), HSQC-TOCSY (mixing time 60 ms). The spectra were assigned with the help of the PRONTO program.[8] Positive-ion mode MALDI TOF mass spectra were obtained using a Perseptive Biosystems Voyager DE STR spectrometer with dihydroxybenzoic acid matrix.

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