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Note

Structure of the O-specific polysaccharide of *Citrobacter braakii* O7a,3b,1c

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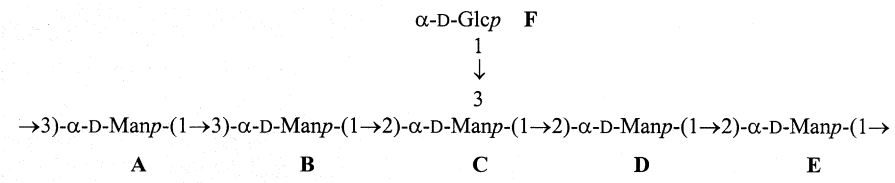
Dedicated to Professor Joachim Thiem on the occasion of his 60th anniversary

Abstract

The following structure of the O-specific polysaccharide of *Citrobacter braakii* O7a,3b,1c was established using sugar and methylation analyses and NMR spectroscopy, including 2D COSY, TOCSY, NOESY, and ^{1}H, ^{13}C heteronuclear single-quantum coherence (HSQC) experiments:



The main D-mannan chain of the polysaccharide studied has the same structure as the O-specific polysaccharide of *Escherichia coli* O9, *Klebsiella pneumoniae* O3, and *Hafnia alvei* PCM 1223.

Keywords: Lipopolysaccharide; O-Specific polysaccharide; Bacterial polysaccharide structure; Mannan; *Citrobacter braakii*

Bacteria of the genus *Citrobacter* from the family *Enterobacteriaceae* are serologically heterogeneous and can be classified in 43 O-serogroups.[1,2] Structures of the O-specific polysaccharide chains (O-antigens) of the lipopolysaccharides from more than 20 *Citrobacter* O-serogroups have been established.[3–7] In this study, we elucidated a new structure of the O-specific polysaccharide from *Citrobacter braakii* O7a,3b,1c.

The polysaccharide was obtained by mild-acid degradation of the lipopolysaccharide, isolated from bacterial cells by the phenol–water procedure,[8] followed by GPC on Sep-

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hadex G-50. Sugar analysis, including determination of the absolute configurations,[9] showed that the polysaccharide contains D-mannose and D-glucose in the ratio 3.7:1.

The ^{13}C NMR spectrum of the polysaccharide (Fig. 1) contained signals for six sugar residues, including signals for anomeric carbons at δ 101.8–103.1, HO*C*H_2–C groups (C-6 of Man and Glc) at δ 62.1–62.5, and other sugar carbons in the region δ 67.5–79.7. Accordingly, the ^1H NMR spectrum of the polysaccharide contained signals for six anomeric protons at δ 5.13–5.35 and other sugar protons at δ 3.43–4.24. The 2D COSY and TOCSY spectra showed that five spin systems belonged to mannose residues and one to a glucose residue.

Therefore, the polysaccharide has a hexasaccharide-repeating unit containing five residues of D-mannose and one residue of D-glucose. A lower than expected mannose-to-glucose ratio in sugar analysis could be accounted for by higher destruction during acid hydrolysis of mannose compared to glucose or/and by origination of some glucose from the core oligosaccharide.[10] Methylation analysis showed that the polysaccharide is branched, glucose is a terminal monosaccharide of the side chain, a mannose residue at the branching point is 2,3-substituted, and from four remaining mannose residues, two are 2-substituted and two 3-substituted (the ratios of the corresponding partially methylated monosaccharides were 0.8:1:2.2:1.7).

The ^1H and ^{13}C NMR spectra of the polysaccharide were assigned using 2D COSY, TOCSY, NOESY, and H-detected ^1H, ^{13}C HSQC experiments (Tables 1 and 2). The presence of strong intraresidue H-1, H-2 cross-peaks and the absence of H-1, H-3,5 cross-peaks in the NOESY spectrum demonstrated the α configuration of the glycosidic linkages of all five mannose residues (units **A**–**E**) and the glucose residue (unit **F**). A $J_{1,2}$ coupling constant value of ~ 3 Hz confirmed the α linkage of Glc.

The glycosylation pattern of the polysaccharide was defined by downfield displacements of the signals for C-2 of units **D** and **E**, C-3 of units **A** and **B**, and both C-2 and C-3 of unit **C** to δ 78.5–79.7 (Table 2), as compared with their position at δ 72.0–72-5 in α-mannopyranose.[11] In accordance with terminal position of unit **F**, the chemical shifts for C-2–C-6 were close to those in α-glucopyranose.[11]

The NOESY spectrum of the polysaccharide showed strong interresidue cross-peaks between the following transglycosidic protons: **A** H-1,**B** H-3; **B** H-1,**C** H-2; **C** H-1,**D** H-2; **D** H-1,**E** H-2; **E** H-1,**A** H-3; and **F** H-1,**C** H-3 at δ 5.14/3.94; 5.13/4.24; 5.26/4.10; 5.26/4.09; 5.35/3.99; and 5.25/4.05, respectively. The 1,2-linkage between units **B** and **C** and between units **D** and **E** was confirmed by H-1,H-1 NOE correlations at δ 5.13/5.26 and 5.26/5.35, respectively. These data defined the

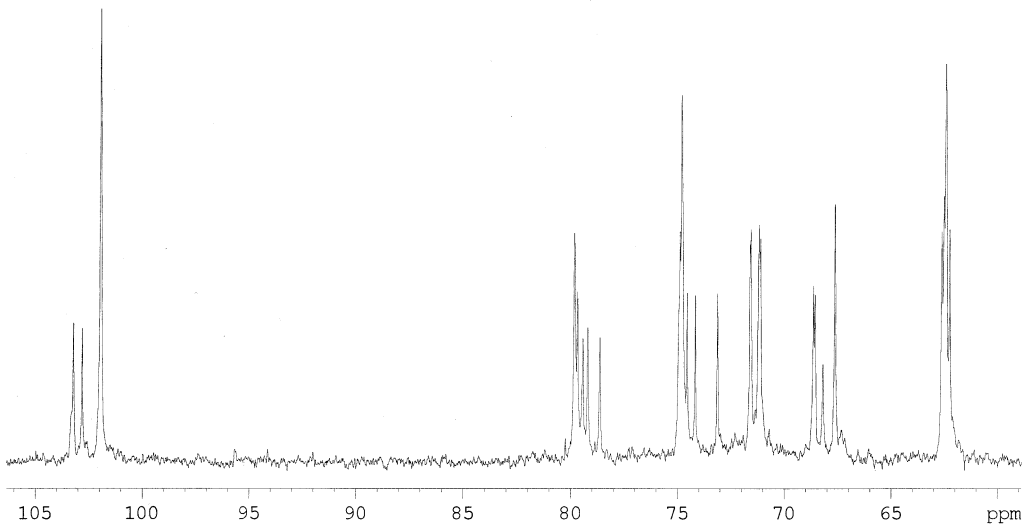


Fig. 1. 125-MHz ^{13}C NMR spectrum of the O-specific polysaccharide.

Table 1
500-MHz ^{1}H NMR chemical shifts of the O-specific polysaccharides (δ, ppm) [a]

Sugar residue		H-1	H-2	H-3	H-4	H-5
Citrobacter braakii O7a,3b,1c						
→3)-α-D-Man*p*-(1→	**(A)**	5.14	4.21	3.99	3.82	3.81
→3)-α-D-Man*p*-(1→	**(B)**	5.13	4.16	3.94	3.79	3.78
→2,3)-α-D-Man*p*-(1→	**(C)**	5.26	4.24	4.05	3.93	3.75
→2)-α-D-Man*p*-(1→	**(D)**	5.26	4.10	3.95	3.71	3.71
→2)-α-D-Man*p*-(1→	**(E)**	5.35	4.09	4.00	3.69	3.72
α-D-Glc*p*-(1→	**(F)**	5.25	3.58	3.68	3.43	3.71
Hafnia alvei PCM 1223[14]						
→3)-α-D-Man*p*-(1→	**(A)**	5.12	4.21	3.99	3.79	3.75
→3)-α-D-Man*p*-(1→	**(B)**	5.04	4.21	3.93	3.78	3.78
→2)-α-D-Man*p*-(1→	**(C)**	5.28	4.10	3.94	3.72	3.72
→2)-α-D-Man*p*-(1→	**(D)**	5.28	4.09	3.95	3.72	3.72
→2)-α-D-Man*p*-(1→	**(E)**	5.35	4.08	3.99	3.69	3.78

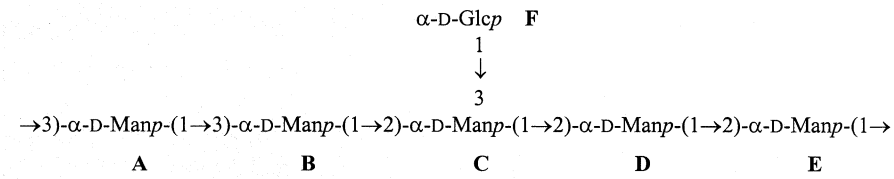
[a] Signals for H-6a and H-6b of Man and Glc in the polysaccharide of *C. braakii* O7a,3b,1c are at δ 3.73–3.81 and 3.85–3.91.

Table 2
125-MHz ^{13}C NMR chemical shifts of the O-specific polysaccharides (δ, ppm) [a]

		C-1	C-2	C-3	C-4	C-5	C-6
Citrobacter braakii O7a,3b,1c							
→3)-α-D-Man*p*-(1→	**(A)**	103.1	71.0	79.7	67.5	74.8	62.3
→3)-α-D-Man*p*-(1→	**(B)**	102.7	71.1	79.1	67.5	74.7	62.3
→2,3)-α-D-Man*p*-(1→	**(C)**	101.8	78.5	79.3	68.1	74.7	62.3
→2)-α-D-Man*p*-(1→	**(D)**	101.8	79.6	71.5	68.4	74.7	62.4
→2)-α-D-Man*p*-(1→	**(E)**	101.8	79.7	71.5	68.5	74.7	62.5
α-D-Glc*p*-(1→	**(F)**	101.8	73.0	74.4	71.1	74.1	62.1
Hafnia alvei PCM 1223[14]							
→3)-α-D-Man*p*-(1→	**(A)**	103.17	70.92	79.60	67.42	74.52	62.34
→3)-α-D-Man*p*-(1→	**(B)**	103.22	70.85	79.25	67.42	74.52	62.34
→2)-α-D-Man*p*-(1→	**(C)**	101.81	79.55	71.32	68.26	74.61	62.26
→2)-α-D-Man*p*-(1→	**(D)**	101.81	79.76	71.38	68.40	74.71	62.22
→2)-α-D-Man*p*-(1→	**(E)**	101.81	79.76	71.32	68.33	74.64	62.26

[a] Assignment of the signals for C-6 could be interchanged.

monosaccharide sequence in the repeating unit and showed that the O-specific polysaccharide of *C. braakii* O7a,3b,1c has the following structure:



Remarkably, the main D-mannan chain of the polysaccharide has the same structure as the O-specific polysaccharide of *Escherichia coli* O9,[12] *Klebsiella pneumoniae* O3,[13] and *Hafnia alvei* PCM 1223.[14] Comparison of the ^{1}H and ^{13}C NMR chemical shifts of the mannose residues in the polysaccharides of *C. braakii* O7a,3b,1c and *H. alvei* PCM 1223 (Tables 1 and 2) showed significant differences for the anomeric signals of residue **B** and non-anomeric signals of residue **C**, thus confirming further the assignment of the NMR

spectra and the structure of the former polysaccharide.

Despite the structural similarity of the O-antigens, no cross-reactivity was observed in Western immunoblotting between anti-*H. alvei* PCM 1223 O-serum and the lipopolysaccharide of *C. braakii* O7a,3b,1c.[14] This could be accounted for by masking of potentially cross-reactive epitope(s) within the D-mannan chain by the lateral glucose residue in the polysaccharide of *C. braakii*.

1. Experimental

Citrobacter O7a,3b,1c (strain Be 59/57) from the Czech National Collection of Type Cultures (Institute of Microbiology and Epidemiology, Prague) was grown in a dense agar medium.[15] Isolation of the lipopolysaccharide[8] and O-specific polysaccharide[15] were performed as described.

The polysaccharide was hydrolysed with 2 M CF_3CO_2H (120 °C, 1 h), the monosaccharides were converted into the alditol acetates[16] and analysed by GLC on a Hewlett–Packard 5890A chromatograph equipped with a capillary column of Ultra 2 using a temperature program of 180–290 °C at 10 °C/min. The absolute configuration of the monosaccharides was determined by GLC of the acetylated (*S*)-2-octyl glycosides according to published method.[9]

Methylation was performed as described,[17] the partially methylated alditol acetates derived were identified by GLC–MS on a Hewlett–Packard 5971A system using a HP-1 glass capillary column and a temperature program of 150–270 °C at 8 °C/min.

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer for a solution in D_2O at 60 °C. Chemical shifts are reported with internal acetone (δ_H 2.225, δ_C 31.45) as reference. A mixing time of 150 and 200 ms was used in TOCSY and NOESY experiments, respectively.

Acknowledgements

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