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Carbohydrate Research 339 (2004) 1809–1812
Carbohydrate RESEARCH

Note

Structure of the O-polysaccharide of the lipopolysaccharide of *Rahnella aquatilis* 1-95

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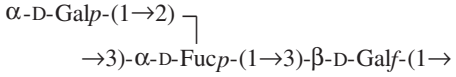
Received 15 April 2004; accepted 17 May 2004

Abstract—The O-polysaccharide was isolated by mild acid hydrolysis of the lipopolysaccharide of *Rahnella aquatilis* 1-95 and studied by sugar and methylation analyses along with ^{1}H and ^{13}C NMR spectroscopy, including NOESY and $^{1}H,^{13}C$ HSQC experiments for linkage and sequence analysis. The following structure of the branched trisaccharide repeating unit of the O-polysaccharide was established:



Keywords: Lipopolysaccharide; O-Antigen; Bacterial polysaccharide structure; *Rahnella aquatilis*

Rahnella aquatilis is a representative of the *Enterobacteriaceae* recognized in 1976–1979.[1,2] Strains of *R. aquatilis* are isolated from open water reservoirs and soil (mostly from the rhizosphere of cereals, including wheat, corn, rice, etc.) as well as from clinical material so far as they are opportunistic pathogens causing a wide spectrum of diseases (rahnelosis) of the gastrointestinal and urinary tracts, respiratory organs, cardiovascular system, and others. Endotoxin (lipopolysaccharide) plays an important role in pathogenesis of diseases caused by gram-negative bacteria. Up to now the lipopolysaccharide of *R. aquatilis* has not been chemically characterized. Here we report, for the first time, the structure of the O-polysaccharide from the lipopolysaccharide of *R. aquatilis* strain 1-95.

Mild acid degradation of the lipopolysaccharide resulted in a high-molecular-mass O-polysaccharide, which was isolated by GPC on Sephadex G-50. Sugar analysis by GLC of the alditol acetates derived after acid hydrolysis (2 M CF_3COOH, 2 h, 120 °C) of the polysaccharide revealed fucose and galactose in the ratio ~1:2. Determination of the absolute configuration by GLC of the acetylated glycosides with a chiral alcohol indicated that both monosaccharides have the D configuration.

Methylation analysis of the polysaccharide, including GLC of the partially methylated alditol acetates, revealed derivatives of 4-*O*-methylfucopyranose, 2,3,4,6-tetra-*O*-methylgalactopyranose and 2,5,6-tri-*O*-methylgalactofuranose in the ratio ~5:2:3.5. Most likely, the nonstoichiometric ratio resulted from destruction of partially methylated monosaccharides during acid hydrolysis. Therefore, the polysaccharide is branched with a terminal galactopyranose residue in the side chain,

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doi:10.1016/j.carres.2004.05.011

a 2,3-disubstituted fucopyranose residue at the branching point, and a 3-substituted galactofuranose residue.

Each of the ^{1}H and ^{13}C NMR spectra of the O-polysaccharide (Figs. 1 and 2) contained signals for three monosaccharide residues and were assigned using $^{1}H,^{1}H$ COSY, TOCSY, NOESY, and H-detected $^{1}H,^{13}C$ HSQC experiments (Table 1). The protons of the (pyranosidic/furanosidic) sugar residues showed spin systems, which were isolated by tracing connectivities in the TOCSY spectrum starting from H-1 of Gal*p* and Gal*f* and from H-1 and H-6 of Fuc*p*. The signals within each spin system were assigned using the COSY spectrum, and the identity of the monosaccharides was established by the characteristic coupling patterns and $^{3}J_{H,H}$ constant values. The α-linkage of Fuc*p* and Gal*p* was established by the characteristic H-1, C-1, and C-5 chemical shifts (Table 1) and from the presence of H-1,H-2 cross-peaks with no H-1,H-5 cross-peaks in the NOESY spectrum. The β-linkage of Gal*f* followed from the C-1 chemical shift of δ 110.3 (the C-1 of α-Gal*f* would resonate near δ 103).[3]

The ^{13}C NMR chemical shift data (Table 1) revealed low-field displacements of the signals for Fuc*p* C-3 and C-2 and Gal*f* C-3, as compared with their positions in the corresponding nonsubstituted monosaccharides.[3] These effects were due to glycosylation and defined the linkage positions in the repeating unit. The C-2–C-6 chemical shifts of Gal*p* were close to the values for unsubstituted α-galactopyranose, which is in agreement with the terminal position of this monosaccharide shown by methylation analysis. The sequence of the monosaccharides in the repeating unit was determined by the following correlations between the anomeric protons and protons at the linkage carbons in the NOESY spectrum: Fuc*p* H-1,Gal*f* H-3; Gal*f* H-1,Fuc*p* H-3; and Gal*p* H-1,Fuc*p* H-2.

Therefore, the O-polysaccharide of the lipopolysaccharide of *R. aquatilis* 1-95 is built up of branched tri-

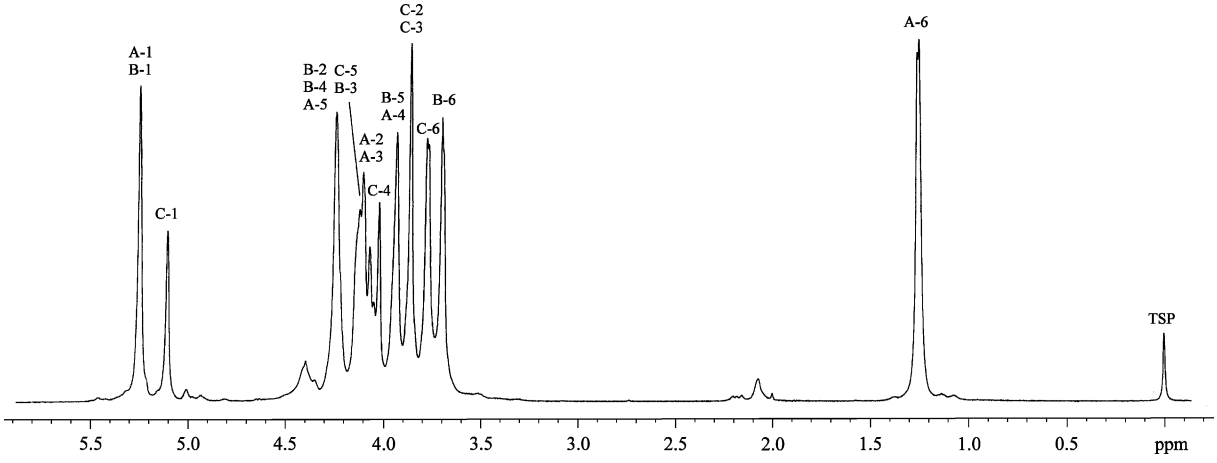

Figure 1. 500-MHz ^{1}H NMR spectrum of the O-polysaccharide of *R. aquatilis* 1-95, where **A** is α-D-Fuc*p*, **B** is β-D-Gal*f*, and **C** is α-D-Gal*p* residues, **TSP** is sodium 3-trimethylsilylpropanoate.

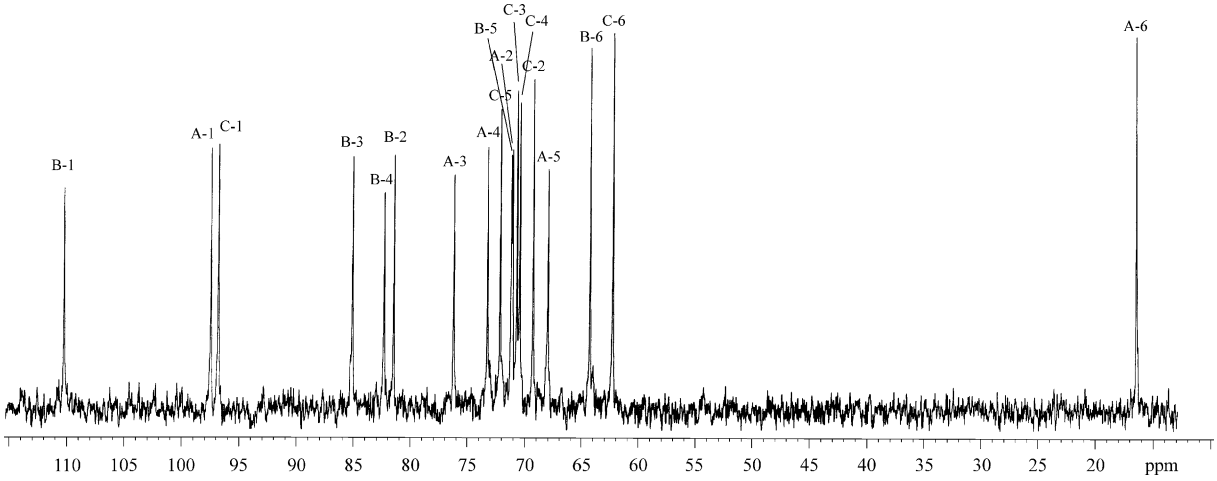

Figure 2. 125-MHz ^{13}C NMR spectrum of the O-polysaccharide of *R. aquatilis* 1-95, where **A** is α-D-Fuc*p*, **B** is β-D-Gal*f* and **C** is α-D-Gal*p* residues.

Table 1. 500-MHz ^{1}H NMR and 125-MHz ^{13}C NMR data (δ, ppm)

Sugar residue	H-1	H-2	H-3	H-4	H-5	H-6a,6b
↓ 2 →3)-α-D-Fuc*p*-(1→	5.25	4.10	4.08	3.92	4.25	1.24
→3)-β-D-Gal*f*-(1→	5.23	4.25	4.13	4.22	3.95	3.68
α-D-Gal*p*-(1→	5.11	3.84	3.85	4.01	4.12	3.76
	C-1	C-2	C-3	C-4	C-5	C-6
↓ 2 →3)-α-D-Fuc*p*-(1→	97.4	71.0	76.2	73.2	67.9	16.4
→3)-β-D-Gal*f*-(1→	110.3	81.4	85.1	82.3	71.1	64.2
α-D-Gal*p*-(1→	96.8	69.2	70.6	70.4	72.1	62.2

saccharide repeating units and has the following structure:

α-D-Gal*p*-(1→2)┐
→3)-α-D-Fuc*p*-(1→3)-β-D-Gal*f*-(1→

1. Experimental

1.1. Growth of bacteria and isolation of the lipopolysaccharide and O-polysaccharide

R. aquatilis strain 1-95 isolated from water of river Uda was kindly provided by Dr. S. Pohyl (Institute of Microbiology and Immunology of Medical Academy of Sciences of Ukraine, Charkov, Ukraine) and was grown at 28 °C for 24 h on a beef-extract agar medium. Cells were centrifuged off and dried by acetone and ether. The lipopolysaccharide was isolated by standard phenol–water procedure[4] followed by removal of nucleic acid by precipitation with aq 50% trichloroacetic acid and ultracentrifugation at 144,000*g*. It was cleaved by hydrolysis with 3% AcOH for 2 h, a lipid residue was removed by centrifugation, and an O-polysaccharide was isolated from the supernatant by GPC on Sephadex G-50.

1.2. Sugar analysis

Hydrolysis of the polysaccharide was performed with 2 M CF_3CO_2H (120 °C, 2 h), the monosaccharides were analyzed by GLC as the alditol acetates[5] on an Ultra 2 capillary column using a Hewlett-Packard 5880 instrument and a temperature gradient of 180 °C (1 min) to 290 °C at 10 °C min^{-1}. The absolute configuration of the monosaccharides was determined by GLC of the acetylated glycosides with (*S*)-2-octanol[6] on the same column at 230 °C (20 min).

1.3. Methylation analysis

Methylation of the polysaccharide was carried out with CH_3I in Me_2SO in the presence of methylsulfinylmethanide.[7] Hydrolysis of the methylated polysaccharide was performed with 2 M CF_3CO_2H (100 °C, 2 h), and the partially methylated monosaccharides were reduced with $NaBH_4$, acetylated, and analyzed by GLC–MS on a Hewlett-Packard HP 5989A instrument equipped with an HP-5ms column using a temperature gradient of 150 °C (3 min) to 320 °C at 5 °C min^{-1}.

1.4. NMR spectroscopy

Prior to the measurements, samples were deuterium-exchanged by freeze-drying from D_2O. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer for solutions in D_2O at 27 °C. Chemical shifts are reported with sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) as internal and acetone (δ_C 31.45) as external standards. A mixing time of 200 and 150 ms was used in TOCSY and NOESY experiments, respectively.

Acknowledgements

Authors thank Dr. S. I. Pohyl for the bacterial culture and Dr. Y. A. Knirel for critical reading of the manuscript. This work was funded in part by the Foundation for Leading Scientific Schools of the Russian Federation (project NSh.1557.2003.3).

References

1. Gavini, T.; Terragut, C.; Lefebre, B.; Leclerk, H. *Ann. Microbiol. Inst. Pasteur.* **1976**, *127*, 317–335.
2. Izard, D.; Gavini, T.; Trinel, P. A.; Leclerk, H. *Ann. Microbiol. Inst. Pasteur.* **1979**, *130*, 163–167.
3. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.

4. Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.
5. Sawardeker, J. S.; Sloneker, J. H.; Jeanes, A. *Anal. Chem.* **1965**, *37*, 1602–1603.
6. Leontein, K.; Lönngren, J. *Methods Carbohydr. Chem.* **1993**, *9*, 87–89.
7. Conrad, H. E. *Methods Carbohydr. Chem.* **1972**, *6*, 361–364.