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Note

The structure of the O-specific polysaccharide from *Salmonella cerro* (serogroup K, O:6,14,18)

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Abstract—The following structure of the *Salmonella cerro* LPS O-chain repeating unit has been determined using NMR and chemical methods:

→4)-α-D-Man-(1→2)-α-D-Man-(1→2)-β-D-Man-(1→3)-α-D-GalNAc-(1→

Keywords: *Salmonella*; *S. cerro*; LPS; O-Chain; Structure

Bacteria of the genus *Salmonella* are a widespread cause of gastrointestinal and other diseases. They are serologically classified on the basis of their O-antigenic factors, located in the lipopolysaccharide O-chains, into Kaufmann–White scheme. This classification is regularly updated and revised with the discovery of new strains.[1,2] Knowledge of the fine structure of the LPS O-chains is essential for understanding of the molecular basis of antigenic serospecific factors.

This paper describes the structure of the O-specific polysaccharide from *S. cerro*, belonging to the serogroup O:18 (K). Most strains of this serogroup exhibit only one O-antigenic factor 18, whereas about one third of group K strains demonstrate additionally factors 6 and 14. *S. cerro* belongs to the latter group.[1]

LPS was isolated by phenol–water extraction and purified by ultracentrifugation. The polysaccharide O-chain was isolated from the LPS by conventional mild acid hydrolysis with 2% AcOH followed by Sephadex G50 gel-filtration chromatography of the water soluble products, yielding a lipid-free high-molecular-mass polysaccharide. GC analysis of alditol acetates prepared from the polysaccharide showed the presence of mannose and galactosamine in the ratio of 3:1. The absolute D-configuration of these monosaccharides was

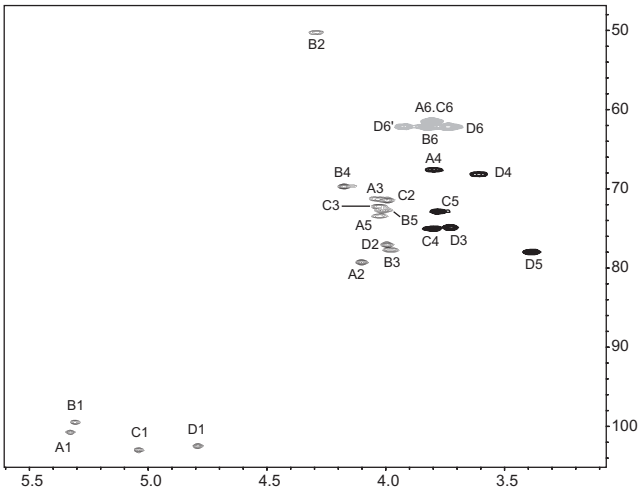

Figure 1. Fragment of the HSQC spectrum of *S. cerro* O-specific polysaccharide.

Abbreviation: LPS, lipopolysaccharide

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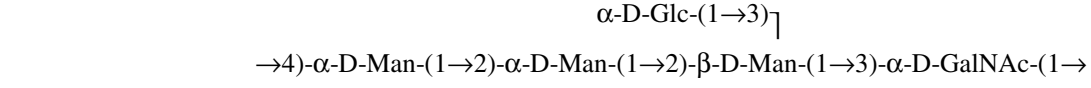
Table 1. NMR data for the *S. cerro* O-specific polysaccharide; acetate signals: Me (^{1}H/^{13}C): 2.05/23.2 ppm

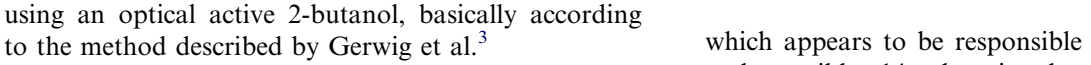
Unit	Nucleus	1	2	3	4	5	6a	6b
α-D-Man A	^{1}H	5.33	4.10	4.03	3.79	4.02	3.80	3.80
	^{13}C	100.6	79.2	71.2	67.5	73.3	61.4	
α-D-GalNAc B	^{1}H	5.31	4.29	3.98	4.17	4.00	3.74	3.74
	^{13}C	99.4	50.1	77.6	69.6	72.6	62.1	
α-D-Man C	^{1}H	5.04	3.99	4.03	3.80	3.78	3.80	3.80
	^{13}C	102.9	71.3	72.1	74.9	72.8	61.4	
β-D-Man D	^{1}H	4.79	3.99	3.72	3.60	3.38	3.73	3.92
	^{13}C	102.4	77.0	74.8	68.1	77.9	62.0	

established by GC of acetylated glycosides prepared using an optical active 2-butanol, basically according to the method described by Gerwig et al.[3]

The set of 2D NMR experiments (COSY, TOCSY, NOESY, HSQC, HMBC) was recorded for the polysaccharide sample. The spectra were completely assigned (Fig. 1, Table 1) using the Pronto program.[4] This led to the identification of spin-systems of four monosaccharides, labeled A–D in decreasing order of their anomeric proton chemical shifts. Monosaccharides were identified on the basis of vicinal proton coupling constants and ^{13}C NMR chemical shifts. Anomeric configurations were deduced from the $J_{1,2}$ coupling constants and chemical shifts of H-1 and C-1 signals. The β-configuration of mannose residue D was confirmed by the observation of interresidual NOE correlations from H-1 to H-3 and H-5. Thus the residues A and C were identified as α-mannopyranosides, B as 2-acetamido-2-deoxy-α-galactopyranoside, and D as β-mannopyranoside. Connections between monosaccharides were identified on the basis of NOE and HMBC correlations. The NOEs A1:D2, A1:D1, B1:C4, C1:A2, C1:A1, D1:B3, as well as transglycosidic HMBC correlations A1:D2, B1:C4, C1:A2, and D1:B3 were observed. Methylation of the polysaccharide[5] with subsequent hydrolysis, $NaBD_4$ reduction, acetylation, and GC–MS analysis led to the identification of the following monosaccharides: 2- and 4-substituted mannopyranose, and 3-substituted Gal*p*NAc. Taken together these data indicate the following structure of the polysaccharide repeating unit:

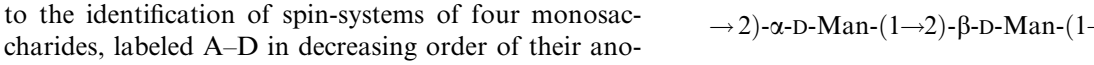


A number of *Salmonella* serovars contain structures built of three or four mannose residues and GlcNAc,[6–10] which always include the substructure:



which appears to be responsible for antigenic factor 6 and possibly 14, that is also present in *S. cerro* polysaccharide.

S. cerro O-chain shares the common trisaccharide fragment



with the O-antigen from *Y. pseudotuberculosis* IIC.[11]

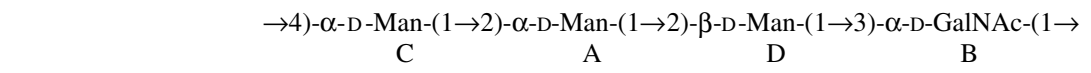
The closest known O-antigen structure to that of *S. cerro* is present in nontyped strain 73-1 of *E. coli*, which has the same as *S. cerro* main chain, substituted by Glc residue:[12]

Serological relationship between these two O-antigens has not been determined.

1. Experimental

^{1}H and ^{13}C NMR spectra were recorded using a Varian Inova 500 spectrometer in D_2O solutions at 40 °C for the polysaccharide and at 25 °C for mono- and oligosaccharides with acetone standard (2.225 ppm for ^{1}H and 31.5 ppm for ^{13}C) using standard pulse sequences COSY, TOCSY (mixing time 120 ms), NOESY (mixing time 200 ms), HSQC, and HMBC (optimized for a 5 Hz coupling constant).

LPS was isolated by the Westphal procedure.[13] LPS was hydrolyzed with 2% AcOH (2 h, 100 °C), precipitate removed, soluble products separated by gel chromatography on a Sephadex G-50 column to give polysaccharide and core fractions.



For monosaccharide analysis, the polysaccharide (0.5 mg) was hydrolyzed (0.2 mL of 3 M TFA, 100 °C, 2 h), followed by evaporation to dryness under a stream of air. The residue was dissolved in water (0.5 mL), reduced with $NaBH_4$ (~5 mg, 1 h), neutralized with concd AcOH (0.3 mL), dried, and MeOH (1 mL) was added. The mixture was dried twice with the addition of MeOH, and the residue was acetylated with Ac_2O (0.5 mL, 100 °C, 30 min), dried, and analyzed by GC on a HP1 capillary column (30 m × 0.25 mm) with a flame ionization detector (Agilent 6850 chromatograph) in a temperature gradient of 170 (4 min) to 260 °C at 4 °C/min or on a Varian Saturn 2000 ion-trap GC–MS instrument in the same conditions.

Gel chromatography was carried out on Sephadex G-50 (2.5 cm × 95 cm) and Sephadex G-15 (1.6 cm × 80 cm) columns using the pyridinium acetate buffer, pH 4.5 (4 mL pyridine and 10 mL AcOH in 1 L water) as eluent, monitored by a refractive index detector.

Acknowledgements

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