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Note

The elucidation of the structure of the core part of the LPS from *Plesiomonas shigelloides* serotype O17 expressing O-polysaccharide chain identical to the *Shigella sonnei* O-chain

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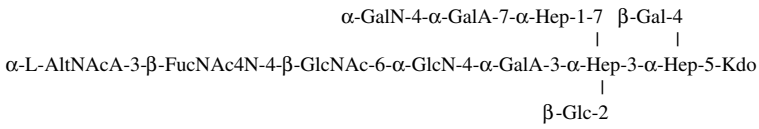
ABSTRACT

Plesiomonas shigelloides O17 LPS contains the same O-antigenic polysaccharide chain as a causative agent of dysentery, *Shigella sonnei*. This polysaccharide can be used as a component of a vaccine against dysentery. Core part of the *P. shigelloides* O17 LPS was studied using NMR and mass spectrometry and the following structure was proposed:



Significant similarity of the *P. shigelloides* O17 LPS core with the structure of the *P. shigelloides* O54 core was observed.

Plesiomonas shigelloides is a gram-negative, flagellated, rod-shaped bacterium. This ubiquitous and facultatively anaerobic organism has been isolated from water and many wild and domestic animals. It may cause diarrhea in humans drinking contaminated water in countries with low sanitary standards. It may also cause various illnesses in immunocompromised hosts and neonates.[1]

The LPS of *P. shigelloides* serotype O17 is of particular interest since its O-antigen structure is identical to that of *Shigella sonnei,* a cause of endemic and epidemic diarrhea and/or dysentery worldwide.[2] The O-antigen gene cluster of both *S. sonnei* and *P. shigelloides* O17 is located on the plasmid Pinv, apparently acquired by *S. sonnei* from *P. shigelloides.*[3,4] This invasion plasmid is essential for penetration of host epithelial cells and is therefore an important virulence factor.[5]

Because of the structural identity of the LPS O-specific polysaccharides (O-SP) of *S. sonnei* and *P. shigelloides* O17, the latter one can be used as an immunogenic component of a vaccine against *S. sonnei.*[6,7] Interpretation of the immunological data and selection of the optimal conjugation conditions require the knowledge of the structure of the LPS core part, which is always present in the O–SP preparations. Here, we present the data on the structure of the core of *P. shigelloides* O17 LPS, including its linkage to the O–SP.

P. shigelloides O17 LPS was hydrolyzed with 2% acetic acid and the core oligosaccharide **1** was isolated by gel chromatography. Its NMR spectra showed the presence of a large number of spin systems (Figs. 1 and 2). Signal spread in such products is usually due to the presence of many isomers of anhydro-Kdo at the reducing end, but in this case it seemed that some additional cause of heterogeneity was present. Attempts to purify the oligosaccharide by anion-exchange chromatography did not result in improved spectra. Some signals were of higher intensity and it was possible to recognize the presence of one O-chain repeat unit, as well as minor quantities of products with two or more repeating units (signals Z′ in Fig. 1).

Monosaccharide analysis (GLC–MS of alditol acetates) showed the presence of LDHep, Glc, Gal, GlcN, and GalN. Methylation analysis by the Ciucanu and Kerek procedure revealed terminal Glc and Gal, 7-, 3,4-, 3,7-, and 2,3,7-substituted Hep, and 4-substituted GlcN.

Abbreviations: LPS, lipopolysaccharide; Hep, L-*glycero*-D-*manno*-heptose; Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid; AltNA, 2-amino-2-deoxy-L-altruronic acid; FucNAc4N, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose; HexN, hexosamine; P, phosphate; HPAEC, high performance anion exchange chromatography.

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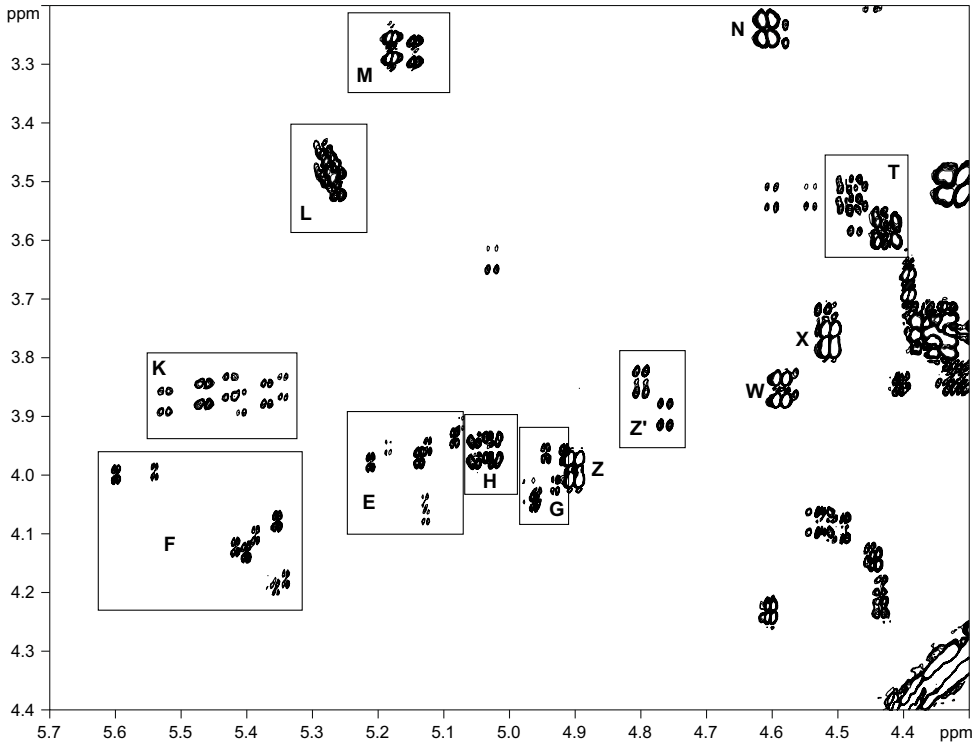


Figure 1. Fragment of the COSY spectrum of oligosaccharide **1** with labeled H-1:H-2 correlations. Z′ belong to the oligosaccharides with several repeat units of the O–SP.

Alkaline deacylation of the LPS with **4** M KOH with subsequent HPAEC separation of the products yielded two major compounds **2** and **3** among a large number of less abundant products. NMR and MS analysis of these products revealed the structures presented in the Scheme 1. Oligosacharides **2** and **3** contained only sugars of the 'inner' part of the core because the outer components were lost by the β-elimination of 4-substituted uronic acids. NMR analysis of these oligosaccharides allowed the determination of the positions of uronic acids H and K, Glc residue N, and Gal residue T, and gave one of the reasons for the oligosaccharide **1** signals spread, the par-

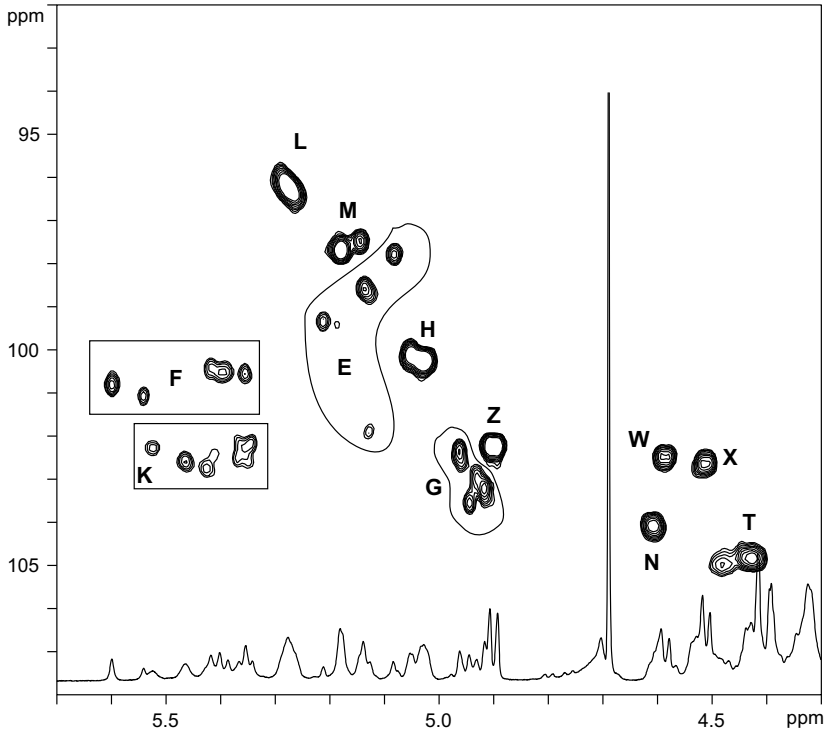
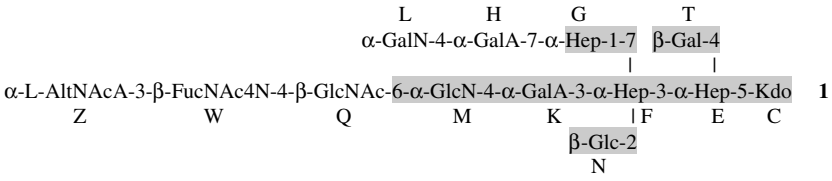
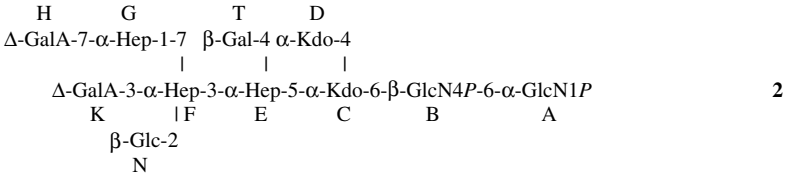
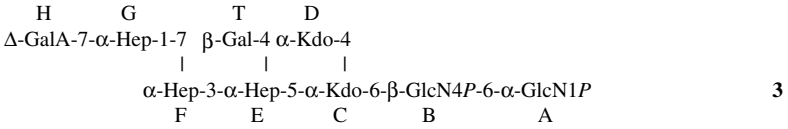


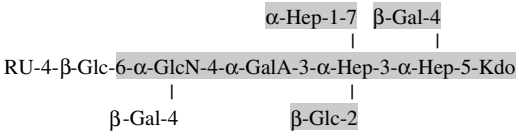
Figure 2. Anomeric region of the HSQC spectrum of oligosaccharide **1** with a corresponding fragment of an ^{1}H NMR spectrum.







Plesiomonas shigelloides O54 acetic acid released core:



Scheme 1. Structures of the isolated oligosaccharides from *P. shigelloides* O17 and *P. shigelloides* O54 cores[5]. Glc residue N in the oligosaccharides **1** and **2** is present ~50%. Δ-GalA is the product of 4,5-β-elimination of α-GalA. RU = O-chain repeat unit. Grey shadowed fragments are common in LPS core from serotypes O17 and O54.

tial absence of GlcN residue. We do not describe oligosaccharides **2** and **3** in detail since similar products were obtained from *P. shigelloides* serotype O54 and were fully characterized.[8]

Elucidation of the inner core structure enabled the analysis of the spectrum of the acid- released oligosaccharide **1**. Spin systems of the repeat unit disaccharide Z–W were identified starting from the easily recognizable H/C-6 (methyl group) of the FucNAc4N residue W. H-1 of the FucNAc4N residue W showed NOE and HMBC correlations to H/C-4 of β-GlcNAc residue Q, indicating the attachment point of the O–SP. Further sequencing was tracked to O-6 of GlcN M and to O-4 of GalA K based on similar evidence. Interestingly, the core contained two similar fragments, α-GalN-4-α-GalA

Table 1
NMR data for oligosaccharide **1** (35 °C, δ, ppm)

Residue	Atom	1	2	3	4	5	6 (6a)	7a (6b)	7b
Hep G	H	4.92	3.96	3.86	3.90	3.63	4.24	3.64	3.83
	C	103.2	71.5	71.8	67.2	73.7	68.8	72.2	
Glc N	H	4.61	3.24	3.53	3.47	3.63	3.81	3.91	
	C	104.1	74.6	76.0	70.4	76.5	61.9		
Gal T	H	4.42	3.58	3.66	3.89	3.68	3.68	3.71	
	C	104.8	72.2	73.8	69.8	76.4	62.9		
GalA H	H	5.03	3.95	4.08	4.52	4.32			
	C	100.0	69.3	70.3	78.3	71.3	177.2		
GalN L	H	5.28	3.47	4.13	4.04	4.34	3.75	3.75	
	C	96.1	52.3	67.6	69.3	72.4	62.0		
GalA K	H	5.46	3.86	4.22	4.43	4.59			
	C	102.6	69.9	68.9	81.0	72.9	176.5		
GlcN M	H	5.17	3.27	3.87	3.50	4.32	3.83	4.08	
	C	97.6	55.3	70.8	70.5	72.4	68.9		
GlcNAc Q	H	4.51	3.77	3.69	3.67	3.51	3.65	3.84	
	C	102.6	56.4	73.7	79.6	75.9	61.2		
FucNAc4N W	H	4.58	3.85	4.18	4.00	4.11	1.34		
	C	102.4	52.1	77.1	55.9	68.6	16.7		
AltNAcA Z	H	4.90	3.99	3.67	4.39	4.41			
	C	102.2	52.7	69.4	70.5	79.2	176.2		

For each monosaccharide only the most intense series of the signals is given (up to 8 variants of each residue were observed). The data for units C-F are not shown since they are similar to the published ones for *P. shigelloides* O54 core.[9]

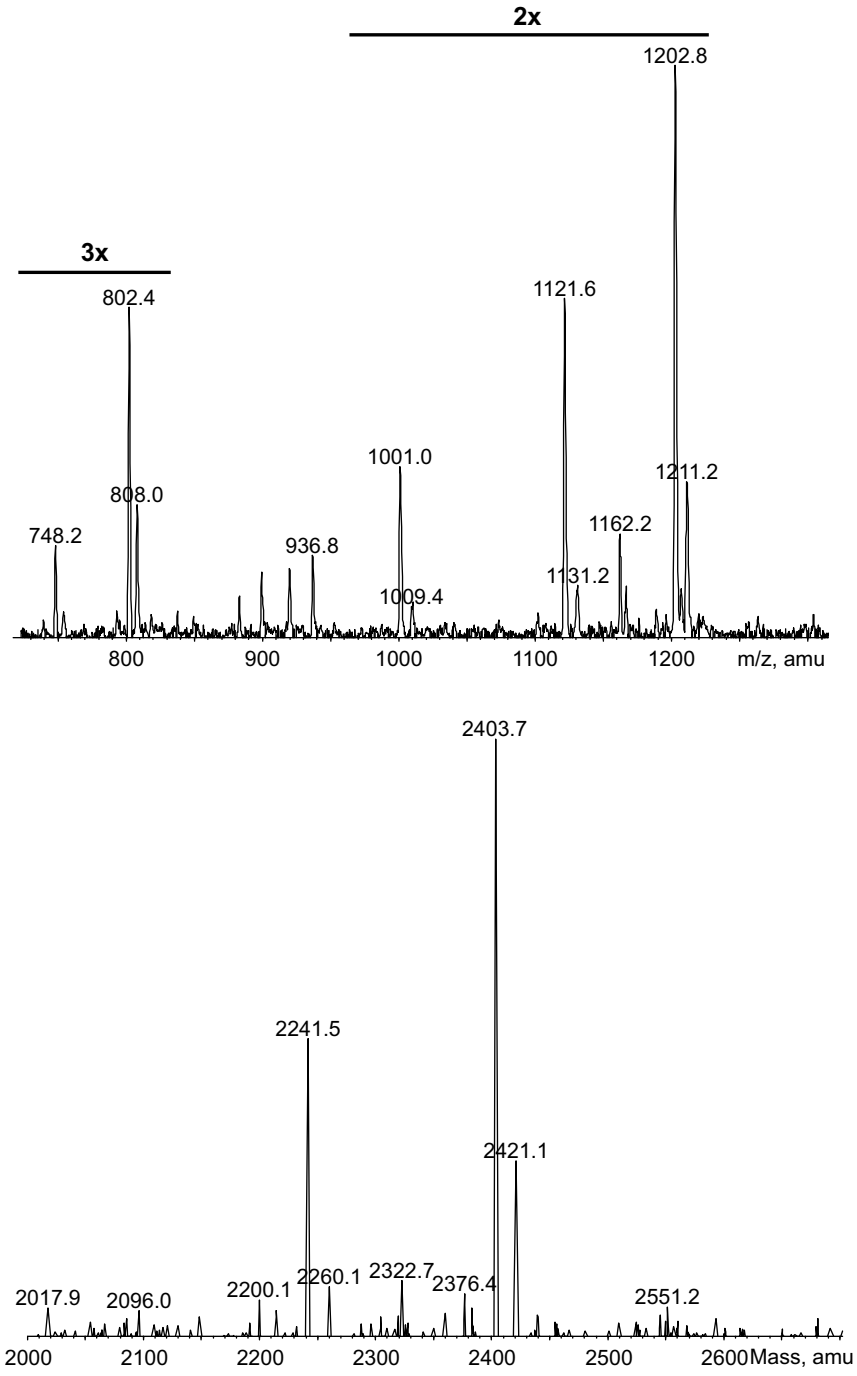


Figure 3. Positive mode ESI mass spectrum of the core oligosaccharide **1** (top) and reconstructed mass spectrum (bottom).

(L–H) and α-GlcN-4-α-GalA (M-K). The aminogroups of GalN L and GlcN M were not acetylated, which followed from the high field position of their H-2 signals (Table 1), the absence of HMBC correlations to acetyl carbonyls, and from mass spectral data.

The structure was confirmed by ESIMS (Fig. 3), where double charged ions at *m/z* 1121.6 and 1202.8 and the respective triple charged ions were observed, corresponding to the molecular masses of 2241.5 and 2403.7 Da; the calculated natural composition mass for the structure $Hex_2Hep_3HexA_2HexN_3HexNA_1dHexNN_1Ac_3anhKdo_1$ is 2402.1 Da, and without Glc N it is 2240 Da. A minor double charged peak at *m/z* 1001 was observed, corresponding to the structure without the Z–W disaccharide (2000 Da, not visible on the reconstructed spectrum, probably because the corresponding triple charged ion intensity was too low). Observed masses were ~1.5 Da higher than calculated, which is nearly always the case with LPS core fragments analyzed by NMR prior to mass spectrometry due to incomplete exchange of D to H.

Overall, the structure of the *P. shigelloides* O17 core was similar to that of serotype O54[8,9] (Scheme 1). The difference being the presence of the additional α-GalN-4-α-GalA (L-H) fragment on O-7 of Hep G and the absence of the side chain β-Gal at O-4 of GlcN M. Attachment of the O-chain in serotype O17 was through O-4 of β-GlcNAc, whereas in the serotype O54 it was through O-4 of β-Glc replacing β-GlcNAc.

1. Experimental

1.1. Bacterial strains and lipopolysaccharide isolation

P. shigelloides strain 7-63 (serotype O17) was grown as described.[4] LPS was extracted by the hot phenol method,[10] ultraspun, and the water phase dialyzed and freeze-dried.

1.2. Oligosaccharides

Oligosaccharide **1** was isolated from LPS hydrolysate (2% AcOH, 100 °C, 3 h) by chromatography on a Sephadex G50SF column (2.5 × 80 cm, pyridine–AcOH buffer), monitored by a Waters refractive index detector. Oligosaccharides **2** and **3** were prepared by treatment of the LPS (50 mg) with 4 mL of 4 M NaOH in a polypropylene vial (120 °C, 16 h). The solution was neutralized by cold 4 M HCl, passed through a Seppak C18 cartridge in water, the oligosaccharide mixture desalted on the Sephadex G50SF column, and further separated on a Dionex Carbopac PA1 column (25 × 0.9 cm) in a gradient of 30–80% of 1 M AcONa in 0.1 M NaOH at 3 mL/min over 1 h with pulsed amperometric detector (Dionex). The oligosaccharides were desalted by Sephadex G15 chromatography.

1.3. NMR spectroscopy, mass spectrometry and GLC

^{1}H and ^{13}C NMR spectra were recorded using a Varian Inova 500 spectrometer in D_2O solutions at 35 °C with acetone standard (2.23 ppm for ^{1}H and 31.5 ppm for ^{13}C) using standard COSY, TOCSY (mixing time 120 ms), NOESY (mixing time 300 ms), HSQC, HMBC (100 ms long-range transfer delay) pulse sequences. ESI mass spectra were obtained using a Micromass Quattro spectrometer in 50% MeCN with 0.2% HCOOH at a flow rate of 15 μL/min with direct injection. GLC, GLC–MS, methylation, and monosaccharide analyses were performed as described.[11]

Acknowledgments

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