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Note

The structure of the polysaccharide part of the LPS from *Serratia marcescens* serotype O19, including linkage region to the core and the residue at the non-reducing end

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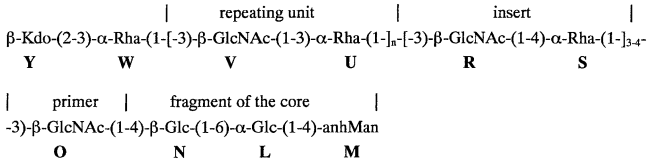
Abstract

The structure of the LPS from *Serratia marcescens* serotype O19 was investigated. Deamination of the LPS released the O-chain polysaccharide together with a fragment of the core oligosaccharide. The following structure of the product was determined by NMR spectroscopy, mass spectrometry, and chemical methods:



The main polymer consists of a repeating disaccharide **V**-**U** and is present on average of 18 units per chain as estimated by integration of signals in the NMR spectra. The residue **O** corresponds to the primer, which initiates biosynthesis of the O-chain, and an oligomer of a disaccharide **R**-**S** is an insert between the primer and the main polymer. The polysaccharide has a β-Kdo residue at the non-reducing end, a feature similar to that observed previously in the LPS from *Klebsiella* O12.

Keywords: LPS; *Serratia*; O-chain; Core

1. Introduction

Serratia marcescens is an opportunistic pathogen, causing urinary tract infections, endocarditis, meningitis and bloodstream infections.[1,2] *Serratia* can invade bloodstream through the intestinal tract in compromised hosts. Being a Gram-negative rod, *Serratia* can cause endotoxin shock and multiple organ failure when a number of bacteria invade the bloodstream. Pathogenicity of these bacteria is due to their complement resistance, production of hemolysins and proteases that degrade some serum proteins, formation of fimbriae, and the presence of the lipopolysaccharide (LPS) in their cell wall.[2]

Abbreviations: ESIMS, electrospray ionization mass spectrometry; LPS, lipopolysaccharide; *P*, phosphate; Hep, L-*glycero*-D-*manno*-heptose; DD-Hep, D-*glycero*-D-*manno*-heptose; GalA, D-galacturonic acid; Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid; Ko, D-*glycero*-D-*talo*-oct-2-ulosonic acid; 2,5-anhMan, 2,5-anhydro-D-mannose; Ara4N, 4-amino-4-deoxy-L-arabinose.

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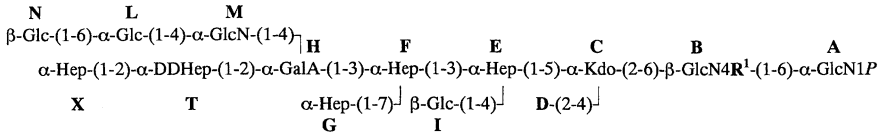


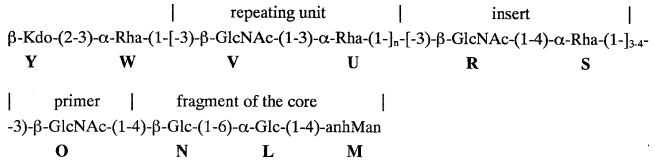
Recently we have determined the following structure of the core part of the LPS from *S. marcescens*:[3]

where **D** = α-Ko or α-Kdo and R^1 = β-Ara4N-1-*P* or *P*. The presence of the GlcN residue with the free amino group enables application of $NaNO_2$ deamination to release the O-chain polysaccharide with the fragment of the core. This procedure has been successfully employed for analysis of the LPS from *Klebsiella* and resulted in determination of the fine polysaccharide structures, including identification of acid-labile Kdo substituents at the non-reducing end of the polymers.[4] Now we report on application of this methodology for a detailed analysis of the O-chain structure in the LPS from *S. marcescens* serotype O19, which has been partially described previously.[5]

2. Results and discussion

S. marcescens O19 LPS was treated with $NaNO_2$–HOAc and the lipid A-containing part was removed by ultracentrifugation. Soluble products were fractionated by GPC, and the polysaccharide fraction was further purified by anion-exchange chromatography. Part of the polysaccharide was reduced with $NaBH_4$ and used for compositional analysis (GC of the alditol acetates or acetylated methanolysis products) and methylation analysis. The following monosaccharides were detected: Glc, Rha, GlcN, 2,5-anhydromannose (anhMan), and Kdo. Methylation analysis revealed the presence of terminal Kdo, 3- and 4-substituted Rha, 3-substituted GlcNAc, 4- and 6-substituted Glc, and a monosubstituted 2,5-anhydrohexose.

The polysaccharide was analyzed by NMR spectroscopy using the methodology outlined elsewhere.[6]The spectra were well resolved and contained a number of signals in addition to the 'repeating unit' signals (Fig. 1). The majority of the signals in the 2D NMR spectra of the polysaccharide (Table 1) were assigned to the following polysaccharide structure:



Interresidual NOE and HMBC correlations were in agreement with this structure.

Several separate regions were found in the polymer. Most of it is built up of repeating disaccharide units, which was previously reported as the main component of the polymer:[5]

[-3)-β-GlcNAc-(1-3)-α-Rha-(1-]
V U

Integration of the ^1H anomeric signals and some selected signals in the HSQC spectrum relative to the signals of units **M** and **L** of the core showed the presence on average of 18 **V**-**U** repeats.

In addition, the polymer includes another repeating structure with an altered substitution position of Rha:

[-3)-β-GlcNAc-(1-4)-α-Rha-(1-]
R S

The presence of a ~4-fold smaller amount of 4-substituted Rha compared to 3-substituted Rha has also been reported previously.[5] **V**-**U** and **R**-**S** units occur as two separate blocks, the **R**-**S** block being located closer to the core region of the LPS. Integration of the NMR spectra showed the presence of three to four **R**-**S** units per chain.

A β-Glc-(1-6)-α-Glc-(1-4)-anhMan (**N**-**L**-**M**) fragment at the reducing end, which originated from the LPS core, was identified based on the core structure determined previously.[3] This fragment is substituted with a β-GlcNAc residue **O**, which carries the **R**-**S** oligomer. That the GlcNAc residue **O** is a linker rather than a component of the repeating unit can be proposed only tentatively since 3-substituted β-GlcNAc is present also in the repeating units. This assumption is based on an analogy with the *Klebsiella* LPS structure, in which a special linker of a single β-GlcNAc residue that intervenes between the first repeating unit and the core has been reliably identified.[4] Usage of this analogy seems to be reasonable since *Klebsiella* has a similar LPS core structure and the same assembly mechanism of the O-chain polysaccharide as *Serratia*.[7–9]

The polysaccharide contains a single β-Kdo residue **Y**, whose anomeric configuration was assigned by the position of the ^1H NMR signals, including the most characteristic one of H-3eq at 2.42 ppm.[10] The attachment site of this residue was determined by a HMBC correlation between C-2 of β-Kdo and H-3 of Rha residue **W**. The terminal position of β-Kdo was confirmed by methylation analysis.

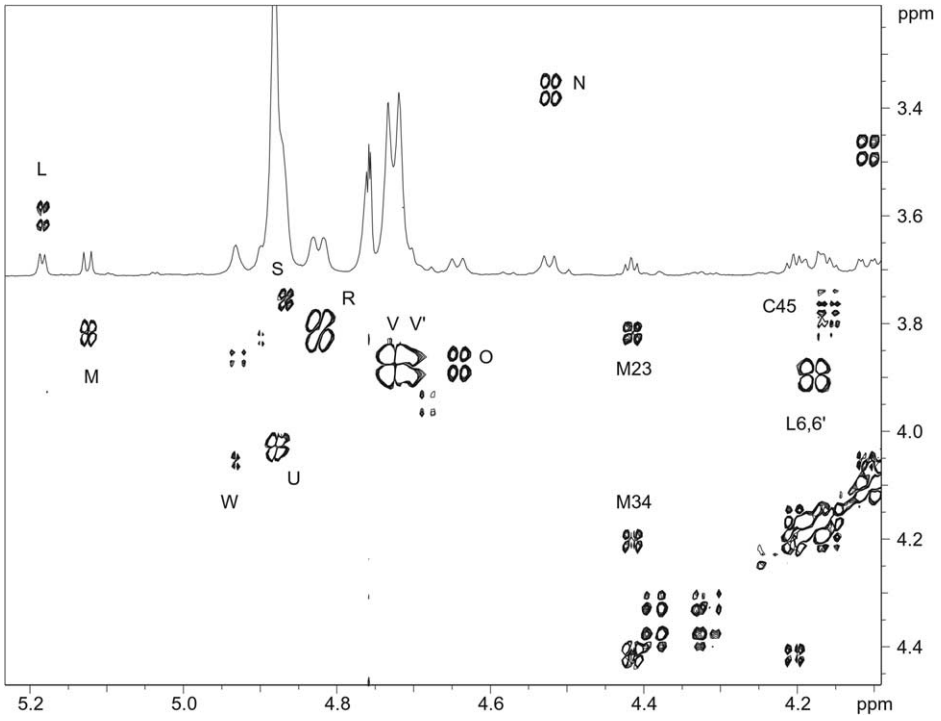


Fig. 1. Fragment of a 800-MHz COSY spectrum of the polysaccharide showing correlations for anomeric protons (designated by single letters) and some adjacent cross-peaks.

The ESI mass spectrum of the non-reduced polymer (Fig. 2) contained peak clusters separated on average by 349 Da, which corresponds to a GlcNAc-Rha unit (calculated value 349.3 Da). In contrast to the NMR spectra, which showed only a hydrated form of anhMan, only species with a non-hydrated form of anhMan

Table 1
NMR data for the *S. marcescens* polysaccharide (ppm)

Unit	Nucleus	1	2(3ax)	3(3ax)	4	5	6(6a)	7(6b)	8a	8b
β-Kdo **Y**	^{1}H		1.81	2.42	3.75	3.93	3.59	3.86	3.85	3.73
	^{13}C		102.8	35.3	68.3	66.0	74.1	69.8	65.0	
α-Rha **W**	^{1}H	4.87	3.99	4.04	3.42	4.01	1.19			
	^{13}C	101.0	72.0	75.4	70.9	69.6	17.0			
β-GlcNAc **V**[a]	^{1}H	4.66 (4.65)	3.81 (3.82)	3.58 (3.64)	3.52 (3.53)	3.42	3.89	3.77		
	^{13}C	102.7	56.3	82.3 (80.0)	69.0	76.4	61.2			
α-Rha **U**	^{1}H	4.81	3.96	3.74	3.44	3.97	1.19			
	^{13}C	101.8	70.9	81.0	71.3	69.7	17.0			
β-GlcNAc **R**	^{1}H	4.76	3.76	3.56	3.47	3.39	3.88	3.72		
	^{13}C	101.6	56.3	82.3	69.0	76.4	61.2			
α-Rha **S**	^{1}H	4.80	3.69	3.77	3.57	3.95	1.23			
	^{13}C	101.8	71.6	71.0	80.7	67.9	17.3			
β-GlcNAc **O**	^{1}H	4.58	3.81	3.57	3.50	3.46	3.90	3.72		
	^{13}C	101.4	56.1	82.3	68.7	76.4	61.2			
β-Glc **N**	^{1}H	4.46	3.30	3.61	3.54	3.48	3.81	3.61		
	^{13}C	103.1	73.4	75.0	79.9	75.1	60.8			
α-Glc **L**	^{1}H	5.12	3.54	3.65	3.48	3.75	4.12	3.83		
	^{13}C	98.7	71.7	73.3	69.9	68.1	69.0			
anhMan **M**	^{1}H	5.06	3.75	4.35	4.14	4.09	3.74	3.70		
	^{13}C	89.9	85.9	77.2	83.7	83.1	62.2			

Signals for NAc are at 2.04 ppm (^{1}H) and 22.8 ppm (^{13}C).
[a] When different, data of the residue substituted with Rha **W** are given in parentheses.

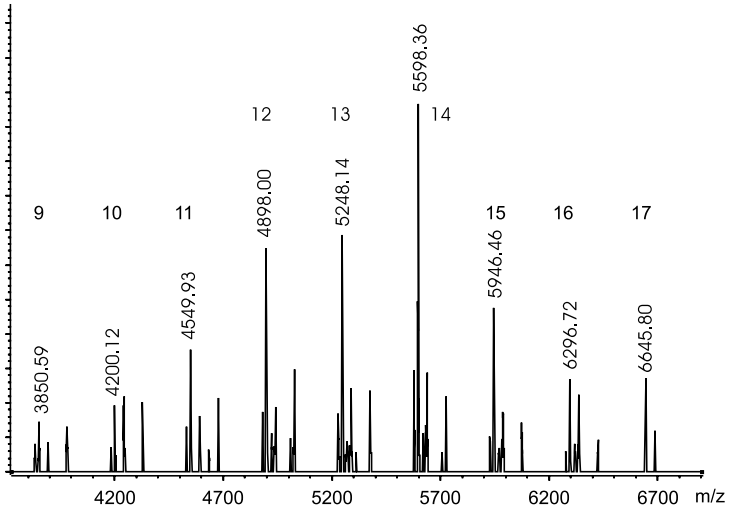


Fig. 2. ESI mass spectrum of the polysaccharide. Numbers above mass numbers indicate the number of the Rha-GlcNAc repeats.

were observed. Absolute mass numbers for the main series of peaks corresponded to the proposed structure (see above) with equal number of Rha and GlcNAc units. There are two explanations for this finding:

1) All Rha and GlcNAc residues belong to repeating units with no primer and no special Rha residues;
2) GlcNAc residue **O** is a 'primer' in the O-chain polysaccharide biosynthesis,[9] and Rha residue **W** is no part of the repeating unit and together with β-Kdo residue **Y** forms the terminating disaccharide.

The second situation has been reported to occur in the *Klebsiella* LPS.[4] Therefore, by analogy the structure of the *Serratia* polysaccharide is presented accordingly.

According to the MS data (Fig. 2), the molecular mass of the polymer ranges between 9 and 17 Rha-GlcNAc units, with the maximum at 14 units. This is less than 21–22 units estimated from the NMR data, which can be accounted for by the presence of a small amount of longer-chain polymers not detectable by MS.

The presence of the repeating units with differently substituted rhamnose residues in the O-chain polysaccharide from *S. marcescens* O17 and O19 has been reported previously,[5] but their distribution remained unknown. The occurrence of separate blocks of different disaccharide units demonstrated in this work is similar to the pattern observed earlier in the *Klebsiella* O1 and O2 polysaccharides.[4]

The presence of the repeating units of two types may account for the serological relatedness of some *S. marcescens* strains from different serotypes, whose O-chain polysaccharides contain similar repeating units. The following group of similar polysaccharides was originally classified into three serotypes, O1, O17, and O19:

	-Y)-β-GlcNAc-(1-X)-α-Rha-(1-	
	Y	**X**
O1	3	4
O17, O19	3	3

The structures of the polysaccharides from serotypes O17 and O19 have been shown to be identical.[5] In the new classification scheme these two serotypes, together with serotype O1, are combined into one serotype O19.[11] The true difference between these strains may be in the lengths and position of the blocks of the repeating units of different structures.

The polysaccharide is terminated with a β-Kdo residue, whose attachment may serve as a signal to stop the chain growth during biosynthesis of the polymer. The terminal β-Kdo may contribute to the interaction of the LPS with antibodies.

3. Experimental

S. marcescens strain IFO 3735 was from the collection of the Institute for Fermentation, Osaka, Japan. Bacteria were cultivated under aerobic conditions in Tryptic Soy Broth medium (Difco) at 37 °C in a fermentor. Bacteria were killed with 1% aq phenol, washed three times with water, then twice with EtOH and finally dried with acetone and Et_2O. The LPS was obtained in a yield of 3% of dry bacterial mass weight by the hot phenol/water extraction according to Westphal et al.[12] and purified by repeated runs in an ultracentrifuge (105,000*g*, three times, 4 h each). Other methods were as described.[4]

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