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The structure of the *Escherichia coli* O148 lipopolysaccharide core region and its linkage to the O-specific polysaccharide

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Abstract

Recently it was demonstrated that *Shigella dysenteriae* type 1, a cause of severe dysentery epidemics, gained its O-specific polysaccharide (O-SP) from *Escherichia coli* O148. The O-SPs of these bacteria differ only by a galactose residue in the repeat unit of *S. dysenteriae* type 1 in place of a glucose residue in *E. coli* O148. Here we analyzed the core structure and its linkage to the O-SP in *E. coli* O148 LPS. Both were found to be identical to those of *S. dysenteriae* type 1 structures, further supporting the relatedness of these two bacteria. The following structure of the core with one repeat unit of the O-SP has been assigned (all have α -configuration except L-Rha):

Keywords

E. coli O148; LPS; O-specific polysaccharide; core; structure

Surface polysaccharides of pathogenic bacteria, including lipopolysaccharides (LPS), serve both as essential virulence factors and as protective antigens. The outer domain of the LPS molecule, termed O-specific polysaccharide (O-SP), shields the bacteria from serum complement killing, similar to the action of capsular polysaccharides.¹ Serum antibodies to the O-SP confer immunity to humans against the homologous bacteria.^{2,3} There are about 170 O-SP types recognized in *Escherichia coli* and about 34 in *Shigella*. At least 13 were reported to be identical in both bacteria. It was postulated recently that as many as 21 of the 34 *Shigella* O-SP and associated gene clusters are identical to or related to those of *E. coli*, indicating a relatedness of these two bacteria.^{4,5} Similarly, the core part of their LPSs is shared between these bacteria, with *Shigella* species having any of the five core types known for *E. coli*.^{4,6,7}

E. coli O148 O-SP structure was reported recently and was found to be similar to that of *S. dysenteriae* type 1 O-SP, bacterium causing severe dysentery epidemics in developing

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countries. Its O-SP differs from that of *S. dysenteriae* type 1 only by having a glucose residue in place of a galactose.⁸ The two bacteria also use the same genes for O-SP synthesis, with the same organization and high level of DNA identity. Based upon these data, it was proposed that *S. dysenteriae* type 1 gained its O-SP from *E. coli* O148 with loss of the glucosyltransferase gene and acquisition of a plasmid-borne galactosyltransferase. We report additional similarity of these two bacteria as they share the same core oligosaccharide structure and its attachment to the O-SP.

Sets of the NMR spectra - COSY, TOCSY, NOESY, HSQC, HMBC, and ^1H - ^{31}P HMQC were recorded for the three fractions obtained after acid hydrolysis of the LPS: F1, containing high molecular mass O-SP, F2, containing core with several repeat units of O-SP and F3, containing core only. All major signals were identified. Relative configurations of the constituent monosaccharides were identified on the basis of vicinal proton coupling constants and ^{13}C NMR chemical shifts. They were in agreement with the standard values for each monosaccharide.^{9,10} Anomeric configurations were deduced from the $J_{1,2}$ coupling constants and chemical shifts of H-1, C-1, and C-5 signals, as well as by observation of intraresidual NOE correlations (H-1:H-3, H-1:H-5) characteristic for the β pyranosides.

The published structure of the O-SP was confirmed (Fig. 1). Spectra of the core part corresponded to the R4 type structure, monosaccharide sequence was identical to that determined for the *S. dysenteriae* type 1 core.⁶ Positions of the signals for the inner part of the core were close to published.¹¹ Hep E and F were incompletely phosphorylated at position 4 with *PP*EtN (or *P*) and *PE*tN groups, respectively (like observed in *S. dysenteriae* type 1 core, data not shown). Analysis of the spectra of F2, containing core plus several repeat units of O-SP (Table 1), showed that the attachment of the O-SP to the core was also identical to that of *S. dysenteriae* type 1. The first repeat unit was bound to the core Gal P and had an inverted anomeric configuration of the GlcNAc residue (α - in polymeric chain and β - in the first repeat unit). Positions of most NMR signals of the F2 fraction were almost identical to that of the corresponding fraction isolated from *S. dysenteriae* type 16 with the only exception of the Glc residue replacing the Gal residue present in *S. dysenteriae* type 1 repeat unit.

The structures of the core and the core plus short O-SP fragments were confirmed by mass-spectrometry analyses. ESI mass spectra of the core contained major double and triple charged ions corresponding to the compound with molecular mass of 1785.2 amu ($\text{Hex}_5\text{Hep}_3\text{Kdo}_1\text{P}_2$), and this compound with the additional *PE*tN (+123 amu). Core substituted with short O-SP fragments showed mainly signals of compounds with two (3099.1 amu) and three (3756.6 amu) repeat units, and peaks of these components with the additional phosphate (+80 amu), *PE*tN (+123 amu), and *PP*EtN (+203 amu) (Fig. 2). The degree of phosphorylation as determined by NMR data seems to be smaller than that detected by mass spectroscopy, probably because of better ionization of the variants containing *PE*tN in negative mode (positive mode spectra were of low quality). Our finding of identical core structures and their linkage to the O-SP further illustrates the association between *S. dysenteriae* type 1 and *E. coli* O148.

1. Experimental

1.1. Growth of bacteria and isolation of LPS

E. coli O148 strain 201 was obtained from the CDC (Atlanta, GA) and cultured in Tryptic Soy Broth (Difco Laboratories) for 20 h at 37 °C with stirring and aeration; the pH was maintained at ~7.5 by addition of ammonium hydroxide. The identity of bacteria was confirmed by agglutination with typing antisera obtained together with the strain from CDC and by routine typing tests performed at Clinical Microbiology Laboratory at NIH, Bethesda,

MD. LPS, extracted by hot phenol, 12 was recovered from the water phase and ultracentrifuged two times at 120 000 g for 5 h, at 4 °C; the content of proteins and nucleic acids in the final LPS preparation was less than 1.5% each.

1.2. Mild hydrolysis of the LPS

The LPS (100 mg) was treated with 10 mL 1% acetic acid at 100 °C for 1.5 h. Lipid A was removed by ultracentrifugation as above, and soluble products were separated by gel chromatography on a BioGel P-10 (1 × 100 cm) column equilibrated with 0.05 M pyridinium acetate buffer, pH 5.5, and monitored with a differential refractometer (Knauer, Germany). The following fractions were separated: high molecular mass O-SP (28 mg), oligosaccharide containing the core plus several O-SP repeat units (5 mg) and core (8 mg).

1.3. NMR spectroscopy

NMR spectra were recorded at 35 °C in D₂O on a Varian UNITY INOVA 500, instrument, using acetone as reference for proton (2.225 ppm) and carbon (31.5 ppm) spectra. Varian standard programs DQCOSY, NOESY (mixing time of 400ms), TOCSY (spinlock time 120 ms), gHSQC, and gHMBC (long-range transfer delay 100 ms) were used.

1.4. Mass spectrometry

Prince CE system (Prince Technologies, The Netherlands) was coupled to a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex, Canada). A sheath solution (isopropanol-methanol, 2:1) was delivered at a flow rate of 1.0 µL/min. Separated fractions were obtained using 90 cm long bare fused-silica capillary and 15 mM ammonium acetate in deionized water, pH 9.0. Five kV or -5 kV electrospray ionization voltage was used for positive and negative ion detection modes, respectively.

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where RU = $[-3\alpha\text{-Rha}-3\alpha\text{-Rha}-2\alpha\text{-Glc}-3\alpha\text{-GlcNAc-}]_n$

Fig. 1.

Structure of the core with one O-SP repeat unit. All sugars are in pyranose form; absolute configuration of rhamnose is L, all other sugars have D-configuration. Phosphorylation is minor, consists mainly of *P* or *PP*EtN on Hep E and *PE*tN on Hep F.

Fig. 2. Deconvoluted negative ion ESI mass spectrum of the core-O-SP fraction (F2) of the acetic acid hydrolyzed LPS isolated from *E. coli* O148. RU, repeat unit (exact mass 657.25 amu).

Table 1

NMR data for *E. coli* type O148 core plus 2-3 O-SP repeat unit. NAc: 2.08/23.4 ppm. Since no actual structure with one RU was present, data for the repeat unit are combined from Z-Y-X sequence of the first repeat, and T from the end of chain.

Unit		H/C-1	H/C-2	H/C-3	H/C-4	H/C-5	H/C-6a/b
Rha T	H	5.08	4.07	3.85	3.47	3.85	1.32
	C	103.5	71.4	71.4	73.3	70.3	17.8
Rha Z	H	5.09	4.17	3.88	3.56	3.88	1.32
	C	102.8	70.8	79.2	72.6	70.5	17.8
Glc Y	H	5.52	3.66	3.77	3.47	3.64	3.77/3.77
	C	98.7	77.9	73.6	70.3	73.5	61.4
GlcNAc X	H	4.63	3.82	3.84	3.70	3.49	3.76/3.93
	C	102.5	55.4	79.2	72.5	77.0	61.7
Gal P	H	4.46	3.55	3.65	3.91	3.81	3.89/3.96
	C	104.3	72.1	73.6	69.3	74.0	68.5
Gal M	H	5.27	3.86	3.97	4.02	4.17	3.76/3.76
	C	97.1	69.5	70.6	71.0	72.5	62.5
Gal L	H	5.58	4.02	4.12	4.07	4.10	3.77/3.77
	C	93.7	73.5	69.0	70.5	72.4	62.4
Glc K	H	5.76	3.78	4.03	3.73	4.18	3.88/4.01
	C	96.1	75.1	71.2	79.3	71.3	60.9
Glc H	H	5.21	3.68	4.08	3.77	3.92	3.82/3.89
	C	102.6	71.4	77.8	71.5	73.6	61.0