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Rapid Communication

The core structure of the lipopolysaccharide from the causative agent of plague, *Yersinia pestis*

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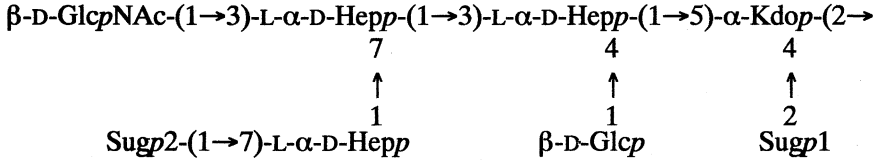
Abstract

The rough-type lipopolysaccharide (LPS) of the plague pathogen, *Yersinia pestis*, was studied after mild-acid and strong-alkaline degradations by chemical analyses, NMR spectroscopy and electrospray-ionization mass spectrometry, and the following structure of the core region was determined:



where L-α-D-Hep stands for L-*glycero*-α-D-*manno*-heptose, Sug1 for either 3-deoxy-α-D-*manno*-oct-2-ulosonic acid (α-Kdo) or D-*glycero*-α-D-*talo*-oct-2-ulosonic acid (α-Ko), and Sug2 for either β-D-galactose or D-*glycero*-α-D-*manno*-heptose. A minority of the LPS molecules lacks GlcNAc.

Keywords: Lipopolysaccharide; *Yersinia pestis*; Core structure

Plague, an acute contagion that comes to pass as a rodent epizootic, was the reason for several devastating pandemics resulting in the ruination of millions of human beings.[1,2] Pathogenicity of the causative agent of plague, the bacterium *Yersinia pestis*, is determined by a number of factors including a rough-type lipopolysaccharide (LPS),[3,4] which mediates serum resistance and infective toxic shock.[1,2] Elucidation of the chemical structure of the LPS may usher in a new era in understanding pathogen–host interactions on a molecular level. Here we report on the structure of the core region of *Y. pestis* LPS, which has not been hitherto studied in detail.

Y. pestis strain KM218, a plasmidless derivative of the Russian vaccine strain EV line NIIEG, was grown at 25 °C in liquid aerated media containing fish-flour hydrolysate and yeast autolysate. The LPS was isolated from dried biomass by phenol–water extraction[5] and purified by treatment with DNAse, RNAse, and Proteinase K, followed by ultracentrifugation (105,000*g*, 4 h).

GLC–MS of the acetylated methyl glycosides prepared after methanolysis of the LPS with 2 M HCl in methanol (85 °C, 16 h) revealed the presence of hexoses, heptoses, 2-amino-2-deoxyhexoses, 4-amino-4-deoxyarabinose, 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), and D-*glycero*-D-*talo*-oct-2-ulosonic acid (Ko). When methanolysis was performed for a shorter time

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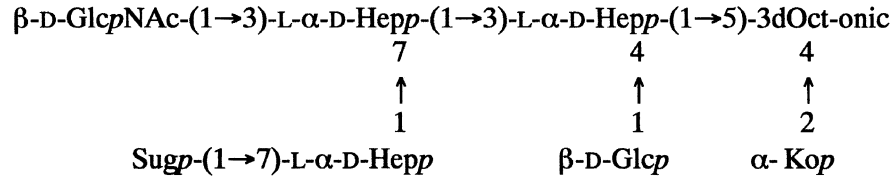
(85 °C, 45 min), a derivative of a Ko–Kdo disaccharide (M_r 852 Da) was identified by GLC–MS by typical fragment ions for Kdo at the reducing end (m/z 375) and Ko at the nonreducing end (m/z 461) in electron-impact MS[6] and an ammonium-adduct ion $[M+18]^+$ at m/z 870 in chemical-ionization MS. The stability of the glycosidic linkage of Ko towards methanolysis has been previously reported.[7]

The LPS was degraded with 2% HOAc (100 °C, 3 h), and a core oligosaccharide (OS) was isolated by GPC of the water-soluble portion on Sephadex G-50 in a yield of 30% of the LPS. Sugar analysis of the OS by GLC–MS of the acetylated alditols derived after hydrolysis with 2 M CF_3CO_2H (100 °C, 4 h) showed the presence of glucose, galactose, L-*glycero*-D-*manno*-heptose (L,D-Hep), D-*glycero*-D-*manno*-heptose (D,D-Hep), and 2-amino-2-deoxyglucose in the ratios 1:0.4:1.9:0.6:0.8, respectively.

The Fourier-transform ion-cyclotron resonance ESI mass spectrum of the OS showed two series of ion peaks with a mass difference of 236 Da corresponding to Ko-containing (OS1) and Ko-lacking (OS2) oligosaccharides. The series for OS1 contained two major ion peaks for octasaccharides $GlcNAcHex_2Hep_3KoKdo$ and $GlcNAcHexHep_4KoKdo$ with M_r 1577.5 and 1607.6 Da (here and below experimental monoisotopic molecular masses are given) and two minor peaks for the corresponding GlcNAc-lacking heptasaccharides with M_r 1374.5 and 1404.5 Da, respectively. Similarly, the series for OS2 included two major peaks for heptasaccharides $GlcNAcHex_2Hep_3Kdo$ and $GlcNAcHexHep_4Kdo$ with M_r 1341.5 and 1371.5, respectively, and two minor peaks for GlcNAc-lacking hexasaccharides with M_r 1138.4 and 1168.4. A peak for a compound with a Kdo anhydro form having a lower molecular mass by 18 Da accompanied each peak in the series for OS2 but not for OS1. Based on these and sugar analysis data, it was concluded that in the LPS of *Y. pestis* there are several core variants, in which Gal interchanges with D,D-Hep and Kdo with Ko. Upon mild-acid degradation, the lateral Kdo residue cleaved completely to give OS2, whereas the lateral Ko residue, whose glycosidic linkage is more stable than that of Kdo, did not cleave, and OS1 was obtained.

The OS was fractionated by anion-exchange chromatography on HiTrap Q using a 0 → 1 M gradient of NaCl in water to give OS1 and OS2. Comparison of the ^1H NMR spectra showed that, in accordance with the ESI MS data, the difference between OS1 and OS2 is restricted to the Kdo (Ko) region. The lesser heterogeneous OS1 was reduced with $NaBH_4$ and further fractionated by high-performance anion-exchange chromatography on CarboPac PA1 (Dionex) in a 0.05 → 0.5 M gradient of AcONa in 0.1 M NaOH to give oligosaccharides **1** and **2** and a number of mixed fractions.

The ^1H and ^{13}C NMR spectra of **1** and **2** were assigned using 2D COSY, TOCSY, HSQC, and ROESY experiments. Based on signal splitting and coupling constant values estimated from the 2D NMR spectra, spin systems for one residue each of β-Glc, β-GlcNAc, Ko, and a 3-deoxyoctonic acid (from Kdo) as well as those for three residues of L-α-D-Hep were identified in both oligosaccharides. In addition, a spin system for β-Gal was identified in **1** and that for D-α-D-Hep in **2**. The ^1H and ^{13}C NMR chemical shifts of the Ko residue were similar to those of α-Ko in the LPS core of *Burkholderia cepacia*.[7] The chemical shifts and ROESY pattern for the region containing one Glc and three L,D-Hep residues were similar to those in the LPS core of *Klebsiella pneumoniae*[8] and indicated the presence of the same tetrasaccharide fragment. The sites of attachment of β-Gal (in **1**), D-α-D-Hep (in **2**), and β-GlcNAc (in **1** and **2**) at the L,D-Hep residues were determined by NOE correlations between the anomeric protons and the protons at the linkage carbons. The glycosylation pattern was confirmed by methylation analysis[9] using a published method for methylation.[10] Based on these data, it was concluded that the oligosaccharides **1** and **2** isolated from OS1 have the following structures:

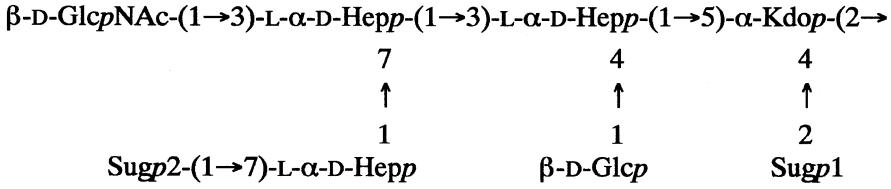


where 3dOct-onic stands for 3-deoxyoctonic acid and Sug for β-D-Gal in **1** or D-α-D-Hep in **2**.

Alkaline degradation of the LPS (4 M NaOH, 100 °C, 4 h) resulted in the expected core-lipid A backbone decasaccharide phosphates $GlcN_3Hex_2Hep_3Kdo_2P$, $GlcN_3Hex_2Hep_3KoKdoP$, $GlcN_3HexHep_4Kdo_2P$, and $GlcN_3HexHep_4KoKdoP$ with M_r 1921.6, 1937.6, 1951.6, and 1967.6 (ESI MS data) as well as the corresponding GlcN-lacking nonasaccharides. However, all these compounds were minor products, whereas the major products gave a similar series of ion peaks in the ESI mass spectrum but had lower molecular masses by 300 Da. Most likely, these compounds resulted from elimination of 4-amino4-deoxyarabinose 1-phosphate from position-4 of the nonreducing-end GlcN residue of lipid A (minor products) and from position-1 of the

reducing-end GlcN residue (major products), in the latter case the elimination being followed by degradation of the lipid A backbone.

Therefore, the data obtained showed that the LPS of *Y. pestis* is distinguished by a high degree of structural heterogeneity, and the LPS core has the following structure:



where Sug1 stands for either α-Kdo or α-Ko and Sug2 for either β-D-Gal or D-α-D-Hep. A minority of the LPS molecules lacks GlcNAc.

This structure shows both similarity to and differences from the LPS core structures of a taxonomically related bacterium, *Yersinia enterocolitica*,[11,12] which is the causative agent of the diarrheal disease yersiniosis.[1,2]

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