Chapter 44

The Core Structure of the Lipopolysaccharide of *Yersinia pestis* Strain KM218

Influence of Growth Temperature

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1. INTRODUCTION

Pathogenicity of the causative agent of plague, the bacterium *Yersinia pestis*, is determined by a number of factors including a rough-type lipopolysaccharide (LPS) (Skurnik *et al.,* 2000), which mediates serum and cationic-antimicrobial-peptides resistance as well as infective toxic shock (Perry and Fetherston, 1997). Elucidation of the chemical structure of the LPS may usher in a new era in understanding pathogen-host interactions on the molecular level. Here we report on the full structure of the core region of *Y. pestis* LPS and the influence of growth temperature on the core structure.

2. ELUCIDATION OF THE LPS CORE STRUCTURE

Y. pestis strain KM218, a plasmidless derivative of the Russian vaccine strain EV line NIIEG, was grown at 25 and 37 °C (flea and mammalian host temperature, respectively) in liquid aerated media containing fish-flour hydrolysate and yeast autolysate. The lipopolysaccharides (LPS-25 and LPS-37) were isolated by phenol-water extraction and purified by treatment with DNAse, RNAse and Proteinase K followed by ultracentrifugation.

Each LPS was degraded with dilute HOAc to cleave the linkage between the core and lipid A moieties. Gel chromatography on Sephadex G-50 afforded core oligosaccharides (fraction II), which were fractionated by anion-exchange chromatography on HiTrap Q (Figure 1) and, after reduction with $NaBH_4$, on CarboPac PA1 (Dionex).

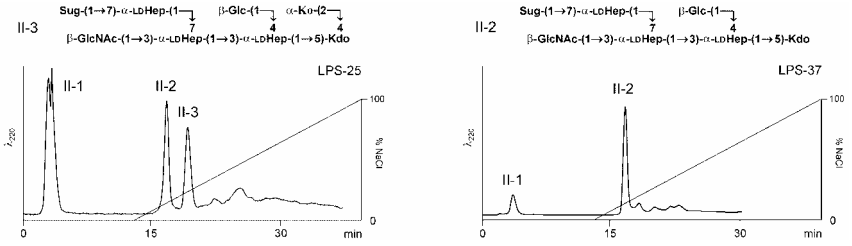

Figure 1. HiTrap Q chromatography of the core oligosaccharides from LPS-25 and LPS-37. II-1 is a contamination; major structures of II-2 and II-3 oligosaccharides are shown on the top. II-2 resulted from the cleavage of the terminal Kdo, whereas Ko in II-3 was not cleaved by acid. Sug is either β-Gal or α-DDHep. For abbreviations of sugars see the legend to Figure 2.

The isolated oligosaccharides were studied by sugar and methylation analyses, ion cyclotron resonance, Fourier transform electrospray ionisation mass spectrometry and one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy. As a result, the structures of the core region of LPS-25 and LPS-37 were determined, and are shown in Figure 2.

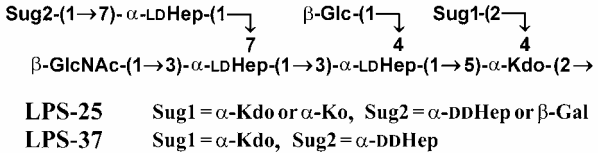

Figure 2. Structures of the core region of LPS-25 and LPS-37. LDHep and DDHep stand for L-*glycero*- and D-*glycero*-D-*manno*-heptose, Kdo stands for 3-deoxy-D-*manno*-octulosonic acid and Ko for D-*glycero*-D-*talo*-octulosonic acid; a minority of the molecules lacks GlcNAc.

3. DISCUSSION

For the first time the full structure of the LPS core of *Y. pestis* has been established. It is distinguished by a structural heterogeneity and variability under different growth temperatures. At 25°C, four major core glycoforms are produced, which differ in the presence of either terminal Kdo or Ko

attached to another Kdo residue and either terminal DDHep or Gal attached to an LDHep residue (LPS-25). At 37°C, there is only one glycoform containing terminal Kdo and DDHep (LPS-37). Hence, at the elevated temperature incorporation of Ko and Gal to the LPS core is suppressed. GlcNAc is present in non-stoichiometric amounts, independent of growth temperature, which further enhances the structural heterogeneity of the core.

Variation in the LPS structure is under the control of the two-component regulatory system PhoPQ (Hitchen *et al.,* 2002), which is involved in the global regulation of virulence gene expression in *Y. pestis*. Together with structural variations of lipid A (Kawahara *et al.,* 2002), the temperature-induced changes in the LPS core structure may protect the bacterium against defence mechanisms of both insect and mammalian hosts.

Remarkably, having one glucose residue less, the core of LPS-37 from *Y. pestis* KM218 represents a truncated form of the LPS core of *Y. enterocolitica* O:8 (Oertelt *et al.,* 2001). This similarity suggests that *Y. pestis* is closely related to not only *Y. pseudotuberculosis* (Skurnik *et al.,* 2000) but also to *Y. enterocolitica.*

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REFERENCES

Hitchen, P. G., Prior, J. L., Oyston, P. C., Panico, M., Wren, B. W., Titball, R. W., Morris, H. R., and Dell, A., 2002, Structural characterization of lipo-oligosaccharide (LOS) from *Yersinia pestis*: regulation of LOS structure by the PhoPQ system. *Mol. Microbiol*. **44**: 1637-1650.

Kawahara, K., Tsukano, H., Watanabe, H., Lindner, B., and Matsuura. M., 2002, Modification of the structure and activity of lipid A in *Yersinia pestis* lipopolysaccharide by growth temperature. *Infect. Immun*. **70**: 4092-4098.

Oertelt, C., Lindner, B., Skurnik, M., and Holst, O., 2001, Isolation and structural characterization of an R-form lipopolysaccharide from *Yersinia enterocolitica* serotype O:8. *Eur. J. Biochem*. **268**: 554-564.

Perry, R. D., and Fetherston, J. D., 1997, *Yersinia pestis* - etiologic agent of plague. *Clin. Microbiol. Rev*. **10**: 35-66.

Skurnik, M., Peippo, A., and Ervelä, E., 2000, Characterization of the O-antigen gene clusters of *Yersinia pseudotuberculosis* and the cryptic O-antigen gene cluster of *Yersinia pestis* shows that the plague bacillus is most closely related to and has evolved from *Y. pseudotuberculosis* serotype O:1b. *Mol. Microbiol*. **37**: 316-330.