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ON THE DETERMINATION OF "KDO-LIKE SUBSTANCE" IN THE LIPOPOLYSACCHARIDE FROM *COXIELLA BURNETII* STRAIN NINE MILE IN PHASE II

R. TOMAN, Ľ. ŠKULTÉTY, J. KAZÁR

Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Slovak Republic

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Summary. – The lipid A proximal carbohydrate region in a lipopolysaccharide (LPS II) isolated from *Coxiella burnetii* strain Nine Mile in avirulent phase II has been reinvestigated. So called "Kdo-like substance", reported to be present in LPS II (Schramek and Mayer, 1982) has been unambigiously identified as the 3-deoxy-D-manno-2-octulosonic acid (Kdo).

Key words: Coxiella burnetii; lipopolysaccharide; Kdo

It has been believed (Schramek and Mayer, 1982; Amano *et al.*, 1987; Mayer *et al.*, 1988) for about a decade that a LPS isolated from *C. burnetii* contains a "Kdo-like substance" as the constituent sugar of lipid A proximal core region. Since Kdo and its derivatives/analogues are important antigenic determinants in bacterial LPSs, it seemed to us of great importance to characterize more deeply the "Kdo-like substance" in *C. burnetii* LPS mainly in connection with our prospective antigen-antibody interaction studies.

C. burnetii strain Nine Mile, serologically in phase II (yolk sac passage 165 in our laboratory), was propagated in chick embryo yolk sacs. The rickettsial cells were killed with formalin and purified as described elsewhere (Schramek *et al.*, 1978). The LPS II was isolated by a modified (Schramek and Brezina, 1979) phenol-chloroform-petroleum ether extraction (Galanos *et al.* 1969).

The LPS II (10 mg) was solubilized in N,N-dimethylformamide (1 ml) and treated with diazomethane in chloroform under stirring for 10 min. After evaporation to dryness, 2 mol/l HCl in methanol (1 ml) was added and the sample was heated at 60 °C for 2 hr. The lipid A precipitate was centrifuged and the supernatant evaporated to dryness, dissolved in methanol and neutralized with silver carbonate. Then the solution (2 ml) was extracted twice with methanol-hexane (1:2.3 ml). The methanolic solution was evaporated and the residue was dissolved in N,N-dimethylformamide (0.5 ml) and methylated (Ciucanu and Kerek, 1984) with methyl iodide (0.2 ml) in the presence of NaH (12 mg) for 1 hr at room temperature. Water (1 ml) and chloroform (1 ml) were then added, and the chloroform layer was washed with water (3 × 5 ml) and dried (Na_2SO_4). Appropriately sized aliquot was taken and analyzed by gas liquid chromatography mass spectrometry (GLC-MS).

GLC-MS was conducted with a Hewlett-Packard Model 5971 A mass selective detector connected to GLC Hewlett-Packard Model 5890 A chromatograph equipped with a chemically bonded SE-54

fused silica capillary column (25 m × 0.32 mm; Weeke, Mülheim, F.R.G.). The column temperature program was 150 °C (3 min) to 305 °C at 5 °C/min. Electron impact (EI) mass spectra were recorded at 70 eV.

In the analysis of "Kdo-like substance" in the LPS II we had to consider several possibilities. The substance to be analyzed could be (a) Kdo, (b) phosphorylated Kdo, and (c) an analogue of Kdo. To elucidate this problem we used the following sequence of reactions. The LPS II was first treated with diazomethane. Any phosphate groups present in the LPS II should be esterified and thus their migration in the further reaction sequence should be prevented. After methanolysis of the LPS II and removal of insoluble lipid A portion, methylation with methyl iodide in N,N - dimethylformamide in the presence of NaH was performed. The methylated material was partitioned between chloroform and water and the organic phase was analyzed by GLC-MS.

Besides a number of undefinable peaks and those assigned to permethylated methyl hexo- and heptosides, one intense and two weak peaks emerged from 30.4 to 30.9 min. The intense peak gave the mass spectrum shown in Fig. 1. This was indistinguishable from that of the standard permethylated methyl ketoside methyl ester of Kdo. The two weak peaks were characterized as the degradation products there of.

In this way, the presence of Kdo in LPS II was unequivocally established. As

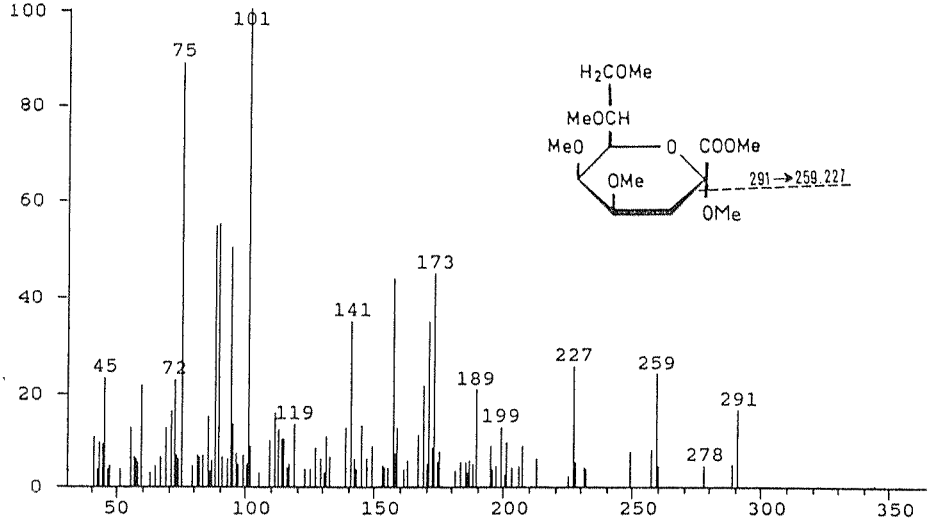

Fig. 1

EI mass spectrum of permethylated methyl ketoside methyl ester of Kdo
Abscissa: mass/charge ratio (m/z); ordinate: relative intensity (%).

no other derivatives or analogues of Kdo could be identified we conclude that "Kdo-like substance" reported to be the constituent of LPS II carbohydrate backbone is indeed the Kdo.

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References

Amano, K., Williams, J. C., Missler, S. R., and Reinhold, V. N. (1987): Structure and biological relationships of *Coxiella burnetii* lipopolysaccharides. *J. Biol. Chem.* **262**, 4740–4747.

Ciucianu, I., and Kerek, F.(1984): A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **131**, 209–217.

Galanos, C., Lüderitz, O., and Westphal, O. (1969): A new method for the extraction of R lipopolysaccharides. *Eur. J. Biochem.* **9**, 245–249.

Mayer, H., Radziejewska-Lebrecht, J., and Schramek, Š. (1988): Chemical and immunochemical studies on lipopolysaccharides of *Coxiella burnetii* phase I and phase II. *Adv. exp.Med. Biol. (Mol. Immunol. Complex. Carbohydr.)* **228**, 577–591.

Schramek, Š. Brezina, R., and Kazár, J. (1978): Influence of mild acid hydrolysis on the antigenic properties of phase I *Coxiella burnetii*. *Acta virol.* **22**, 302–308.

Schramek, Š., and Brezina, R. (1979): Isolation of endotoxic lipopolysaccharide from phase II *Coxiella burnetii*. *Acta virol.* **23**, 349.

Schramek, Š., and Mayer, H. (1982): Different sugar compositions of lipopolysaccharides isolated from phase I and pure phase II cells of *Coxiella burnetii*. *Infect. Immun.* **38**, 53–57.