ANALYSIS OF THE AMINO SUGAR COMPOSITION OF *CHLAMYDIA PSITTACI* LIPOPOLYSACCHARIDE

R. TOMAN

Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Slovak Republic

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Chlamydia psittaci is a widely distributed obligatory intracellular bacterium which exhibits a broad pathogenic potential for birds, ruminants, other animals and man (*1*). A lipopolysaccharide (LPS) isolated from *C. psittaci* PK 5082, associated with enzootic abortion in ewes, was already investigated (*2*). The amino sugar analysis revealed D-galactosamine (GalN) and D-glucosamine (GlcN) in the molar ratio of approximately 1:2. This result is in contrast with the findings reported for the LPSs isolated from other chlamydial species, i.e. *C. trachomatis* (*3*) and *C. pneumoniae* (*4*), in which GlcN was the only hexosamine detected. Therefore, it was decided to re-examine the amino sugar composition of *C. psittaci* LPS.

The ewe abortion strain PK 5082 of *C. psittaci* was propagated in chick embryo yolk sacs (*5*). The elementary bodies (EBs) were purified as reported (*2*). The crude LPS was extracted from EBs (161 mg) by the hot phenol-water extraction (*6*). The LPS (6.5 mg), collected from aqueous phase, was solubilized in 50 mmol/l Tris-HCl pH 7.5 and treated at 37 °C simultaneously with RNase and DNase, and then with proteinase K. After dialysis, it was purified by ultracentrifugation at 120,000 × g for 4 hrs. The final yield was 4.5 mg of LPS corresponding to 2.8% of EBs. Fatty acids were released from the LPS by the combined acid and alkaline hydrolyses (*7*). After extraction of fatty acids with chloroform-ethyl acetate (1:1, v/v), the dried residue (1.5 mg) was dissolved in water (0.3 ml), treated with 33% acetic acid (0.5 ml) and 5% aqueous sodium nitrite (0.5 ml) and kept at 20 °C for 90 mins. The mixture was deionized and the carbohydrate residue was reduced with $NaBH_4$ and acetylated prior to analysis by gas chromatography-mass spectrometry (GC-MS). The latter was performed using a Hewlett-Packard Model 5971 A mass selective detector connected to a Hewlett-Packard Model 5890 A chromatograph equipped with a chemically bonded SE-54 fused silica capillary column (25 m × 0.32 mm; Weeke, Muehlheim, FRG). The column temperature program was 160 °C (3 mins) to 260 °C at 2 °C/min. After sample injection, an intense peak emerged at 13.3 mins. It had the retention time and gave the mass spectrum which was indistinguishable from that of the standard peracetylated 2,5-anhydro-D-mannitol. The compound was evidently obtained from GlcN, present in *C. psittaci* LPS as the product of nitrous acid deamination followed by reduction and acetylation. Using this method, no evidence for the presence of GalN in the LPS investigated could be obtained.

In conclusion, the results indicate clearly that the *C. psittaci* LPS contains solely GlcN in its macromolecule in agreement with the previous findings reported for other chlamydial rough type LPSs isolated from *C. trachomatis* and *C. pneumoniae* (*3,4*). In addition, it is well known that the beta-1,6-linked glucosamine disaccharide constitutes the hydrophylic part of lipid A moiety in chlamydial LPSs.

References

1. Storz, J., and Krauss, H., Chlamydial infections. In H. Blobel and Th. Schliesser (Eds): *Handbuch der bakteriellen Infektionen bei Tieren.* Band V, Gustav Fischer Verlag, Jena, pp. 447–531, 1985.

2. Brade, L., Schramek, Š., Schade, U., and Brade, H., *Infect. Immun.* **54,** 568, 1986.

3. Nurminen, M., Rietschel, E., Th., and Brade, H., *Infect. Immun.* **48**, 573, 1985.

4. Toman, R., Leinonen, M., and Saikku, P., Preliminary structure of lipopolysaccharide of *Chlamydia pneumoniae.* In P.A. Mardh, M. La Placa and M. Ward (Eds): *Proc. Eur. Soc. Chlamydia Res.*, University of Uppsala, Uppsala, p. 14, 1992.

5. Sádecký, E., Trávniček, M., Balaščák, J., Brezina, R., Kazár, J., Urvölgyi, J., Flešár, I., and Čižmár, J., *Vet. Med.* (Prague) **23**, 25, 1978.

6. Westphal, O., and Jann, K., *Methods Carbohydr. Chem.* **5**, 83, 1965.

7. Haeffner, N., Chaby, R., and Szabo, L., *Eur. J. Biochem.* **77**, 535, 1977.