

Structural Analysis of the Lipopolysaccharide from *Chlamydia trachomatis* Serotype L2*

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The lipopolysaccharide (LPS) of *Chlamydia trachomatis* L2 was isolated from tissue culture-grown elementary bodies using a modified phenol/water procedure followed by extraction with phenol/chloroform/light petroleum. From a total of 5×10^4 cm² of infected monolayers, 22.3 mg of LPS were obtained. Compositional analysis indicated the presence of 3-deoxy-D-manno-oct-2-ulopyranosonic acid (Kdo), GlcN, phosphorus, and fatty acids in a molar ratio of 2.8:2.2:1:4.5. Matrix-assisted laser-desorption ionization mass spectrometry performed on the de-O-acylated LPS gave a major molecular ion peak at *m/z* 1781.1 corresponding to a molecule of 3 Kdo, 2 GlcN, 2 phosphates, and two 3-hydroxyeicosanoic acid residues. The structure of deacylated LPS obtained after successive treatment with hydrazine and potassium hydroxide was determined by 600 MHz NMR spectroscopy as Kdo α 2 \rightarrow 8Kdo α 2 \rightarrow 4Kdo α 2 \rightarrow 6D-Glc-pN β 1 \rightarrow 6D-GlcP α 1,4'-bisphosphate. These data, together with those published recently on the acylation pattern of chlamydial lipid A (Qureshi, N., Kaltashov, I., Walker, K., Doroshenko, V., Cotter, R. J., Takayama, K., Sievert, T. R., Rice, P. A., Lin, J.-S. L., and Golenbock, D. T. (1997) *J. Biol. Chem.* 272, 10594–10600) allow us to present for the first time the complete structure of a major molecular species of a chlamydial LPS.

Chlamydia are obligatory intracellular bacteria (1) causing acute and chronic infections in animals and humans (2, 3). *Chlamydia trachomatis* is the world's leading cause of preventable blindness in developing countries in North Africa and Asia. It is also a predominant pathogen in sexually transmitted urogenital infections in developed countries and is considered as the major cause of secondary infertility in women resulting from tubal damage and occlusion. Reactive arthritis is another syndrome resulting from primary genital *C. trachomatis* infection.

Chlamydia pneumoniae is a pathogen of the respiratory tract with which more than half of the adult population is infected in different geographic areas all over the world (4). It is well established that the infection is usually mild in immunocompetent hosts, but severe pneumonias are observed in immunocompromised patients. There is an ongoing discussion on the putative association of *C. pneumoniae* infections with atherosclerosis in general and myocardial infarction in particular (5). If this hypothesis would be verified it is necessary to identify the mechanisms by which chlamydiae can provide a continuous

stimulus of inflammation, which at the same time would help to understand the chronic inflammatory processes in trachoma and pelvic inflammatory disease.

Chlamydiae possess a lipopolysaccharide (LPS)¹ of low endotoxic activity (6, 7), which is attributed to the higher hydrophobicity of its lipid A moiety with fatty acids of longer chain length and the presence of nonhydroxylated fatty acids ester-linked to the sugar backbone. Chlamydial LPS harbors also a genus-specific epitope composed of a 3-deoxy-D-manno-oct-2-ulopyranosonic acid (Kdo) trisaccharide with the sequence Kdo α 2 \rightarrow 8Kdo α 2 \rightarrow 4Kdo α 2 \rightarrow , which is surface-exposed and highly immunogenic (8). It is used in clinical microbiology laboratories as a marker for the whole genus and can be detected with monoclonal antibodies against it. This genus-specific Kdo trisaccharide is biosynthetically assembled by a single Kdo transferase and can be synthesized by recombinant *Escherichia coli* bacteria expressing chlamydial Kdo transferase genes (9). The availability of LPS of such recombinant bacteria actually allowed us to determine the chemical and antigenic structure of the Kdo region (10), because chlamydial LPS was, due to its obligate intracellular growth, so far only available in quantities not allowing a structural analysis with the methods used at that time. With the improvements in analytical chemistry, particularly in NMR spectroscopy and mass spectrometry, the required amounts of LPS were reduced to levels that can now be prepared even from tissue culture-grown chlamydiae. Recently, Qureshi *et al.* (11) have investigated by mass spectrometry the acylation pattern of the lipid A moiety of the LPS of *C. trachomatis* serotype F. Here, we report the structure of the phosphorylated carbohydrate backbone of chlamydial LPS as determined by NMR spectroscopy, which, together with compositional analysis, mass spectrometric data on the de-O-acylated LPS, and the data of Qureshi *et al.* (11), show the first complete structure of a major LPS molecular species in *C. trachomatis*.

EXPERIMENTAL PROCEDURES

General—The following experiments were performed as described: the determination of the absolute configuration of GlcN (12), analytical procedures for the quantification of Kdo, GlcN, and phosphate (12), and high-performance anion-exchange chromatography (13), with the modification that in semi-preparative high-performance anion-exchange chromatography, the column was eluted with a linear gradient of 30–70% 1 M sodium acetate in 0.1 M NaOH for 16 min, and then eluted isocratically with 70% 1 M sodium acetate in 0.1 M NaOH, and that fractions were desalted by gel-permeation chromatography (P5-Econo-Pac cartridges, Bio-Rad, Munich, Germany). The fatty acid analyses were performed according to Wollenweber and Rietschel (14).

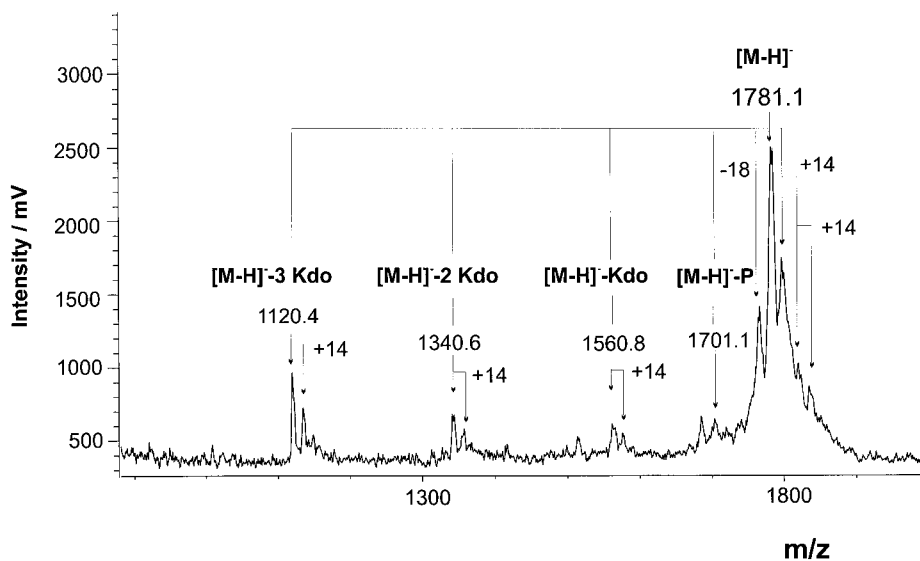
Cultivation of Bacteria—*C. trachomatis* serotype L2 was grown in monolayer cultures of mycoplasma-free L929 cells in multilayer trays (NUNC, 10×600 -cm²) for 2 days. The cultures were killed by the

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¹ The abbreviations used are: LPS, lipopolysaccharide; Kdo, 3-deoxy-D-manno-oct-2-ulopyranosonic acid; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry.

FIG. 1. Negative-ion MALDI mass spectrum of de-*O*-acylated LPS from *C. trachomatis* L2.



addition of 0.5% (w/v) phenol, and the chlamydial elementary bodies were sedimented with the cell debris by centrifugation (Beckman J2–21 centrifuge, JA10 rotor, $9,000 \times g$) yielding 2.88 g from 50,000 cm² of infected cells. LPS was obtained by using the phenol/water method (15), modified as follows. The sediment was suspended in 75 ml of 45% phenol, containing 2% *N*-lauroylsarcosine sodium salt in water (w/v), heated at 68 °C for 10 min, and cooled on ice. After centrifugation ($3,000 \times g$, 30 min), the water phase was removed and the phenol phase was extracted again with 35 ml of 2% aqueous *N*-lauroylsarcosine sodium. After centrifugation and separation as before, the extraction was repeated again. The water layers were combined, dialyzed against water, and lyophilized (yield 261 mg), then extracted twice with 90% aqueous phenol/chloroform/light petroleum (boiling point 40–60 °C) 2:5:8 (v/v/v). The organic solvents were evaporated, and the LPS was precipitated from the phenol phase by dropwise addition of water. The precipitated LPS was washed with acetone (yield 25.6 mg) dissolved in water (5 mg/ml) and precipitated with 10 volumes of ethanol/acetone 9:1 (v/v), dried, dissolved in water (5 mg/ml), and freeze-dried (yield 22.3 mg).

Deacylation Procedure—Deacylation of LPS (2 mg) was according to the method of Holst *et al.* (16) with the modifications that reactions were performed in the same reaction tube and that, after extraction of the fatty acids, the water phase was dialyzed using a dialyzer system (volume, 1 or 1.5 ml, Sialomed, CA) with cellulose acetate membranes (molecular weight cut off, 500).

Matrix-assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS)—MALDI-MS of LPS and de-*O*-acylated LPS was performed with a Bruker-ReflexII (Bruker-Franzen Analytik, Bremen, Germany) in the linear time-of-flight configuration, with continuous ion extraction in the negative ion mode at an acceleration voltage of 30 kV. Samples (<10 nmol) were dispersed in 15 μ l of aqueous triethylamine solution (0.18 M) and treated with small amounts of Amberlite IR-120 (H⁺) cation-exchanger (Merck, Darmstadt, Germany) to remove excess sodium and potassium ions. The solutions were mixed with an equal volume of matrix solution (0.5 M 2,4,6-trihydroxyacetophenone in methanol; Aldrich, Steinheim, Germany) and aliquots of 0.5 μ l were deposited on a metallic sample holder and analyzed immediately after drying in a stream of air. The mass spectra shown are the sum of at least 50 laser shots.

NMR Spectroscopy—One-dimensional ¹H-NMR and two-dimensional ¹H, ¹H and ¹H, ¹³C NMR (pD 5.0) spectra were recorded with a Bruker AMX-600 spectrometer, using a microprobe head (Bruker PHTXI 600SB H-C/N-D-02.5) and Bruker standard software. The ¹H resonances were measured relative to internal acetone (2.225 ppm), and coupling constants were determined on the first-order basis (± 0.5 Hz). The assignment of the proton chemical shifts was achieved by correlation spectroscopy, total correlation spectroscopy, and double-quantum-filtered correlation spectroscopy experiments. The assignment of carbon chemical shifts was achieved by ¹H, ¹³C heteronuclear multiple quantum coherence experiments and by comparison to published ¹³C NMR data (13, 17, 18). Nuclear Overhauser effect contacts were identified using rotating frame nuclear Overhauser effect spectroscopy and nuclear Overhauser effect spectroscopy experiments. ¹³C resonances were determined relative to internal dioxane (67.4 ppm).

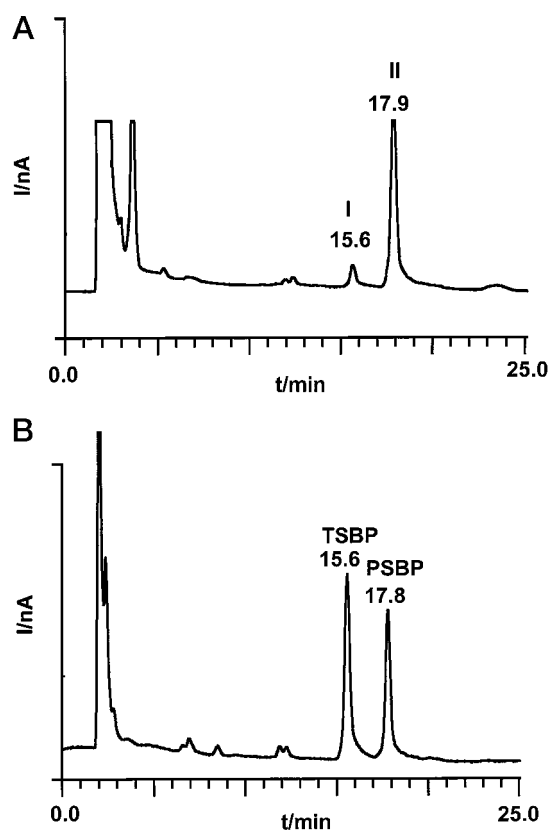


FIG. 2. Analytical high-performance anion exchange chromatogram of deacylated LPS from *C. trachomatis* L2 (A) and *E. coli* F515–207 (B). TSBP, tetrasaccharide bisphosphate; PSBP, pentasaccharide bisphosphate.

RESULTS

Isolation and Characterization of the LPS—Preliminary experiments had shown that the extraction of chlamydial elementary bodies together with the cell debris gave higher yields of LPS than applying the phenol/chloroform/light petroleum method to purified elementary bodies. However, the LPS was equally distributed between the water and phenol phase. When 2% of *N*-lauroyl sarcosyl sodium were added to the phenol/water mixture, the large majority (>90%) of the LPS was found in the water phase (data not shown). Because phenol/water-extracted LPS contains a considerable amount of protein and nucleic acid, the extracted material was further purified by the

TABLE I
¹H and ¹³C chemical shifts of oligosaccharide II isolated from LPS of *C. trachomatis* L2

Spectra were recorded at 600 MHz (¹H) and 125.7 MHz (¹³C) in ²H₂O relative to acetone (¹H, 2.225 ppm) or dioxane (¹³C, 67.4 ppm). For residues A–E see Fig. 4.

Atom	Chemical shift in residue				
	A	B	C	D	E
	<i>ppm</i>				
H-1	5.64 ^a	4.85 ^b			
H-2	3.41	3.01			
H-3 _{ax}	3.84	3.76	1.87 ^c	1.78 ^d	1.79 ^e
H-3 _{eq}			1.97 ^f	2.12 ^g	2.01 ^h
H-4	3.59	3.70	4.15	4.06	4.05
H-5	4.12	3.68	4.09	4.08	4.07
H-6a	4.25	3.43	3.77	3.76	3.66
H-6b	3.69	3.74			
H-7			3.88	4.15	3.88
H-8a			3.89	3.60	3.92
H-8b			3.64	3.59	3.73
C-1	91.0				
C-2	55.1	56.4			
C-3	72.9	74.4	34.3	35.3	34.9
C-4	70.2	73.4	70.9	66.9	66.9
C-5	72.7	74.7	65.9	67.5	67.2
C-6	70.9	63.2	71.9	72.2	71.8
C-7			70.6	70.9	70.2
C-8			63.8	63.8	63.3

^a ³J_{H-1,H-2} 4 Hz, ³J_{H-1,P} 7 Hz.

^b ³J_{H-1,H-2} 7 Hz.

^c Doublet of doublets, ²J_{H-3ax,H-3eq} 11.7 Hz, ³J_{H-3ax,H-4} 4.4 Hz.

^d Doublet of doublets, ²J_{H-3ax,H-3eq} 12.8 Hz, ³J_{H-3ax,H-4} 4.8 Hz.

^e Doublet of doublets, ²J_{H-3ax,H-3eq} 12.5 Hz, ³J_{H-3ax,H-4} 5.1 Hz.

^f Triplet, ²J_{H-3eq,H-3ax} 2.3 Hz.

^g Triplet, ²J_{H-3eq,H-3ax} 12.1 Hz.

^h Triplet, ²J_{H-3eq,H-3ax} 12.1 Hz.

phenol/chloroform/light petroleum method and by precipitation with ethanol/acetone from an aqueous solution. Sugar and phosphate analyses of the LPS identified Kdo, GlcN, and phosphate in the molar ratio of approximately 2.8:2.0:2.1, respectively. Fatty acid analysis revealed the presence of tetradeconoic, iso- and anteiso-branched pentadecanoic, hexadecanoic, octadecanoic, and eicosanoic acids as major fatty acids. Hexadecanoic, heptadecanoic, nonadecanoic, and heneicosanoic acids (all present as iso- and anteiso-branched fatty acids) are minor ester-linked fatty acids. It should be noted that no ester-linked 3-hydroxy fatty acids were identified. (*R*)-3-Hydroxyeicosanoic acid (158 nmol/mg LPS) was found to be the major amide-linked fatty acid, and (*R*)-3-hydroxyoctadecanoic (47 nmol/mg), (*R*)-3-hydroxynonadecanoic (traces), iso- and anteiso-branched (*R*)-3-hydroxyeicosanoic (traces), iso- (43 nmol/mg) and anteiso- (71 nmol/mg) branched (*R*)-3-hydroxyheneicosanoic, and (*R*)-3-hydroxydodecansanoic (16 nmol/mg) acids are present as minor amide-linked fatty acids.

MALDI-MS Analysis—Molecular mass analysis of the purified LPS by MALDI-MS revealed the presence of molecular species consisting of lipid A (two GlcN and two phosphate residues, and 4–5 fatty acid residues) alone or substituted by 1, 2, or 3 Kdo residues (data not shown). No higher masses were detected. Because the mass resolution was low due to a considerable heterogeneity in fatty acid substitution and cation attachment, de-*O*-acylated LPS was prepared and analyzed using MALDI-MS (Fig. 1). The mass spectrum exhibited one major ion at *m/z* 1781.1 corresponding to an [M-H]⁻ ion consisting of 3 Kdo, 2 GlcN, 2 phosphate, and 2 amide-bound (*R*)-3-hydroxyeicosanoic acid residues. Less intense signals with mass differences of ±14 (CH₂) were additionally identified, indicating also a heterogeneity of amide-bound fatty acids. This was confirmed by analysis of amide-bound fatty acids after HCl hydrolysis of de-*O*-acylated LPS. Minor ions were observed that represent molecular species representing de-*O*-acylated lipid A alone or substituted by 1 or 2 Kdo residues. However, it could

not be decided to which extent these ions reflect intrinsic biological heterogeneity or are fragment ions produced in the ion source during laser desorption. In addition, an ion of low intensity at *m/z* 1701.1 corresponding to monophosphorylated de-*O*-acylated LPS was found.

Deacylation of LPS and Characterization of the Products—To analyze the carbohydrate backbone of the LPS from *C. trachomatis* L2, we applied the deacylation procedure of LPS published in Ref. 18 that comprises successive hydrazinolysis and hot KOH treatment. Fig. 2 shows the high-performance anion-exchange chromatography analyses of aliquots of the obtained products from LPS of *C. trachomatis* L2 (Fig. 2A) and recombinant *E. coli* F515–207 (Fig. 2B) (12). The major peak (*peak II*) in Fig. 2A possesses a retention time identical to that of Kdoα2→8Kdoα2→4Kdoα2→6D-GlcpNβ1→6D-GlcpNα 1,4'-bisphosphate (pentasaccharide bisphosphate), which has been isolated previously from LPS of *E. coli* F515–207 (12). In addition, a small peak (Fig. 2A, *peak I*) was detected in the chromatogram with a retention time identical to that of Kdoα2→4Kdoα2→6D-GlcpNβ1→6D-GlcpNα 1,4'-bisphosphate (tetrasaccharide bisphosphate) of LPS from *E. coli* F515–207 (12). From deacylated LPS, oligosaccharide II (Fig. 2A, 110 μg, 5.5% LPS) was isolated using high-performance anion-exchange chromatography and investigated by NMR spectroscopy. The ¹H and ¹³C chemical shift assignments (Table I) are based on two-dimensional correlation spectroscopy, nuclear Overhauser effect spectroscopy, rotating frame nuclear Overhauser effect spectroscopy, total correlation spectroscopy (Fig. 3), and a heteronuclear multiple quantum coherence experiment. Chemical shifts were similar to those of pentasaccharide bisphosphate (13). Larger differences were observed in the chemical shifts of H-1 and C-1 of residue A (see Fig. 4 for labeling of residues), caused by a different pD of the sample in Ref. 13. In the ¹H NMR spectrum, two signals were found in the anomeric region (at 5.64 and 4.65 ppm) that could be assigned to one α-linked [³J_{H-1,H-2} 4.0 Hz] and one β-linked [³J_{H-1,H-2} 7.0 Hz] GlcN residue (A and B), respectively. The H, P-coupling constant [³J_{H-1,P} 7.0 Hz] indicated the substitution of

FIG. 3. Total correlation spectroscopy spectrum of Kdo α 2 \rightarrow 8Kdo α 2 \rightarrow 4Kdo α 2 \rightarrow 6D-GlcpN β 1 \rightarrow 6D-GlcpN α 1,4'-bisphosphate isolated from LPS of *C. trachomatis* L2. The spectrum was recorded at 600 MHz and 27 °C. The letters refer to the carbohydrate residues as shown in Fig. 4, and the Arabic numerals to the protons in the respective residue.

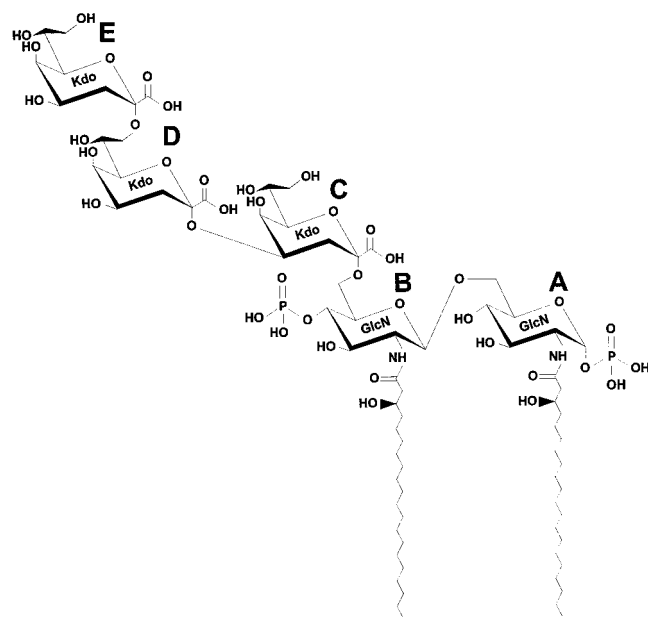
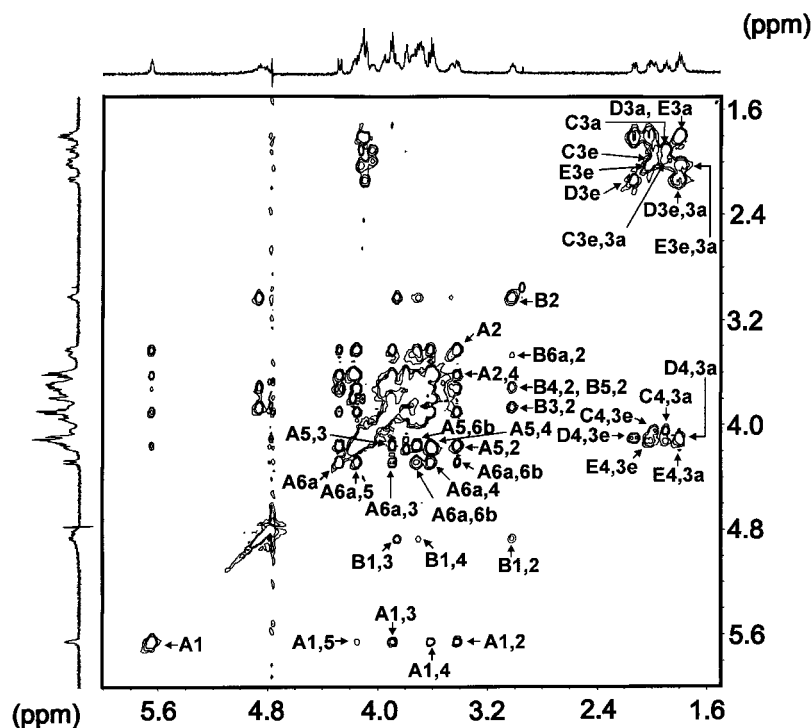


FIG. 4. Structure of de-O-acylated LPS from *C. trachomatis* L2. This structure corresponds to the major peak at $m/z = 1781$ in Fig. 1 and was determined by chemical analysis, MALDI-MS, and NMR spectroscopy.

O-1 of the reducing GlcN by a phosphate residue. The α -pyranosidic configuration of Kdo residues C, D, and E was deduced from the proton signals of H-3_{ax} and H-3_{eq}, which were in the region 1.7–2.2 ppm, characteristic for deoxyprotons (16, 19). This was further confirmed by the chemical shifts of the H-4 protons of residues C, D, and E, which were identified between 4.05–4.15 ppm. Comparison of the proton signals with published data (13, 18) identified E as a terminal Kdo residue. The chemical shifts of protons H-8a,b of residue D were shifted upfield proving (13, 17) the substitution of Kdo D at O-8 by another Kdo residue. Also, the chemical shifts of H-6a,b of GlcN residues A and B were shifted downfield, indicating the substitution of both sugars at O-6. In the ¹³C NMR spectrum, the signal of C-4 of Kdo C was shifted downfield (about 4 ppm),

whereas a slight β -shift of about 1 ppm was found for C-5 indicating the substitution of residue C at position O-4. The second phosphate residue was linked to O-4 of residue B, as shown by the downfield chemical shift of C-4 (73.4 ppm). The downfield shifts of the C-6 signals of residues A and B confirmed their substitution at O-6; that of residue A was also proven by a nuclear Overhauser effect contact between H-1 of residue B and H-6 of residue A. The observed contacts between protons H-3_{eq} of residue C and H-6 of residue D proved the sequence Kdo α 2 \rightarrow 4Kdo α 2 \rightarrow (C-D) (13). No other nuclear Overhauser effect contacts were identified.

Taken together, our data establish (i) the structure of oligosaccharide II as Kdo α 2 \rightarrow 8Kdo α 2 \rightarrow 4Kdo α 2 \rightarrow 6D-GlcpN β 1 \rightarrow 6D-GlcpN α 1,4'-bisphosphate, which is identical to pentasaccharide bisphosphate isolated from LPS of recombinant *E. coli* F515–207 (12) and (ii) the structure of de-O-acylated LPS as shown in Fig. 4.

DISCUSSION

The increasing biomedical interest in *Chlamydia* has initiated investigations more than a decade ago on the molecular organization of this obligatory intracellular bacterium. Because of its intracellular growth, these studies were hampered by the availability of only minute quantities of the microorganism and isolated compounds. When it was found that chlamydial genes can be expressed in *E. coli* (20), the knowledge on a number of immunogenic proteins grew rapidly. A complex glycolipid antigen was also recognized at that time as a major surface antigen with genus specificity but nothing was known on its chemical or antigenic structure. In the 1980s, independent reports were published on the relatedness of this antigen to enterobacterial rough LPS of the Re-chemotype (containing Kdo as the only core sugar), which was based on compositional analysis, polyacrylamide gel electrophoresis profiles and serology (7, 21, 23). Finally, the preparation of monoclonal antibodies against the genus-specific epitope allowed the identification of a cloned chlamydial gene as a Kdo transferase, which was able to synthesize the chlamydial epitope in an *E. coli* background (9). These recombinant bacteria gave us access to a structural analysis of the genus-specific epitope that turned out to be a Kdo trisaccharide of the sequence Kdo α 2 \rightarrow 8Kdo-

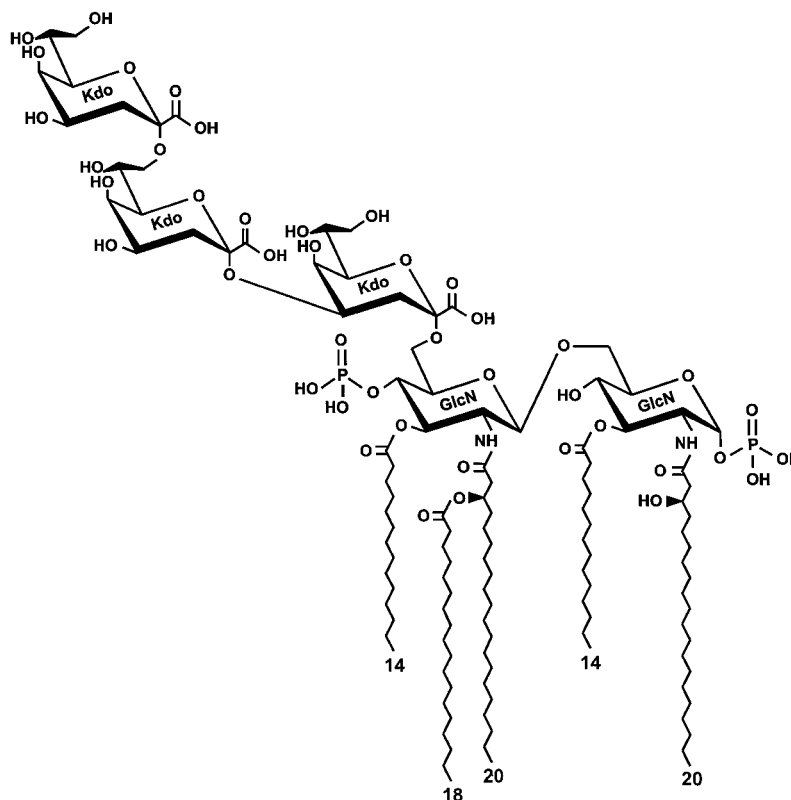


FIG. 5. Chemical structure of a major molecular species of LPS from *C. trachomatis* L2. The structure of the phosphorylated carbohydrate backbone was determined by NMR spectroscopy; the acylation pattern was determined by fatty acid analysis and MALDI-MS; the position of the acyloxyacyl residue is drawn in analogy to the data obtained for lipid A of serovar F (11).

$\alpha 2 \rightarrow 4 \text{Kdo} \alpha 2 \rightarrow$ (10). The assumption that the same structure occurs also in chlamydial LPS was based only on the reactivity of monoclonal antibodies and on a mass spectrometric “fingerprint” of a chlamydial LPS fragment (10). There was never the slightest doubt from our or other groups on the correctness of this assumption, but when we and others became interested in the chlamydial LPS as an endotoxin and in its biosynthesis, it was necessary to investigate again chlamydial LPS to determine the following: (i) the structure of the lipid A moiety (which in the LPS of the recombinant bacteria was of the *E. coli* type); (ii) the linkage point of the Kdo to the lipid A region; and (iii) whether components other than the Kdo trisaccharide were present in the core region. The latter point was of particular interest because meanwhile it became known that the Kdo transferase of *Chlamydia psittaci* was able to assemble a branched Kdo tetrasaccharide in the *E. coli* background (13).

Because the endotoxic activity of LPS depends mainly on the structure of the lipid A moiety (22), we first discuss this part of the molecule. It is known from earlier reports that chlamydial lipid A is of less endotoxic potency than enterobacterial lipid A, which was attributed to the lower number of acyl chains and to their longer chain length (6, 7). Compositional analyses had indicated that there were on the average two hydroxy and 3.2 nonhydroxy fatty acids in amide- and ester-linkage, respectively, and two phosphate groups/2 mol of glucosamine suggesting, in analogy to many lipid A structures, the presence of a bisphosphorylated glucosamine backbone but the lack of ester-linked hydroxy fatty acids (7, 23). Recently, Qureshi *et al.* (11) showed in a detailed mass spectrometric analysis of a major molecular monophosphoryl lipid A species that indeed only two hydroxy fatty acids are present in chlamydial lipid A and that these are exclusively amide-linked. After the analysis of hundreds of lipid A structures, this is the first example that positions 3 and 3' of the lipid A backbone are not substituted with 3-hydroxy fatty acids. These authors also determined the number and distribution of fatty acids showing that a tetra- and pentaacyl species predominate, the latter containing a single

acyloxyacyl residue in amide-linkage on the distal sugar unit. This work also confirmed the enormous heterogeneity of the acyl chains described earlier (7, 23). The structural analysis of the lipid A backbone and its substitution with phosphate was not determined. Therefore, we isolated the complete carbohydrate backbone after successive deacylation with hydrazine and potassium hydroxide and investigated the purified product by NMR spectroscopy. The data clearly identified the lipid A backbone as $\text{GlcN} \beta 1 \rightarrow 6 \text{GlcN} \alpha 1,4' P_2$. Because glycosidic linkages are not cleaved under the conditions used for deacylation, the sugar composition of the core region could be analyzed on the same compound. The Kdo trisaccharide of the sequence $\text{Kdo} \alpha 2 \rightarrow 8 \text{Kdo} \alpha 2 \rightarrow 4 \text{Kdo} \alpha 2 \rightarrow$ was identified by various one- and two-dimensional NMR experiments in comparison to reference compounds (12), and the linkage of this trisaccharide to the lipid A moiety was determined as $2 \rightarrow 6$. MALDI-MS analysis of de-*O*-acylated chlamydial LPS identified as a major fraction a molecular species composed of Kdo, GlcN, phosphate, and 3-hydroxyeicosanoic acid in a ratio of 3:2:2:2, respectively. This indicates that the major portion of chlamydial LPS does not contain components other than those described here, unless these would be present in hydrazine-labile linkage.

In summary, our data are in agreement with those published by us and others and add important structural details not known before. Taken together, the structure of LPS of *C. trachomatis* serotype L2 is as shown in Fig. 5. It is noted that the data on fatty acid composition and distribution determined here for serotype L2 are similar to those described by Qureshi *et al.* (11) for serotype F. Based on this knowledge, we have recently started to synthesize the chlamydial lipid A to study its endotoxic activity and its function as an acceptor for chlamydial Kdo transferases, studies which so far could not be done on homogenous compounds.

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