Structure of the Monophosphoryl Lipid A Moiety Obtained from the Lipopolysaccharide of \textit{Chlamydia trachomatis}\textsuperscript{*}

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Monophosphoryl lipid A was prepared from the lipopolysaccharide of \textit{Chlamydia trachomatis}, converted to the methyl ester, and fractionated by reverse-phase high-performance liquid chromatography. The peak fractions were collected and analyzed by mass spectrometry. Matrix-assisted laser desorption/ionization and liquid secondary ion mass spectrometry of the first of two major high-performance liquid chromatographic fractions showed multiple quasi-molecular ions of Mn\textsuperscript{+} at \textit{m/z} 1780, 1794, 1808, 1822, and 1836. The positive-ion liquid secondary ion mass spectrometry spectrum also showed a minor series of peaks at \textit{m/z} 1916, 1930, 1944, 1958, and 1971, consistent with the formation of matrix adducts with 3-nitrobenzyl alcohol. Oxonium ions representing the distal subunit were observed at \textit{m/z} 1057, 1071, 1085, 1099, and 1113. The second fraction was similarly analyzed and found to contain structural homologs of the first fraction. Based on this study, the major lipid A component of chlamydial lipopolysaccharide is a glucosamine disaccharide that contains five fatty acids and a phosphate in the distal segment. Three fatty acyl groups are in the distal segment, and two are in the reducing end segment. The acyloxyacyl group is located in the distal segment in amide linkage. Two structural series, differing by 14 atomic mass units in the reducing subunit, were observed. Chlamydial lipid A is complex and consists of at least 20 homologous structural components. The relatively low potency of \textit{Chlamydia trachomatis}\textsuperscript{1} lipopolysaccharide in activating lipopolysaccharide-responsive cells might be related to the unusual fatty acid composition of the lipid A moiety.

Bacteria of the genus \textit{Chlamydia} are pathogenic, obligate phagosomal intracellular parasites that cause acute and chronic diseases in animals and humans \cite{1, 2}. \textit{Chlamydia pneumoniae} is the major pathogen responsible for pelvic inflammatory disease, a major cause of female infertility in the United States \cite{3}. Chlamydial pelvic infection is often subacute, and infected females may not seek medical attention until many years after infection, perhaps during an examination for reproductive difficulties. The lipopolysaccharide (LPS)\textsuperscript{1} from this microorganism appears to be responsible for the generation of cytokines in which \textit{C. trachomatis} interacts with tissue phagocytes during the initiation of infection \cite{4}. In comparison with other bacterial LPS, \textit{C. trachomatis} LPS is ~100-fold less stimulatory. The low potency of \textit{C. trachomatis} LPS provides a biological explanation for the clinical observation that infected females may be asymptomatic. When \textit{C. trachomatis} is grown in tissue culture, chlamydial LPS appears to lack the characteristic O antigen that is observed in LPS from other Gram-negative bacteria. Thus, only two regions of the LPS have been examined, i.e. the core and lipid A. We have focused our efforts on the lipid A moiety because the acute cytokine response to other LPS results from the interaction of lipid A with phagocyte receptors for LPS \cite{4}.

Chlamydial LPS is similar to the rough forms of enterobacterial LPS. It consists of the lipid A and core regions. The core region of chlamydial LPS is composed of a unique trisaccharide of \textit{Kdo(2→8)Kdo(2→8)Kdo(2→8)Kdo(2→8)} (5, 6). The LPS of \textit{C. trachomatis} contains D-glucosamine, fatty acids, Kdo, and phosphorus in a molar ratio of ~2:5:3:2.6, respectively \cite{7}. There is a complex mixture of long-chain, normal, 3-hydroxylated, and branched fatty acids. The most prominent fatty acids are tetradecanoic, iso- and anteiso-branched tetradecanoic, hexadecanoic, and 3-hydroxyicosanoic acids. Minor fatty acids are 3-hydroxyeicosanoic acid and 3-hydroxyicosanoic acid. Nurminen et al. \cite{7} concluded that all hydroxy fatty acids are amide-linked and all nonhydroxylated fatty acids are ester-linked. However, essentially no structural information is presently available on the lipid A moiety of chlamydial LPS, perhaps because of difficulty in obtaining sufficient bacteria for LPS preparation.

We previously determined the structures of the LPS from the rough mutants of \textit{Escherichia coli} \cite{8, 9} and the lipid A from \textit{Salmonella typhimurium} \cite{10, 11}, \textit{Salmonella minnesota} \cite{12}, \textit{Neisseria gonorrhoeae} \cite{13}, \textit{Rhodobacter sphaeroides} \cite{14, 15}, and \textit{Brucella abortus} \cite{16}. We now report the preparation, purification, and characterization of the lipid A derived from

\textsuperscript{1} The abbreviations used are: LPS, lipopolysaccharide(s); Kdo, 2-keto-3-deoxyoctonate; HPLC, high-performance liquid chromatography; MPLA, monophosphoryl lipid A; MS, mass spectrometry; LSIMS, liquid secondary ion mass spectrometry; CID, collision-induced dissociation; MALDI/TOF, matrix-assisted laser desorption/ionization/time-of-flight; MALDI/IT, matrix-assisted laser desorption/ionization/ion trap.

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the LPS of *C. trachomatis* using a similar approach. The methyl derivative was purified by high-performance liquid chromatography (HPLC) and characterized by using soft ionization mass spectrometry. The fatty acyl composition of chlamydial lipid A is complex, but follows a defined structural pattern.

**EXPERIMENTAL PROCEDURES**

**Materials**—HPLC-grade chloroform, methanol, acetone, and isopropyl alcohol were purchased from Burdick & Jackson (Muskegon, MI). Silica Gel H thin layer plates (250 μm) were purchased from Analabs Inc. (North Haven, CT). Dowex 50W-X8, Chelex 100, and HSC buffer as described previously (18). Purified elementary bodies were obtained from Bio-Rad.

**Growth of Bacteria and Preparation of LPS**—Cells of *C. trachomatis* serovar F (gift of H. Caldwell, Rocky Mountain Laboratories, Hamilton, MT) were grown in McCoy suspension culture on microcarrier beads as described previously (12). Briefly, 4.5 × 10^13 elementary bodies were extracted sequentially with ethanol (5 ml, three times), acetone (5 ml, twice), and ether (3 ml, once). Dry cells (0.39 g) were added. This mixture was centrifuged to yield the rough chemotype. The lower organic layer was recovered, filtered, and evaporated to dryness. The MPLA (6 mg) was converted into the free acid form by passing through Chelex 100 (Na⁺) and Dowex 50 (H⁺) columns, as described previously (12), and methylated with diazomethane. HPLC was performed with two Waters 600A solvent delivery systems, a Waters 660 solvent programmer, a U6K universal liquid chromatography (MS). The fatty acids were extracted and derivatized to the methyl esters of fatty acids as described previously (16). The methyl esters were analysed by reverse-phase HPLC as described previously (16). The chromatogram was background-corrected. Peak fractions 1–9 were collected for further analysis.

**Preparation of M PLA**—Chlamydial LPS (12 mg) was suspended in 0.1 M HCl and heated to 100 °C for 30 min. The resulting crude MPLA was centrifuged at 8000 × g for 15 min. The pellet was suspended in a two-phase chloroform/methanol/water solvent (10:5:8) and centrifuged. The lower organic layer was recovered, filtered, and evaporated to dryness. The MPLA (6 mg) was converted into the free acid form by passing through Chelex 100 (Na⁺) and Dowex 50 (H⁺) columns, as described previously (12), and methylated with diazomethane. HPLC was performed with two Waters 600A solvent delivery systems, a Waters 660 solvent programmer, a USK universal liquid chromatograph injector, a variable wavelength detector (Model LC-55B, Perkin-Elmer), and a Nova-Pak cartridge (8 mm × 10 cm, C18-bonded, 5 μm, silica; Waters Associates, Milford, MA) used at a flow rate of 2 ml/min. A linear gradient of 30–100% isopropyl alcohol in acetone was used over a period of 60 min. The wavelength of the detector was set at 210 nm. The peak fractions were recovered and analyzed by mass spectrometry (MS).

**Fatty Acid Analysis**—HPLC-purified methylated MPLA (100 μg) was hydrolyzed in 2.5 ml of 4 M HCl at 100 °C for 5 and 18 h for fatty acid analysis. The fatty acids were extracted and derivatized to the methyl or converted to the p-bromophenacyl esters as described previously (16). Gas chromatography-MS of the methyl esters of fatty acids was carried out on a Carlo Erba Fractovap® 4161 gas chromatograph with a DB5 capillary column (60 m, 1-μm film, 0.32-mm inner diameter). The effluent was coupled directly into an ion source of a Kratos MS-25 mass spectrometer at a resolution of 600 (1 s/decade from 544 to 45 atomic mass units, 2-kV acceleration for 600-atomic mass unit mass range, 50-eV electron energy using electron impact ion source). The injector temperature was 280 °C. The column was programmed at 100–300 °C. The interface and source temperatures were 350 and 300 °C, respectively, as described previously (16). The p-bromophenacyl esters of the fatty acids were analyzed by reverse-phase HPLC as described previously (16). The fractions were recovered and analyzed by electron impact MS.

**MS of Purified Dimethyl-M PLA**—Liquid secondary ion mass spectrometry (LSIMS) spectra of HPLC-purified dimethylated MPLA were acquired on a Concept IH two-sector mass spectrometer (Kratos Ana-
lytical, Manchester, United Kingdom) at a resolution of 1000. A 1-μl aliquot of sample solution in methanol/chloroform (1:2) was mixed with the matrix (either 3-nitrobenzyl alcohol or monothioglycerol) on the tip of the probe. Analyte ions were desorbed from the matrix by an 8-keV Cs+ primary ion beam. The mass spectra were acquired by scanning the magnet in the range of 100–2500 atomic mass units at a scan rate of 10 s/decade. The B/E-linked scans were employed to acquire the spectra of the product ions following collision-induced dissociation (CID; xenon, 50% attenuation) of the precursor ions in the first field-free region of the mass spectrometer (tandem MS utilizing a sector instrument). About 10–20 scans were averaged per spectrum.

All matrix-assisted laser desorption/ionization/time-of-flight (MALDI/TOF) mass spectra were acquired on a Kompact III time-of-flight mass spectrometer (Kratos Analytical) with a dual-stage reflectron. In most cases, gentisic acid (2,5-dihydroxybenzoic acid) was used as a matrix. Analyte ions were desorbed from the matrix with a 337-nm nitrogen laser irradiation. Each spectrum was an average of 50 scans.

RESULTS

Preparation and Characterization of MPLA Derived from LPS of C. trachomatis—The LPS of C. trachomatis was acid-hydrolyzed to yield crude MPLA, which was converted to the free acid and methylated with diazomethane. Dimethyl-MPLA was fractionated by reverse-phase HPLC. A representative profile of such a fractionation is shown in Fig. 1. The results of HPLC suggested that this dimethyl-MPLA preparation is an extremely complex mixture of several structural series. By comparison, the HPLC profile of the dimethyl-MPLA derived from the LPS of S. minnesota R595 under similar conditions gave a simple pattern (12). C. trachomatis dimethyl-MPLA revealed one major peak (peak 7) and two other prominent peaks (complex peak 6 and simple peak 8) within the elution time of 36–42 min. Smaller amounts of lower structural series were found within 27–36 min (peaks 1–5), as well as a minor lipid A of higher structural series (peak 9). All of these peak fractions were collected and analyzed for size and fragmentation by secondary ion mass spectrometry. The fatty acyl contents were determined by HPLC. One major peak and several minor peaks were observed at 30–40 min. These peaks were collected and analyzed. Each peak was composed of a very complex mixture of a homologous series of lipid A.

Gas chromatography-MS of the methyl esters of the fatty acids obtained from either chlamydial LPS or HPLC peak 7 showed that the pentaacyl-MPLA contains C20OH. The normal fatty acids were C14, C15, C16, C18, C20, and C21, of which C14, C15, and C18 were the most prominent in lipid A. HPLC peak fraction 7 contained traces of C16, C21OH and C22OH were minor components in this fraction. Our results were essentially consistent with those of Nurminen et al. (7).

MALDI/TOF MS, MALDI/IT MS, and LSIMS Analysis of Dimethyl-MPLA—The major HPLC peak fraction 7 showed multiple quasi-molecular ions of MNa+ at m/z 1780, 1794, 1808, 1822, and 1836 in each of the mass spectra acquired using MALDI MS and LSIMS (Fig. 2). The positive-ion MALDI/TOF MS and MALDI/IT mass spectra showed that the distal phosphate group was cleaved, giving rise to MNa+–PO4H(CH3)2 peaks at m/z 1654, 1668, 1682, 1696, and 1710. The loss of the ester-linked C14 was observed in the positive-ion MALDI/IT spectrum at m/z 1553, 1567, 1581, 1595, and 1609. The loss of both phosphate groups and C14 gave rise to MNa+–PO4H(CH3)2–C14 peaks at m/z 1426, 1440, 1454, 1468, and 1482. These results show that the major lipid A of the LPS of C. trachomatis is a complex mixture of structural homologs containing five fatty acids (pentaacyl lipid A).
The positive-ion LSIMS spectrum also showed a minor series of peaks at \( m/z \) 1916, 1930, 1944, 1958, and 1971. These peaks were consistent with the formation of matrix adducts with 3-nitrobenzyl alcohol (Fig. 2C). The negative-ion LSIMS spectrum (Fig. 3) showed even more facile cluster ion formation when using 3-nitrobenzyl alcohol as a matrix, and the major high-mass peaks were observed at \( m/z \) 1909, 1923, 1937, 1951, and 1965. It should be noted that \( [M - H]^- \) and \( [M - 2H + Na]^+ \) ions were also present in the negative-ion LSIMS spectrum, but were not as abundant as the matrix adducts. Only \( [M - H]^- \) ion peaks could be seen in the high-mass region of the negative-ion LSIMS spectrum when monothioglycerol was used as a matrix (data not shown).

The positive-ion LSIMS spectrum contained structurally informative fragment ions in the lower mass region (Fig. 2C). The five most abundant peaks in the 800–1500-atomic mass unit mass region were \( m/z \) 1057, 1071, 1085, 1099, and 1113, corresponding to oxonium ions that represented the distal subunit \( \text{B}_1 \) fragment ions (Fig. 4) (12, 21). The number of peaks and their intensity distribution within these oxonium ions suggested that they are derived from the corresponding quasi-molecular ions \( [MNa]^+ \) and that these subunits are the source of structural heterogeneity of HPLC peak fraction 7 (variation in the fatty acyl content). This fragmentation showed that the...
distal subunit contains three fatty acyl groups, while the reducing subunit has just two fatty acyl groups.

Structural information regarding the kinds of fatty acids present in lipid A was obtained from the low-mass portion of the negative-ion LSIMS spectrum (data not shown). An abundant peak at 125 atomic mass units corresponded to a phosphate fragment ion of PO₄H(CH₃)₂, while peaks at 227, 241, and 255 atomic mass units indicated the presence of C₁₄, C₁₅, and C₁₆ fatty acyl groups. Interestingly, a peak at 269 atomic mass units due to C₁₇ was not present in the spectrum, while peaks at 283, 297, 311, and 326 atomic mass units suggested the presence of C₁₈, C₁₉, C₂₀, and C₂₁ fatty acyl groups. There was no evidence of ester-linked hydroxy fatty acids. These observations are in good agreement with the results of normal fatty acid analysis by gas chromatography-MS. No peak corresponding to hydroxy fatty acid was detected in the low-mass region of the negative-ion LSIMS spectrum, suggesting that these groups are linked to the sugar moiety via stable amide bonds, while normal fatty acyl groups are linked via labile ester bonds.

To provide further insight into the structure of lipid A, product-ion spectra of high-energy CID of MNa⁺ quasi-molecular ions were acquired on a sector instrument using B/E-linked scans. Since the mass window in these experiments is rather wide, products of more than just one precursor were detected in each experiment. Nevertheless, these experiments provided very useful structural information about chlamydiad lipid A. Although CID spectra were acquired for four precursor ions in HPLC peak fraction 7 (at m/z 1780, 1794, 1808, and 1822), we will only discuss the CID spectrum of the precursor ion at m/z 1794 (Fig. 5). The high-mass fragments corresponded to loss of a C₁₅ fatty acyl group as well as consecutive losses of a phosphate group and a C₁₄ fatty acyl group (loss of C₁₅ was also possible). The loss of only one phosphate group was observed in the spectrum, confirming that this lipid A preparation is monophosphorylated. The CID spectra also gave information regarding the structures of the distal subunit of methylated MPLA. The oxonium ion resulting from the dissociation of lipid A with MNA⁺ at m/z 1794 was at m/z 1071. The appearance of a fragment ion peak at 787 atomic mass units showed that the C₁₅ fatty acyl group is present in the distal subunit of lipid A (1071-C₁₅). The other fatty acyl group was C₁₄, since we observed a peak at m/z 419, atomic mass units, probably corresponding to B₁₅-C₁₅ PO₄H(CH₃)₂. There was no evidence of the presence of C₁₇. These fragment ions also established that the phosphate group is present at the distal subunit of lipid A. This suggests that the fatty acyl groups in the distal segment are probably C₁₂ (R₂) and C₂₀OC₁₈ (R₂). A minor component containing C₁₄ (R₁) and C₂₁OC₁₈ (R₂) is also possible. The other alternative would be the presence of C₁₉ (R₁) and C₂₀OC₁₅ (R₂).

### Table I

**Proposed fatty acyl distribution of dimethyl-MPLA components of HPLC peak fraction 7**

| m/z     | Oxonium ion | R₁ | R₂ | R₃ | R₄ |
|---------|-------------|----|----|----|----|
| 1780    | 1057        | C₁₄| C₁₈OC₁₈| C₁₄| C₂₀OH |
| 1794    | 1071        | C₁₅| C₂₀OC₁₉| C₁₄| C₂₀OH |
| 1808    | 1085        | C₁₅| C₂₀OC₁₉| C₁₄| C₂₀OH |
| 1822    | 1099        | C₁₄| C₂₀OC₂₁| C₁₄| C₂₀OH |
| 1836    | 1113        | C₁₄| C₂₀OC₂₀| C₁₄| C₂₀OH |

*The location of the normal fatty acyl groups in the distal segment (in R₁ and R₂) for each molecular ion is given as one possibility based on structural symmetry. The other possibility is to have the normal fatty acyl groups in reverse order at these two positions.*

The reducing end segment of lipid A would then contain C₁₄ (R₂) and C₂₀OH (R₄) (Table I). The two remaining fragment ion peaks at 696 and 710 atomic mass units can now be assigned as Y₁⁻ and 0.1X₁⁻ (22), respectively (Fig. 4). The proposed structures for the major homologous series present in HPLC peak fraction 7 are listed in Table I. These results allow us to propose the fatty acyl distribution in the reducing and distal subunits of a major dimethyl-MPLA component. It is not presently possible to determine which structure represents the major component; however, it is clear that we have a mixture.

### Figure 6

**Positive-ion MALDI/TOF MS (A) and LSIMS (B) spectra of HPLC peak fraction 8** (see Fig. 1).

The reducing end of lipid A would then contain C₁₄ (R₂) and C₂₀OH (R₄) (Table I). The two remaining fragment ion peaks at 696 and 710 atomic mass units can now be assigned as Y₁⁻ and 0.1X₁⁻ (22), respectively (Fig. 4). The proposed structures for the major homologous series present in HPLC peak fraction 7 are listed in Table I. These results allow us to propose the fatty acyl distribution in the reducing and distal subunits of a major dimethyl-MPLA component. It is not presently possible to determine which structure represents the major component; however, it is clear that we have a mixture.
simply analyzed and found to be tetraacyl-MPLA. No further work was done on these fractions. It is not clear whether some tetraacyl-LPS existed in the original sample since treatment of LPS with 0.1 M HCl at 100 °C causes the release of ester-linked fatty acids (22).

**DISCUSSION**

The literature on the structure of the LPS from *C. trachomatis* is meager. One significant study directly relates to the structure of the lipid A moiety (7). In this study, chemical analyses of the LPS revealed the presence of β-glucosamine, normal and hydroxy fatty acids, Kdo, and phosphate. This composition suggested that it is a rough chemotype LPS. Since CID analysis was not done on molecular ions at *m/z* 1808, 1822, 1850, and 1864, the assignments of fatty acyl groups at *R*₁ and *R*₂ on these are more speculative.

By a combination of scale-up and growing organisms over a long period, we were able to obtain enough elementary bodies to prepare a sufficient amount of LPS. This allowed us to begin to examine the structure of the lipid A moiety of the LPS. About 12 mg of LPS were hydrolyzed under mild acid conditions to yield MPLA (with ~50% yield); this was converted to the methyl esters (phosphate esters) and purified by reverse-phase HPLC. The HPLC elution pattern immediately suggested that the lipid A derived from this LPS is a complex mixture (Fig. 1). We collected nine peak fractions, examined them by mass spectrometry, and identified peak fractions 1–6 as tetraacyl-MPLA, peak fractions 7 and 8 as pentaacyl-MPLA, and peak fraction 9 as the incompletely hydrolyzed bisphosphoryl lipid A. We selected the major HPLC peak fractions 7 and 8 to study the chemical structure of the lipid A moiety of chlamydial LPS.

Fatty acid analysis of these HPLC peak fractions after acid hydrolysis by gas chromatography-MS revealed the composition to be similar to that found by Nurminen et al. (7). We then examined these two select fractions by MALDI MS and LSIMS. The negative-ion spectra showed the presence of quasi-molecular ions ([MNa⁺]) listed in Table I) that gave us the molecular masses of each of the dimethyl-MPLA components. Each of the HPLC peak fractions 7 and 8 showed the presence of five different structural components based on size. Furthermore, the spectra showed the presence of oxonium ions that represented the distal fragment of dimethyl-MPLA. Each oxonium ion was structurally related to the corresponding molecular ion. This allowed us to estimate the size of the reducing segment of each dimethyl-MPLA.

We showed the results of analysis of the quasi-molecular ion (MNa⁺) at *m/z* 1794 (a major component of HPLC peak fraction 7) by high-energy CID. High-mass fragments were present in the spectrum, showing the loss of C₁₄, C₁₅, and C₁₆ fatty acids. With this knowledge, we were able to assign the R values for the distal segment of this component (Table I). We proposed similar structures for the distal segment of this representative dimethyl-MPLA. Similar CID analysis was performed on the other molecular ions in HPLC peak fractions 7 and 8. Because HPLC peak fractions 7 and 8 showed identical structural series for the oxonium ions (Tables I and II), suggesting that the distal segments of the two series are also identical, the structural differences between the two fractions must be due to differences in the reducing segments.

Because we now knew the sizes of the reducing segments of dimethyl-MPLAs and the fatty acid composition of lipid A (i.e., normal fatty acids are ester-linked, the hydroxy fatty acids are amide-linked, and the reducing segment has only two fatty acids), we were able to determine the fatty acid distribution in the reducing segment of chlamydial lipid A (Tables I and II). In HPLC peak fraction 7, the most probable structure is where R₃ is C₁₅ and R₄ is C₂₀OH. In HPLC peak fraction 8, the major component might have R₃ as C₁₅ and R₄ as C₂₀OH. It is to be noted that these two structural series differ by 14 atomic mass units. Thus, the structural series representing HPLC peak fractions 7 and 8 only differ by one methylene in the fatty acid in the reducing segment.

Based on this study, the major lipid A component of chlamydial LPS is a glucosamine disaccharide that contains five fatty acids (pentaacyl) and a phosphate group in the distal segment. A minor lipid A component might be the tetraacyl lipid A that we identified in the acid-hydrolyzed product. Although not studied (at the bisphosphoryl lipid A level), we suspect the presence of a second phosphate group, presumably at the anomic carbon of the disaccharide. Three fatty acyl groups were in the distal segment, and two were in the reducing segment. The acyloxyacyl group was located in the distal segment in amide linkage. All normal fatty acids were ester-linked, and all hydroxy fatty acids were amide-linked. These results are consistent with the classical structure of lipid A derived from LPS of other Gram-negative bacteria (22). The position of the 4'-phosphate and the (β1→6) linkage of the glucosamine disaccharide will be confirmed by nuclear magnetic resonance spectroscopy. The proposed general structure of the dimethyl-MPLA derived from chlamydial LPS is shown in Fig. 4, along with the assignment of the R (fatty acyl) groups in Tables I and II.

A general pattern of the structure of lipid A of chlamydial LPS has emerged from this study. There are two basic struc-

| *m/z* | Oxonium ion | R₁ | R₂ | R₃ | R₄ |
|------|-------------|----|----|----|----|
| 1808 | C₁₄         | C₁₅| C₁₅| C₂₀OH |
| 1822 | C₁₄         | C₁₅| C₁₅| C₂₀OH |
| 1836 | C₁₄         | C₁₅| C₁₅| C₂₀OH |
| 1850 | C₁₅         | C₁₅| C₁₅| C₂₀OH |
| 1864 | C₁₆         | C₁₅| C₁₅| C₂₀OH |

The location of the normal fatty acyl groups in the distal segment (in R₁ and R₂) for each molecular ion is given as one possibility based on structural symmetry. Since CID analysis was not done on molecular ions at *m/z* 1808, 1822, 1850, and 1864, the assignments of fatty acyl groups at R₁ and R₂ on these are more speculative.

**Table II**

Proposed fatty acyl distribution of dimethyl-MPLA components of HPLC peak fraction 8

Only the quasi-molecular ion at *m/z* 1836 was subjected to high-energy CID, and the spectrum of product ions was acquired (data not shown). The fragment identified as the oxonium ion allowed us to determine the ester-linked fatty acyl content in the distal segment. Since the loss of the acyloxyacyl and hydroxy fatty acyl groups was not observed, we concluded that they are amide-linked.
Chlamydial LPS was less potent than Enterobacteriaceae LPS, as defined by its ability to activate mononuclear phagocytes and a Chinese hamster ovary cell line transfected with the putative LPS receptor CD14 (4). The structural basis for this low potency might be due to the following differences in the lipid A structure of chlamydial LPS. (a) It has five rather than six fatty acyl groups; (b) its fatty acyl groups are longer than C_{14}; and (c) it contains normal fatty acids rather than hydroxy fatty acids attached directly to the glucosamine disaccharide in ester linkage. Such structural information provides a meaningful explanation for the clinical syndrome associated with Chlamydia, which often results in severe infections without significant local and systemic symptoms.

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Structure of the Monophosphoryl Lipid A Moiety Obtained from the Lipopolysaccharide of *Chlamydia trachomatis*

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