Chemical Structure of Lipid A Isolated from Flavobacterium meningosepticum Lipopolysaccharide

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The chemical structure of the lipid A of the lipopolysaccharide component isolated from Flavobacterium meningosepticum IFO 12535 was elucidated. Methylation and nuclear magnetic resonance analyses showed that two kinds of hydrophilic backbone exist in the free lipid A: a β(1→6)-linked 2-amino-2-deoxy-N-glucose, which is usually present in enterobacterial lipid A's, and a 2-amino-6-O-(2,3-diamino-2,3-dideoxy-b-N-glucopyranosyl)-2-deoxy-N-glucose, in a molar ratio of 1.00:0.35. Both backbones were α-glycosidically phosphorylated in position 1, and the hydroxyl groups at positions 4, 4′, and 6′ were unsubstituted. Liquid secondary ion-mass spectrometry revealed a pseudomolecular ion at m/z 1673 [M-H]⁻ as a major monophosphoryl lipid A component carrying five acyl groups. Fatty acid analysis showed that the lipid A contained 1 mol each of amide-linked (R)-3-OH iC₁₅:₀, ester-linked (R)-3-OH iC₁₅:₀ and amide-linked (R)-3-OH iC₁₅:₀, respectively in a molar ratio of 1:3:1. Both amide- and ester-linked (R)-3-OH iC₁₆:₀. Fatty acid distribution analyses using several mass spectrometry determinations demonstrated that the former two constituents were distributed on positions 2 and 3 of the reducing terminal unit of the backbones and that the latter two were attached to the 2′ and 3′ positions in the nonreducing terminal residue.

Lipopolysaccharide (LPS) is known to act as an endotoxin that mediates pathophysiological changes such as fever and shock which occur in the course of severe gram-negative bacterial infection (5, 18, 27). The pathophysiological activity of LPS depends on the chemical structure of the hydrophilic portion called lipid A, the biologically active center of LPS (12, 14), which generally consists of a β(1→6)-linked 2-amino-2-deoxy-N-glucose (GlcN) disaccharide carrying phosphate and fatty acid residues; many fine structural variations are observed in different bacterial families (38).

Since many of the LPSs from various gram-negative bacteria cause similar endotoxic effects despite differences in chemical composition and positions of substitution, the chemical structure required for the activity does not seem to be very strict. It has been reported, however, that several lipid A forms, isolated from the LPSs of Porphyromonas gingivalis (16), Rodobacter sphæroides (22, 26) and Rhodobacter capsulatus (20), as well as chemically synthesized lipid A analogs (6, 12), which are structurally similar to the active-type lipid A, exhibit dramatically low endotoxicity. Biologically active lipid A has been found to be changed to completely nontoxic derivatives by simple chemical modifications (28, 29). These findings indicate that the biological activity of lipid A is controlled by the fine structural variations. The nontoxic or low-toxicity lipid A preparations are very important for the determination of the relationship between the chemical structure and biological activity of lipid A, and also for the systematic development of LPS antagonists. However, the essential structural requirements for the complete activity or nontoxicity of lipid A are still uncertain. It is, therefore, meaningful to study the chemical and biological properties of naturally occurring lipid A’s which possess a unique structure.

Flavobacterium meningosepticum is an aerobic gram-negative rod which is known to cause meningitis and septicemia in newborn infants (4, 21). Interestingly, the bacterium does not induce Limulus gelation activity when tested with whole cells (32), strongly suggesting that the LPS is of low toxicity or nontoxic and that the lipid A must have a unique structure relative to other enterobacterial lipid A’s.

In the present study, the chemical structure of lipid A isolated from F. meningosepticum LPS was characterized by compositional study, methylation analysis, mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy.

MATERIALS AND METHODS

Bacteria and preparation of LPS. F. meningosepticum IFO 12535 strain was obtained from the Institute for Fermentation Osaka. The bacterium was cultivated in a fermentor at 30°C for 16 h in a medium consisting of 1% (wt/vol) polypeptone, 0.2% (wt/vol) beef extract, and 0.1% (wt/vol) MgSO₄·7H₂O, at pH 7.0. The heat-killed cells were harvested by centrifugation and washed with distilled water, acetone, ethanol, and diethyl ether, then acetone dried (yield, 0.5 g of cells/liter).

LPS (7.1 mg/g [dry weight] of cells) was extracted from the acetone-dried cells with a mixture of phenol-chloroform-petroleum ether (2:5:8 [vol/vol/vol]) according to the method described by Galanos et al. (7) and was purified by RNase and DNase (Sigma) treatments (36) and repeated ultracentrifugation (105,000 × g, 3 h, six times).

Isolation of lipid A. Purified LPS (1.23 g) was hydrolyzed with 1% [vol/vol] aqueous acetic acid at 100°C for 2 h, followed by centrifugation (14,000 × g, 10 min). The sediment was washed with distilled water and crude lipid A was obtained after lyophilization. The crude lipid A (708.6 mg) was purified by Sephadex LH-20 column chromatography according to the method described in reference 16 to yield 420 mg of purified lipid A.

Chemical modification of LPS and lipid A. Lipid A backbone was prepared according to the method of Hase and Rietschel (11). Briefly, LPS (20 mg) was treated with 0.17 M NaOH (100°C, 1 h) and 0.1 M HCl (100°C, 30 min), followed by reduction with NaBH₄ (37°C, 16 h), complete hydrazinolysis (103°C, 40 h), and N acetylation to obtain the reduced and N-acetylated lipid A backbone. Escherichia coli F654 R3 LPS was treated by the same method as reference material.

De-O-acylation of LPS and lipid A was performed by treatment with anhydrous hydrazine at 60°C for 30 min, and the de-O-acylated preparations were recovered by a method reported previously (13).

De-O-acylated LPS was hydrolyzed in 0.1 M HCl at 100°C for 30 min. The centrifugal sediment recovered was treated with acetic anhydride-pyridine (1:1...
TMS (0.00 ppm) was used as an internal standard for 1H and 13C NMR experiments. Canonic acid methyl ester was used as a reference compound. The presence of a shift reagent (1 mg), Tris-[3-(heptafluoroprolyl-hydroxymethylene)-imidazolium], was used as the matrix. For the negative-ion mode, each containing a small amount of Kryptofix 222 (Aldrich), was used for the determination of the absolute configurations of amino sugars with temperature program E (180°C for 3 min, increasing to 250°C at 3°C/min). Nitrogen was used as the carrier gas.

Analytical methods. Each temperature program is described under “GLC conditions” above. Total fatty acids were determined by means of GLC and GLC-MS using temperature program A, described under “GLC conditions” above, as the methyl ester according to the method of Haeffner et al. (8). 2-Hydroxydecanoic acid and nona decanoic acid (GL Science) were used as internal standards. Ester- and amide-linked fatty acids were also investigated by 1H,13C-GLC and TC-MS using temperature programs A and B, respectively, according to methods described elsewhere (13, 16, 24, 37).

Neutral and amino sugars were analyzed by GLC and GLC-MS (program C) as the alditol acetate derivatives (9). The absolute configurations of amino sugar constituents were determined by GLC analysis (program E) of the peracetylated S-2-butylglycoside derivatives according to the method described in reference 13. Amino sugars present in the lipid A possessed a α-configuration.

Analysis of lipid A backbone. The chemical composition of lipid A isolated from F. meningosepticum LPS is shown in Table 1. The lipid A also contained 467.0 nmol of total phosphate/mg, 416.7 nmol of GlcN/mg, and 86.5 nmol of 2,3-diamino-2,3-dideoxy-3,4,6-tri-O-methyl-2-(N-methylacetylamo) glucose (2,3-Diamino-hexose) was identified as 2,3-dideoxy-2,3-di-amino-glucose by NMR analyses.
glucopyranosyl)-2-deoxy-1,3,4,5-tetra-O-methyl-2-(N-methylacetamido)-D-glucitol, which originated from the usual GlcN disaccharide backbone, because the EI-mass spectrum and retention time were identical to those of the same derivative prepared from *E. coli* R3 LPS possessing a \(\beta\)(1→6)-linked GlcN disaccharide lipid A backbone. On the other hand, the latter peak was identified as 6-O-[2,3-dideoxy-4,6-di-O-methyl-2,3-di-(N-methylacetamido)-D-glucopyranosyl]-2-deoxy-1,3,4,5-tetra-O-methyl-2-(N-methylacetamido)-D-glucitol. In the EI-mass spectrum (Fig. 3), fragment ions representing cleavage of the glycosidic linkage of the hybrid disaccharide were recognized at \(m/z\) 276 and 301. A significant fragment ion at \(m/z\) 218, corresponding to cleavage of the C-4–C-5 bond, which was characteristic of the derivative of \(\beta\)(1→6)-linked GlcN disaccharide (3), was also detected in the spectrum. As shown in Fig. 3, other characteristic fragment ions were obtained at \(m/z\) 130 (C1-C2 fragment) and \(m/z\) 174 (C1-C3 fragment), as well as several daughter ions based on the loss of methanol (−32), acetic acid (−60), or an N-acetyl group (−42). This EI-mass spectrum was almost identical to that of the same derivative of \(\beta\)(1→6)-linked GlcN3N-GlcN disaccharide reported by Moran et al. (19). These results indicate that two kinds of lipid A backbones exist in the *F. meningosepticum* lipid A: one \(\beta\)(1→6)-linked GlcN disaccharide backbone that is normally present in enterobacterial lipid A’s and a (1→6)-linked GlcN3N-GlcN disaccharide, the GlcN3N residue of which was found to possess a β configuration by NMR analysis as described below.

**Distribution of fatty acid and phosphate residues.** The distribution pattern of fatty acid and phosphate residues on the *F. meningosepticum* lipid A backbone was determined by LSI-MS and FAB-MS/MS. The LSI-mass spectrum of *F. meningosepticum* lipid A in the negative-ion mode is shown in Fig. 4. A predominant ion observed at \(m/z\) 1673 [M-H]− corresponds to monophosphoryl lipid A species carrying 1 mol each of \((R)\)-3-OH iC\(_{15:0}\), \((R)\)-3-OH C\(_{16:0}\), \((R)\)-3-OH iC\(_{17:0}\), and \((R)\)-3-O-(iC\(_{15:0}\))iC\(_{17:0}\) on the lipid A backbone. Characteristic ions originating from the reducing terminal unit of the lipid A were also detected at \(m/z\) 767 and 795, which arise from the cleavage of the glycosidic linkage and C-1′–C-2′—C-1′–O bond, respectively (Fig. 4). The fragment ion at \(m/z\) 749 corresponds to the daughter ion caused by elimination of H\(_2\)O from the ion at \(m/z\) 767. These fragment ions indicate that the reducing terminal unit of *F. meningosepticum* lipid A consists of monophosphoryl GlcN replaced by 1 mol each of \((R)\)-iC\(_{15:0}\) and \((R)\)-3-OH iC\(_{17:0}\) and that a nonreducing terminal residue contains 1 mol each of GlcN (or GlcN3N), \((R)\)-3-OH C\(_{16:0}\), and \((R)\)-3-O-(iC\(_{15:0}\))iC\(_{17:0}\), respectively. These results also showed that the hydroxyl groups at positions 4 and 6 in the nonreducing terminal unit of the lipid A species having a GlcN3N-GlcN hybrid backbone exist in the free form, because the amide-linked \((R)\)-3-OH C\(_{16:0}\) and \((R)\)-3-O-(iC\(_{15:0}\))iC\(_{17:0}\) are attached to positions 3 and 2 of the GlcN3N residue, respectively. Several species based on the loss of acyl and phosphate residues were usually observed in LSI-mass spectra in most of the lipid A.
preparations, but *F. meningosepticum* lipid A appears to be extremely homogeneous.

Since the difference in molecular mass between a GlcN disaccharide backbone and an identically acylated hybrid backbone is only 1 Da, which is within the uncertainty of mass scale calibration, it was necessary to degrade the material further to differentiate between the two backbone disaccharides. As shown in Fig. 5, two peaks were predominantly detected in the LSI-mass spectrum of the de-O-acylated lipid A of *F. meningosepticum*. A molecular ion was observed at m/z 1209 [M-H]⁻; it corresponds to the monophosphoryl lipid A species carrying three N-acyl residues, 1 mol of (R)-3-OH C₁₆:0, and 2 mol of (R)-3-OH iC₁₇:0 on the β(1→6)-linked GlcN₃N-GlcN hybrid backbone. Another ion at m/z 956 [M-H]⁻ was identified as a monophosphoryl GlcN disaccharide replaced by 2 mol of amide-linked (R)-3-OH iC₁₇:0. Thus, the presence of a β(1→6)-linked GlcN₃N-GlcN hybrid backbone in addition to a GlcN disaccharide backbone was again recognized in the LSI-mass spectrum of the de-O-acylated lipid A.

The pattern of distribution of acyl residues was also confirmed by FAB-MS/MS of the molecular ions at m/z 1209 [M-H]⁻ and 956 [M-H]⁻, detected in the LSI-mass spectrum of the de-O-acylated lipid A (Fig. 5). The tandem spectrum of the ion at m/z 1209 [M-H]⁻ is shown in Fig. 6. A fragment ion at m/z 406.2 caused by the cleavage of the C-1-O-C-2-C-3 bond of the reducing terminal unit of the backbone revealed that a phosphate residue was linked to position 1 of the backbone and that an amino group at position 2 was N acylated with (R)-3-OH iC₁₇:0 (Fig. 6). Other characteristic fragment ions were observed at m/z 508.3 and 524.3 and m/z 526.3 and 554.3; they were generated by the cleavage of the glycosidic linkage and C-1′-C-2′—C-5′-O bond, respectively (Fig. 6). The tandem spectrum of the ion at m/z 956 [M-H]⁻ was almost identical to that of the ion at m/z 1209 [M-H]⁻ (data not shown).

**Structure of the peracetylated derivative of de-O-acylated lipid A having a hybrid backbone.** LSI-MS and NMR analyses of the peracetylated derivative of the hybrid backbone carrying three N-acyl and no phosphate residues were performed. In positive-ion mode LSI-MS, a molecular ion was detected at m/z 1467 [M+H]⁺, and a fragment ion originating from the non-reducing terminal unit was also observed at m/z 853, indicating that 1 mol each of (R)-3-OH C₁₆:0 and (R)-3-OH iC₁₇:0 was distributed on the nonreducing terminal unit and the remaining 1 mol of (R)-3-OH iC₁₇:0 was present in the reducing terminal residue. The ¹H and ¹³C NMR data are shown in Tables 2 and 3. In the ¹H NMR analysis (Table 2), the J₂,₃ (10.4 Hz), J₃,₄ (9.9 Hz), and J₄,₅ (9.9 Hz) values of the nonreducing terminal unit indicated that this sugar constituent possesses the gluco-conformation, and the J₁,₂ value (8.4 Hz) revealed the β-configuration. Two amide protons were assigned at 6.48 ppm and 6.37 ppm, and they were cross-coupled with H-2 (3.96 ppm) and H-3 (4.09 ppm), respectively, indicating that this sugar unit possesses two amino groups at positions 2 and 3. On the other hand, the α- and β-anomers of the reducing terminal unit, which were produced during acetylation, were assigned. In both anomers, the J₂,₃, J₃,₄, and J₄,₅ values indicated the gluco-conformation, and an amino group exists at position 2, because

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**FIG. 2.** EI-mass spectrum and fragmentation pattern of methyl ester derivatives of (R)-3-O-(13-methyltetradecanoyl)-15-methylhexadecanoic acid found in *F. meningosepticum* lipid A.
the amide proton cross-coupled with H-2 was detected at 6.04 ppm for the α-anomer and 6.34 ppm for the β-anomer. H-6α and H-6β were slightly shifted to a higher field, because the nonreducing terminal unit was linked to position 6. These findings were also supported by the 13C NMR data shown in Table 3. These results clearly showed that the hybrid backbone present in *F. meningosepticum* lipid A consists of a β(1→6)-linked GlcN3N-GlcN hybrid disaccharide.

NMR analysis of monophosphoryl-methylated lipid A. In order to determine the positions of free-hydroxyl groups and the attachment site of phosphate residue, one- and two-dimensional 1H NMR analyses of the monophosphoryl-methylated derivative of *F. meningosepticum* lipid A were performed (Table 4). The H-1 signal (6.01 ppm; J_{1,2} = 3.12 Hz) of the reducing terminal unit of the lipid A was shifted at 0.55 ppm to a lower field in comparison to the unsubstituted H-1 signal (5.46 ppm) (23), indicating that the phosphate residue was α-glycosidically linked to position 1. Since direct J coupling of the hydroxyl proton with H-4 (3.98 ppm) was detected at 5.86 ppm, the hydroxyl group at position 4 of the reducing terminal
unit was identified as being free form. Furthermore, an amide proton was assigned at 8.12 ppm (2-NH; $J_{\text{NH},2} = 9.34$ Hz), and it was cross-coupled with the proton at position 2 (4.68 ppm). The other signals that originated from the unit were detected at 4.10 ppm (H-6b), 4.19 ppm (H-5), 4.39 ppm (H-6a), and 5.64 ppm (H-3). An H-1 proton was detected at 5.05 ppm ($J_{1},2 = 7.51$ Hz) as a signal originating from the nonreducing terminal unit of the lipid A. The resonance was shifted to a higher field (0.41 ppm) by glycosidical substitution, and the $J_{1},2$ value (7.51 Hz) revealed a $\beta$-configuration. However, no other signals were able to be clearly assigned because of the heterogeneity.

The location of phosphate groups was directly determined by $^{31}$P NMR analysis. One signal was predominantly observed at −0.669 ppm in the $^{31}$P NMR spectrum. This signal was cross-coupled with the H-1 proton in $^{31}$P,$^1$H-HMQC analysis, proving that the phosphate residues are attached to position 1 of the lipid A backbone.

**DISCUSSION**

The proposed chemical structure of *F. meningosepticum* lipid A, determined by chemical and physicochemical analysis in the present study, is shown in Fig. 7. It was noted that the hydrophilic backbone consisted of an unusual hybrid backbone iden-

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**FIG. 4.** LSI-mass spectrum of *F. meningosepticum* lipid A in the negative-ion mode. A mixture of triethanolamine and 3-nitrobenzyl alcohol (1:1 [vol/vol]) containing a small amount of Kryptofix 222 was used as the matrix.

**FIG. 5.** LSI-mass spectrum of *F. meningosepticum* de-O-acylated lipid A in the negative-ion mode. A mixture of triethanolamine and 3-nitrobenzyl alcohol (1:1 [vol/vol]) containing a small amount of Kryptofix 222 was used as the matrix.
tified as a β(1→6)-linked GlcN3N-GlcN disaccharide in addition to a β(1→6)-linked GlcN disaccharide backbone, which is widely distributed in many LPS molecules (38). Both backbones are 1-O-α-glycosidically phosphorylated, and (R)-3-OH iC15:0 and (R)-3-OH iC17:0 attached to the reducing terminal unit are linked to positions 3 and 2, respectively. The hydroxyl group at position 4 of the unit exists in the free form in the lipid A molecule, and position 6 is the site to which the nonreducing terminal unit is linked. Although the exact attachment sites of (R)-3-OH C16:0 and (R)-3-OH-iC15:0-iC17:0 on the nonreducing terminal unit were not determined, they are assumed to link to positions 3' and 2' of the unit based on the following results: (i) all (R)-3-O-(iC15:0)-iC17:0 existed as an amide-linked acyl residue, while (R)-3-OH C16:0 was detected as both amide- and ester-linked residues; (ii) monophosphoryl GlcN disaccharide carrying 2 mol of amide-linked (R)-3-OH iC17:0 at positions 2 and 2' was determined by LSI-MS to be a single component of de-O-acylated lipid A; and (iii) the hydroxyl groups at positions 4' and 6' of lipid GlcA species having N3N-GlcN hybrid backbones were identified as free form by LSI-MS.

_F. meningosepticum_ lipid A has a number of chemically unique characteristics compared to other enterobacterial lipid A's (12, 14, 38). _F. meningosepticum_ lipid A mainly contains relatively longer-chain and isoform fatty acids, in contrast to the enterobacterial lipid A's, which contain (R)-3-hydroxytetradecanoic acid as the main constituent of acyl residues. Regarding the location of fatty acids, _F. meningosepticum_ lipid A contains only 1 mol of an acyloxyacyl group at position 2',

![FIG. 6. Negative-ion mode FAB-MS/MS of the molecular ion at m/z 1209 [M-H]− detected in the LSI-mass spectrum of _F. meningosepticum_ de-O-acylated lipid A.](image)

| Table 2. 1H NMR data for the peracetylated derivative of _F. meningosepticum_ de-O-acylated lipid A-HCl containing a hybrid backbonea |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Unit            | Value for chemical shift (coupling constant)b          |                  |                  |                  |                  |                  |
|                 | H-1            | H-2            | H-3            | H-4            | H-5            | H-6a           | H-6b           |
| Reducing terminal unit |                  |                  |                  |                  |                  |                  |                  |
| α-D-Glucosamine | 6.10 (3.3)     | 4.41 (10.8)    | 5.25 (9.4)     | 5.15 (9.9)     | 3.91 (2.2)     | 3.41 (11.7)    | 4.00 (4.8)     |
| β-D-Glucosamine | 5.68 (8.8)     | 4.29 (10.8)    | 5.20 (9.2)     | 4.91 (9.7)     | 3.79 (2.3)     | 3.68 (12.5)    | 3.80 (NDc)     |
| Nonreducing terminal unit | 4.38 (8.4)     | 3.96 (10.4)    | 4.09 (9.9)     | 4.83 (9.9)     | 3.70 (2.2)     | 4.05 (12.6)    | 4.24 (3.1)     |
| 2,3-di-amino-glucose                  | 6.48 (7.7)     | 6.37 (8.4)     |                  |                  |                  |                  |                  |

a Spectra were recorded at 600 MHz in CDCl3 relative to TMS (0.00 ppm). Assignments were made by 1H,1H-COSY NMR. The α-, β-, γ-, methylene, and methyl protons of fatty acid residues were detected at 2.15 to 2.60, 4.94 to 5.24, 1.50 to 1.70, 1.15 to 1.35, and 0.85 to 0.90 ppm, respectively, and methyl protons of acetyl groups were detected at 2.00 to 2.25 ppm.

b Chemical shift is expressed in parts per million; coupling constant (J) is expressed in hertz.

c ND, not defined.
while two or sometimes three acyloxyacyl residues are present at positions 2' and 3', or additionally at position 2 (in the case of Salmonella), of the nonreducing terminal in enterobacterial lipid A's. A pattern of distribution of phosphate groups different from that of enterobacterial lipid A's was also recognized in F. meningosepticum lipid A, which completely lacked an ester-linked phosphate residue attached to position 4 of the lipid-A backbone. Interestingly, a quite similar structure has been found in the lipid A isolated from oral anaerobic gram-negative bacteria such as Bacteroides fragilis (34) and P. gingivalis (16). These lipid A's have the same fatty acid composition and phosphate group distribution pattern as F. meningosepticum lipid A, although a small amount of ester-linked phosphate group was recognized in P. gingivalis lipid A. However,

### TABLE 3. $^{13}$C NMR data for the peracetylated derivative of F. meningosepticum de-O-acylated lipid A-HCl containing a hybrid backbone$^a$

<table>
<thead>
<tr>
<th>Unit</th>
<th>Chemical shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-1</td>
</tr>
<tr>
<td>Reducing terminal unit</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-GlcN</td>
<td>91.4</td>
</tr>
<tr>
<td>$\beta$-GlcN</td>
<td>94.3</td>
</tr>
<tr>
<td>Nonreducing terminal unit, 2,3-di-amino-glucose</td>
<td>102.9</td>
</tr>
</tbody>
</table>

$^a$ Spectra were recorded at 150.8 MHz in CDCl$_3$ relative to TMS (0.00 ppm). Assignments were made by HMOC experiment. The $\beta$, $\gamma$, and methyl carbons of fatty acid residues were detected at 71.7 to 72.1, 27.4 to 39.1, and 20.3 to 26.5 ppm, respectively, and the methyl and carbonyl carbons of acetyl groups were detected at 19.2 to 25.0 and 168.5 to 172.4 ppm, respectively.

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**F. meningosepticum** lipid A could be obviously distinguished chemically from these oral bacterial lipid A's based on its hybrid backbone consisting of $\beta$(1→6)-linked GlcN3N-GlcN disaccharide in addition to the usual $\beta$(1→6)-linked GlcN disaccharide. Such an unusual GlcN3N has been found to be a component of the lipid A backbone in Brevundimonas (Pseudomonas) diminuta, Brevundimonas vesicularis, Rhodopseudomonas viridis, Rhodopseudomonas sulfuroxidans, Legionella pneumophila, and Campylobacter jejuni (1, 15, 19, 33, 35). The backbones of R. diminuta and B. vesicularis consist of the disaccharide of the diamino sugar, in which position 1 may be replaced by $\alpha$-glucuronic acid. R. viridis, R. sulfuroxidans, and R. palustris lipid A's have unique backbones consisting of the diamino monosaccharide only. The backbone of C. jejuni lipid A is similar to that of F. meningosepticum, which contains a $\beta$(1→6)-linked GlcN3N-GlcN disaccharide forming the lipid A backbone in addition to a $\beta$(1→6)-linked GlcN disaccharide and a GlcN3N disaccharide. With the exception of these structural similarities, F. meningosepticum lipid A has a unique fatty acid composition, phosphate distribution, and hybrid backbone. Moreover, the lipid A preparation seems to contain unknown compounds in the backbone structure, because the total amount of GlcN3N recovered by the compositional analysis does not match the theoretical quantity obtained from the other structural analysis, which indicates the existence of a hybrid backbone in the lipid A. Recovery was not increased by any hydrolysis or degradation procedures tested. The reason for this is not clear, but it may be based on the tight linkage of the unknown compounds to the GlcN3N residue, which may make the detection of the amino sugar impossible.

We have recently proposed the complete lipid A structure of P. gingivalis (16). Using the lipid A, we demonstrated that the lipid A moiety of P. gingivalis LPS, which exhibited relatively lower activity in LPS-responsive mice than lipid A moieties from enteric bacteria, specifically mediates the activation of LPS-unresponsive C3H/HeJ mice (30, 31). Since the chemical structure of F. meningosepticum has similarities to that of P. gingivalis, as found in the present study, the biological prop-
erties of this lipid A are of especially great interest, and studies are currently in progress in our laboratory.

REFERENCES


