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Partial Characterization of the Major Lipooligosaccharide from a Strain of \textit{Haemophilus ducreyi}, the Causative Agent of Chancroid, a Genital Ulcer Disease*  

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The first preliminary structure of a surface lipooligosaccharide from \textit{Haemophilus ducreyi} has been determined. The major oligosaccharide was released by mild acid hydrolysis and analyzed by liquid secondary ion and tandem mass spectrometry. The mass spectral data combined with composition and methylation analysis yielded the most probable structure: GlcA1→4GlcNAc1→3Gal1→4Hepl→6Glc1→(Hepl→2Hepl \rightarrow 3)4Hepl→KDO, where the reducing terminal 3-deoxy-d-manno-octulosonic acid (or KDO) exists in an anhydro form. This anhydro species results from the elimination of a phosphate from C-4 of KDO during mild acid hydrolysis. The core heptose trisaccharide consists of L-glycero-D-manno-heptose, but analysis of the peracetylated sugars indicated that the 1,4-linked heptose is likely D-glycero-D-manno-heptose. The monoclonal antibody 3F11 generated against \textit{Neisseria gonorrhoeae} also binds to this lipooligosaccharide and suggests that the terminal trisaccharide is Galβ1→4GlcNAcβ1→3Galβ1→, an epitope found in the glycose moiety of the human erythrocyte glycosphingolipid lactone tetraglycosylceramide. Mass spectrometric and composition analysis of the lipid A moiety shows that it is similar to the lipid A of \textit{Haemophilus influenzae} strain I-69 Rd− in its carbohydrate composition, suggesting that it is a lipid A of the \textit{Haemophilus ducreyi} strain has been previously reported to be virulent, although a well defined criterion for virulence or avirulence is currently unavailable for this organism (17, 18).

\textit{Haemophilus ducreyi} is a Gram-negative human mucosal pathogen that is the principle cause of genital ulcer disease in developing countries (1-3). \textit{H. ducreyi} infection (or chancroid) is endemic in large parts of Africa and Asia, and it has recently been increasing in prevalence in the United States (4-6). Recent studies involving intradermal injection of \textit{H. ducreyi} in rabbits and mice suggest that the surface lipooligosaccharides (LOS) may play an important role in establishing infection (10, 11). Electrophoretic analyses of the LOS indicate that they lack the repeating O-antigenic side chains that are characteristic of lipopolysaccharides (or LPS) from Gram-negative enteric bacteria, and instead exhibit the migration patterns similar to the LOS of other \textit{Haemophilus} and \textit{Neisseria} species (12, 13). Western blot studies using monoclonal antibodies directed to the LOS of \textit{Neisseria gonorrhoeae} have identified similar epitopes on \textit{H. ducreyi} LOS (14). One of these epitopes is immunochemically similar to the terminal tetrasaccharide of paragloboside, a precursor to a major human blood group antigen (15). The significance of this epitope is still unclear, however, the expression of human antigens on bacterial surface components may allow these organisms to evade the immune system or provide a means to attach to human mucosal epithelial cells (15, 16).

Although the LOS of \textit{H. ducreyi} appears to be important in the pathogenesis of chancroid, limited structural data is currently available. Elucidation of the molecular structure of \textit{H. ducreyi} LOS may provide critical information pertaining to the roles these components play in the pathogenic and immunologic processes. In this report we present the preliminary structure of the major LOS from \textit{H. ducreyi} strain 35000. This strain has been previously reported to be virulent, although a well defined criterion for virulence or avirulence is currently unavailable for this organism (17, 18).

\textbf{EXPERIMENTAL PROCEDURES}

\textit{Materials}—LPS from \textit{Salmonella typhimurium} TV119 Ra mutant, glucose, galactose, glucosamine, galactosamine, KDO, and anhydro

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1 The abbreviations used are: LOS, lipooligosaccharide; Gal, galactosamine; GaINaC, N-acetylgalactosamine; GC/MS, gas chromatography/mass spectrometry; Glc, glucose; GlcNAc, N-acetylglucosamine; Hep, L-glycero-d-manno-heptose or D-glycero-d-manno-heptose; Hex, hexose; HexNAc, N-acetylhexosamine; HPLC, high performance liquid chromatography; KDO, 3-deoxy-d-manno-octulosonic acid; LPS, lipopolysaccharide; LSIMS, liquid secondary ion mass spectrometry; (M−H)−, deprotonated molecular ion; MS/MS, tandem mass spectrometry; PMMA, partially methylated alditol acetates; TLC, thin layer chromatography; BPH, butyl phenylhydrazine.
The Lipo-oligosaccharides of _H. ducreyi_

were all obtained from Sigma. An alditol acetate mixture prepared from the LPS of _Aermonas liquefaciens_ serotype SJ19 was kindly provided by G. Aspinall (University of York, Canada) and butyl phenylhydrazine by J. Webb (Illinois State University). Aqueous HF (48%) was purchased from Mallinckrodt (Moskow, MI) and Aladdin (Skokie, IL) and dilute hydroxide (98%) from Aldrich. Water and methanol were obtained from Burdick and Jackson (Muskegon, MI). Acetic anhydride was purchased from Supelco (Bellefonte, PA), methylhydride from Fluka (Switzerland), and the standard test mixture of partially methylated alditol acetates from BioCarb (Lund, Sweden). All other reagents and solvents were used as reagent grade.

**Isolation and Purification of LPS**—The LPS from _H. ducreyi_ strain 35000 was isolated using a modified phenol/water extraction procedure of Westphal and Jahn (19). Briefly, the dried organisms were suspended in a mixture of hot water/phenol which was kept at 65 °C for 10–15 min. The mixture was then cooled to 10 °C and centrifuged at 80,000 × g for 45 min and the top aqueous layer saved. The bottom phenol layer was heated to 65 °C, hot water added, and the mixture shaken vigorously at 65 °C for 15 min. The mixture was centrifuged as before and the aqueous layers combined and dialyzed against running water for 72 h. The LOS sample was then centrifuged for 8 h at 80,000 × g. The pellet was re-suspended in water, centrifuged at 105,000 × g for 3 h, and lyophilized.

For mass spectrometric analysis, a small amount of LOS was O-deacylated according to the procedure of Helander et al. (20). Briefly, 1 mg of LOS was incubated with 200 μl of anhydrous hydroxide for 20 min at 90 °C. The sample was then cooled to −20 °C and chilled acetone added dropwise to precipitate the O-deacylated LOS which was then centrifuged at 12,000 × g for 20 min. The supernatant was removed and the pellet washed again with cold acetone and centrifuged. The precipitated O-deacylated LOS was then resuspended in 500 μl of water and lyophylized. In one experiment, the O-deacylated LOS was further treated with 48% aqueous HF to remove phosphate.

**Isolation and Purification of Oligosaccharide Fraction**—Twenty mg of LOS from _H. ducreyi_ strain 35000 was hydrolyzed in 10 ml of 1% acetic acid for 2 h at 100 °C. The hydrolysate was centrifuged at 5,000 × g for 10 min and the supernatant removed. The precipitate was washed twice with 5 ml of H2O followed by centrifugation at 5,000 × g for 20 min at 4 °C. The supernatant and washings were pooled and lyophilized.

Approximately 10 mg of the dried oligosaccharide was dissolved in 300 μl of 0.05M pyridinium acetate (pH 5.2), centrifuge filtered, and loaded onto two Bio-Gel P-4 columns connected in series (1.6 × 50 cm, <400 mesh, 30 °C). Samples were eluted in the pyridinium acetate buffer at a flow rate of 10–12 ml/h. Fractions were collected every 10 min and dried down on a Speed-Vac concentrator.

For composition analysis, small aliquots of the oligosaccharide fraction were hydrolyzed in 2 ml trichloroacetic acid (TCA) 6N, 100 °C. The hydrolysate was evaporated to dryness in a Speed-Vac concentrator, redissolved in 20 μl of H2O, and dried. Mono- and disaccharide separations were carried out by high performance liquid chromatography with pulsed amperometric detection as previously described (21). To remove phosphoester moieties, approximately 100 μg of oligosaccharide was placed in a 1.5-mL polypropylene tube and cold 45% aqueous HF was added to make a 10 μg/μl solution. The reaction mixture was incubated for 18 h at 4 °C. Excess HF was removed under a stream of nitrogen in a polypropylene desiccator in vacuo. Solid NaOH was used as a desiccant and an in-line NaOH trap was connected to a water aspirator.

**Preparation of Butyl Phenylhydrazine-Oligosaccharide**—Bio-Gel purified oligosaccharide (~100 μg) was dissolved in water, transferred to 1 ml glass Reacti-Vials (Pierce Chemical Co.) and dried. To each sample approximately 3 ml of butyl phenylhydrazine (BPH) in 40 μl of methanol (22) was added followed by the addition of 10 μl of water and 0.5 μl of glacial acetic acid for every 100 μg of butyl phenylhydrazine. The reaction mixture was placed in a heating block at 80 °C for 30 min, cooled, and dried under a stream of nitrogen. The BPH derivatives were redisolved in water and separated by high performance liquid chromatography (HPLC) with a Vydac C-18 column (25 cm × 4.6-mm inner diameter). The oligosaccharides were eluted with a linear gradient of 0–60% acetonitrile in 60 min at a flow rate of 1 ml/min in a Rainin gradient HPLC system, using a pulse attenuator (solvent B) and a constant 0.05% trifluoroacetic acid (solvent A) and monitored at 530 nm. The derivatized BPH-oligosaccharides were detected at 335 nm with a Kratos 783 variable wavelength detector, and dried prior to mass spectrometric analysis.

**Fractionation and Characterization of Lipid A**—Crude lipid A preparations from _H. ducreyi_ strain 35000 and _S. typhimurium_ Ra mutant (10 mg) were suspended in 0.1 N HCl at a concentration of 2.5 mg/ml and sonicated for 10 min. The mixture was heated at 100 °C for 20 min and cooled. Five volumes of chloroform/methanol (2/1, v/v) were added followed by the addition of 100 ml of chloroform/methanol/water (10/5/1, v/v). The lipid A mixture was then centrifuged at 12,000 for 25 min and dried down on a Speed-Vac concentrator.

**Methylation Analysis**—Linkage analysis was performed on purified oligosaccharide using the microscale method of Levery and Hakomori (23), which was modified by replacing NaH with NaOH as previously described (21). Final analysis of the partially methylated alditol acetates were carried out using a VG70SE mass spectrometer equipped with a Hewlett-Packard 5980 gas chromatograph and an on-column injector (J&W Scientific). Samples were separated on a 30-m DB-1 column with a 0.1-μm film thickness (J&W Scientific). For comparison, a purified HF-treated _S. typhimurium_ Ra oligosaccharide mixture was injected that contained equimolar amounts of the following sugars: terminal Gal, terminal Hep, 1,2-linked Gal, 1,2-linked Glc, 1,3,6-linked Glc, 1,3,6-linked Hep, and 1,3,7-linked Hep.

**Mass Spectrometric Analysis**—Both underivatized and derivatized oligosaccharides were directly analyzed by liquid secondary ion mass spectrometry (LSIMS). The oligosaccharides and their corresponding BPH derivatives were dissolved in water, dried on the probe under vacuum, and redissolved in thioglycolic/glycerol (1/2, v/v). Lipid samples were dissolved in chloroform and 1 ml of 1:1 nitrobenzyl alcohol/triethanolamine (1/1, v/v) was applied as the liquid matrix. Samples were then analyzed on a Kratos MS 505 mass spectrometer retrofitted with a cesium ion source (26) and operating at a resolution of 1500–2000 (m/z m, 10% valley). A primary ion beam of 10 keV was used to ionize the samples and secondary ions were accelerated at 6 kV. Scans were acquired at 500 s/decade and recorded on a Gould electrostatic recorder. Ultramark6 1206 was used for manual calibration to an accuracy better than ±0.2 Da.

**Results**

**Oligosaccharide Composition and Structure**—Size exclusion chromatography of the oligosaccharide fraction from _H. ducreyi_ strain 35000 showed the elution profile shown in Fig. 1. Analysis of the most abundant fractions (57 and 58) by LSIMS identified a single major oligosaccharide with a deprotonated molecular ion, (M−H)−, at m/z 1676 (M, 1,677). Aqueous HF treatment of this oligosaccharide and subsequent LSIMS analysis did not change its mass, indicating the lack of an oligosaccharide. 

2 Nominal masses are given throughout the text for both ions and molecular weights unless otherwise noted. Both values are based on the isotopically pure 13C component of the natural isotopic distribution.
of phosphate or phosphoethanolamine. Composition analysis of the major fraction using high pH anion exchange chromatography identified glucose (Glc), galactose (Gal), glucosamine (GlcN), and L-glycerol-D-manno-heptose (Hep) in a molar ratio of 1/2.5/1.1/2.7, but no KDO was observed. In addition to these components, a peak was observed eluting just prior to the peak for heptose of ≤30% the relative area. Consistent with this information, a computer calculation1 yielded a single overall composition of Hex, HexNAc, Hep, (KDO-H2O), with this last KDO species representing an anhydro form that would not be readily identified in the composition analysis.

The presence of a modified KDO in the LOS of *H. ducreyi* was not unexpected as we had previously encountered an anhydro-KDO in our studies of the LOS from the related bacteria *Haemophilus influenzae*, strain 2019 (21). In *H. influenzae* 2019 LOS, the formation of the anhydro-KDO form was linked to the presence of a phosphate group on the C-4 position that undergoes β-elimination during mild acid hydrolysis (21). To confirm the presence of a similar labile phosphate on the KDO of *H. ducreyi* LOS, the intact LOS was O-deacylated in mild hydrazine and treated with aqueous HF to remove any phosphoester moieties prior to mild acid hydrolysis. LSIMS analysis of the released oligosaccharide yielded a (M-H)- ion at m/z 1884, 18 Da higher in mass than that previously observed.

In order to obtain sequence information of this major oligosaccharide, the corresponding BPH derivative(s) of this major oligosaccharide was prepared and separated by HPLC. The HPLC elution profile of the corresponding BPH derivative(s) of this major oligosaccharide showed seven peaks of near equal abundance. LSIMS analysis yielded the expected molecular ion for the hydrazine derivative at m/z 1822 for all seven peaks, and each peak produced a more or less identical spectrum to that shown in Fig. 2. This result was consistent with the presence of anhydro-KDO at the reducing terminus which has previously been shown to produce a series of diastereomers upon reaction with substituted hydrazines.4 Analysis of the accompanying fragment ions in these LSIMS spectra identified at least two branches: a Hex→HexNAc→Hex→Hep→Hex branch defined by an abundant reducing terminal Y-type ion series (m/z 1660, 1457, 1295, 1103, and 941) and a nonreducing terminal Hep (m/z 1630). Y-type ions are formed by cleavage of the glycosidic bonds with charge retention on the reducing terminal fragment (28). These data allowed us to construct the following partial structure: Hex→HexNAc→Hex→Hep→Hex→(Hep)→Hep→KDO, where the inner core heptose arrangement was still undefined.

To both confirm and complete this preliminary structure, tandem mass spectrometry was performed on both the undervatized oligosaccharide and the corresponding BPH derivative(s). After collision-induced dissociation in the intermediary collision cell, an array of ion fragments is generated from a single isotopic molecular ion that provides more detailed structural information than that typically seen in a conventional LSIMS experiment. One such MS/MS spectrum is shown in Fig. 3 for the undervatized oligosaccharide at m/z 1676. In this spectrum, Y-ions at m/z 795, 1292, and 1484 now define the inner core region as a heptose trisaccharide attached to anhydro-KDO. The remaining fragment ions are
consistent with the structure determined from the BPH oligosaccharide detailed in Fig. 2. Given that this structure contains an unusual branch heptose that is not part of the core region, MS/MS analysis was also carried out on the two Y-ions representing cleavage on either side of this heptose in the underivatized oligosaccharide, i.e. m/z 1149 (Y-1) and 957 (Y-2), to make sure that no other interpretation exists. These two spectra (data not shown) yielded fragment ions that were consistent with the structure as presented in Fig. 3. Specifically, the MS/MS spectrum of the fragment ion at m/z 1149 showed only a loss of heptose from the nonreducing terminus at m/z 957 (Am 192 Da) while the MS/MS spectrum of m/z 957 gave losses for both hexose at m/z 795 (Am 162 Da) and heptose at m/z 765 (Am 192 Da). Taken together, a complete biantennary structure containing nine sugars can now be proposed as Hex→HexNAc→Hex→Hep→Hex→Hep→Hex→Hep→Hep→KDO.

To identify the monosaccharides and their linkages, methylation analysis of the major oligosaccharide fraction was performed. The partially methylated alditol acetates (PMAAs) were analyzed by GC/MS in both electron impact and chemical ionization modes. As shown in Fig. 4, this oligosaccharide is comprised of terminal galactose (1,5-di-O-acetyl-2,3,4,5-tetra-O-methylgalactitol), 1,3-linked galactose (1,3,5-tri-O-acetyl-2,4,6-tri-O-methylgalactitol), 1,6-linked glucose (1,5,6-tri-O-acetyl-2,3,4-tri-O-methylglucitol), terminal heptose (1,5-di-O-acetyl-2,3,4,6,7-penta-O-methylheptitol), 1,4-linked heptose (1,4,5-tri-O-acetyl-2,3,4,6,7-tetra-O-methylheptitol), 1,2-linked heptose (1,2,5-tri-O-acetyl-3,4,6,7-tetra-O-methylheptitol), 1,3,4-linked heptose (1,3,4,5-tetra-O-acetyl-2,6,7-tri-O-methylheptitol), and 1,4-linked N-acetylglucosamine (2-acetamido-2-deoxy-1,4,5-tri-O-acetyl-3,6-di-O-methylglucitol). With the exception of KDO, which would not be expected to form a stable PMAA under these conditions, eight monosaccharides were identified in roughly equimolar amounts and are consistent with those expected from our preliminary structure. When this data is combined with the previous mass spectrometric data, the oligosaccharide structure can now be narrowed down to only a few possibilities, with the most likely one shown below.

\[
\text{Gal} \rightarrow 4\text{GlcNAc} \rightarrow 3\text{Gal} \rightarrow \text{Hep} \rightarrow 4\text{Glc} \rightarrow 3,4\text{Hep} \rightarrow \text{KDO}
\]

Although the precise placement of the two monosubstituted hexoses (1,3-Gal and 1,6-Glc) and heptoses (1,2-Hep and 1,4-Hep) in this oligosaccharide cannot be made at this point, several lines of evidence point to the structure containing the assignments as shown. First, the terminal Gal→4GlcNAc→3Gal→sequence is consistent with Galβ1→4GlcNAc1→3Galβ1→sequence of paragloboside. As previously reported, the monoclonal antibody 3F11, which recognizes the terminal saccharide region of paragloboside, binds strongly to the LOS of \textit{H. ducreyi} strain 35000 (14). This high binding suggests that this paragloboside structure is contained, at least in part, in the nonreducing terminal region of this LOS. Second, the PMAAs for terminal heptose, 1,2-linked heptose, and 1,3,4-linked heptose eluted at the exact positions as the corresponding heptoses of \textit{H. influenzae} 2019, suggesting that the core of \textit{H. ducreyi} 35000 is analogous to that of \textit{H. influenzae} 2019 which contains a Hep1→2Hep1→3(4)Hep1→core, all consisting of L-glycero-D-manno-heptose (29). If so, the heptose on the nonreducing terminal branch is 1,4-linked. Furthermore, compositional analysis revealed the presence of a heptose that is not L-glycero-D-manno-heptose. To verify this, the oligosaccharide was hydrolyzed to its monosaccharides, which were then reduced to their alditols and peracetylated. When these peracetylated monosaccharides were analyzed by GC/MS, a second earlier eluting heptose was observed with a characteristic (M+NH4)+ ion at m/z 926 of approximately one-third the abundance of the peracetylated L-glycero-D-manno-heptose (data not shown). Comparative GC/MS analysis of the alditol acetates from \textit{H. ducreyi} and from \textit{A. liquefaciens} serotype SJ19, whose oligosaccharide contains both L-glycero-D-manno-heptose and D-glycero-D-manno-heptose, clearly identified D-glycero-D-manno-heptose as this less abundant heptose component. Therefore, we made the tentative assignment that this D-glycero-D-manno-heptose is the 1,4-linked heptose on the branch region.

\textbf{Partial Structure of Lipid A}—The lipid A fraction from \textit{H. ducreyi} LOS was analyzed by LSIMS after first partitioning in CHCl3, methanol, 0.1 N HCl (2/1/2, v/v/v) (see Fig. 5a). The molecular ions for both a diphasphorylated (m/z 1823) and monophosphorylated (m/z 1743) species are clearly prominent. Also evident in the spectrum are ions corresponding to loss of myristoyl groups (m/z 1613) and the elimination of an ester-linked hydroxymyristoyl (m/z 1597) and myristoylmysto- rystoyl (m/z 1387) group as their corresponding ketenes. The X-, Y-, Z-type ions, indicative of glycosidic bond cleavage between the two glucosamines (which were independently identified in the high pH anion exchange chromatography composition analysis) are also evident at m/z 738, 710, and 692, respectively. Losses of phosphates from many of these ions were also observed, but it is not clear whether this represents heterogeneity in the lipid or part of the LSIMS fragmentation process.

To further explore details of the lipid A structure, tandem mass spectrometry was performed on the major lipid A fragment ions at m/z 1823, 1597, 1387, and 710. The MS/MS spectrum of the m/z 710 ion is shown in Fig. 5b. In this spectrum, the peak at m/z 466 represents the elimination of

![Fig. 4. Total GC/MS ion chromatogram in the electron impact mode of PMAAs from \textit{H. ducreyi} strain 35000. Peak areas relative to terminal galactose are follows: 1-linked Gal (1.0), 1,3-linked Gal (1.5), 1,6-linked Glc (1.7), 1-linked Hep (0.65), 1,4-linked Hep (0.57), 1,2-linked Hep (0.94), 1,3,4-linked Hep (0.70), and 1,4-linked GlcNAc (1.8). By determining the relative molar response factors for the PMAAs that were also present in the oligosaccharide isolated from \textit{S. typhimurium} Ra LPS (i.e. terminal Gal and terminal Hep), the molar ratio of these same two sugars in the \textit{H. ducreyi} oligosaccharide was found to be 0.96, close to the predicted molar ratio of 1.0.](image-url)
the O-linked hydroxymyristoyl group as the free acid. The peak at m/z 240 probably indicates a two-bond cleavage of the O-linked hydroxymyristoyl group as an acid and loss of the N-linked hydroxymyristoyl group, this time as a ketene. At low mass, abundant ions at m/z 97 (H₂PO₄⁻) and 79 (PO₄⁻) are seen that arise from elimination of phosphate. This spectrum and the others (data not shown) are all consistent with the lipid A structure as detailed in Fig. 5.

To better assay for heterogeneity in the lipid preparation, the crude sample was then partially separated by TLC. Three minor spots appeared at RF values of approximately 0.35, 0.5, and 0.6 with a major (multiple) band at RF = 0.8. The LSIMS spectrum of the major band gave the same (M-H)⁻ ion at m/z 1823 seen previously in the crude fraction, as well as a less abundant ion at m/z 1991. This latter ion could not be readily accounted for, but by mass difference might correspond to further substitution of the M, 1824 lipid with an unusual 0-linked fatty acid (AM, 168 Da). Fatty acid analysis of the intact LOS, however, revealed the presence of only myristic and β-hydroxymyristic acids. While more structural information is needed to definitively place the fatty acids and phosphates on the glucosamine disaccharide, this preliminary analysis shows that lipid A from H. ducreyi is similar to the lipid A of H. influenzae strain I-69 Rd⁻/β⁺ (20).

Analysis of Intact LOS—At this point, the precise nature of the linkage between the oligosaccharide and lipid A moieties had not been established, nor the identity of the acid labile group attached to KDO. Mass spectrometric analysis of the intact LOS would provide this information, but the aggregated state and low solubility of LOS has generally precluded such a measurement. However, removal of the base labile O-linked fatty acids on the lipid A moiety greatly reduces these problems and enables an “intact” LOS to be analyzed (21). Therefore, O-deacylated HF-treated LOS of H. ducreyi 35000 was prepared and analyzed by LSIMS. A molecular ion was observed at m/z 2468 (average M, 2,470) which corresponds to a species containing the previously determined nonasaccharide, Gal₁→4GlcNAc₁→3Gal₁→4Glc₁→2Hepl₁→β,3,4Hepl₁→KDO, directly linked to the lipid moiety consisting of a glucosamine disaccharide containing the two (hydrate resistant) N-linked β-OH myristoyl groups. When O-deacylated (non-HF treated) LOS was analyzed, the electrospray mass spectrum produced two abundant multiply charged ions, (M-4H)⁻ at m/z 6767 and (M-3H)⁻ at m/z 902.2, yielding an average M, of 2,710. This mass is 240 Da larger than the average mass of the O-deacylated HF-treated LOS, and signifies the presence of three phosphate groups, two of which have been previously assigned to the lipid A moiety. This third phosphate group must therefore be the acid labile moiety on the KDO.

DISCUSSION

In this study we have presented a partial structure of the LOS of H. ducreyi strain 35000 (Fig. 6). Previous reports have analyzed the chemical composition of the LOS from various strains of H. ducreyi, but no structural data was presented (12). Elucidation of the molecular structure of H. ducreyi LOS may provide critical information pertaining to the role these components play in the pathology and host immunochemical response to H. ducreyi infection.

The proposed structure of the major H. ducreyi LOS form
contains some unusual features. The lack of phosphate or phosphoethanolamine in the core heptose region is in direct contrast to virtually all other LOS of related mucosal bacteria (30). However, the presence of phosphate on the KDO may in some way compensate for the lack of other phosphoester moieties in this core region, although the significance of these phosphate moieties is unknown. The presence of a 1,4-linked heptose, most likely D-glycero-D-manno-heptose, in the branch region is also atypical of both enteric and non-enteric bacterial LOS (30), although a terminal D-glycero-D-manno-heptose has been reported in the outer core LOS region of a strain of Proteus mirabilis (31). If this sugar proves to be a common structural feature in the LOS of other strains of H. ducreyi, this heptose may provide a unique target for therapeutic intervention, or possibly as part of a more specific diagnostic indicator. We are currently analyzing LOS from several other strains of H. ducreyi to determine if this is a common feature shared among these organisms.

Immunochromical studies have previously shown that the LOS of most H. ducreyi strains express an epitope which is also present on the LOS of many N. gonorrhoeae and Neisseria meningitidis strains (14). This epitope is immunochromically similar to the terminal tetrasaccharide of paragloboside (Galβ1→GlcNAcβ1→3Galβ1→Glc1→ceramide), a precursor to a major human blood group antigen (15). Our structural studies suggest that this epitope is similar to the terminal tetrasaccharide exists in the terminal trisaccharide of the LOS from H. ducreyi 35000. Monoclonal antibody 3F11 reacts with this terminal saccharide and also binds to the terminal region of porphobiloside (15). The significance of this epitope on the LOS of these mucosal pathogens is unclear, but the presence of this structure on microbial surfaces could mask the pathogen on the mucosal surface and blunt the host immune response. Previous studies involving humans recovering from gonococcal infection suggest that this is the case since the human response is limited primarily to serotypic LOS determinants rather than the conserved antigenic sites (30). It has also been suggested that this region may function as an adherence factor or as a receptor which could bind to membrane lectin-like structures (15, 16).

The similarity between the LOS of H. ducreyi and Neisseria is also evident in their biological activities. Recent animal studies involving the intradermal injection of LOS from H. ducreyi and N. gonorrhoeae show that these organisms express LOS with similar toxic activity (10, 11). Since the lipid A moiety of N. gonorrhoeae LOS mediates damage to fallopian tube mucosa in vitro (33), it is likely that the lipid A of H. ducreyi plays an analogous role in a typical skin lesion (or ulcer). Studies are now in progress that will directly address this issue.

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32. Deleu in proof