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# The Lipooligosaccharides of *Haemophilus ducreyi* Are Highly Sialylated

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**The major lipooligosaccharides of the sexually transmitted pathogen *Haemophilus ducreyi* 35000 have been previously found to terminate in *N*-acetyllactosamine and sialyl-*N*-acetyllactosamine, Neu5Acα2→3Galβ1→4GlcNAc (W. Melaugh, N. J. Phillips, A. A. Campagnari, M. V. Tullius, and B. W. Gibson, Biochemistry 33: 13070–13078, 1994). In this study, mass spectrometry and composition analyses have shown that the lipooligosaccharides from three other *H. ducreyi* strains also contain *N*-acetyllactosamine and are highly sialylated (≈30 to 50%), although one African strain was found to contain neither of these structural features.**

The lipooligosaccharides (LOS) of *Haemophilus ducreyi*, like LOS of the mucosal pathogens *Haemophilus influenzae* and *Neisseria gonorrheae*, consist of a lipid A moiety embedded in the outer membrane of the bacterium attached to a variable oligosaccharide (13, 14, 21). It is thought that LOS is responsible for much of the cytotoxicity of *H. ducreyi* infection (4), but the role of the variable oligosaccharide has been difficult to define. Electrophoretic and mass spectrometric studies of LOS from numerous *H. ducreyi* strains have shown them to be highly heterogeneous (3, 7, 14). Previously, we had determined the structure of the major oligosaccharide from the LOS of *H. ducreyi* 35000 (14) and found it to contain terminal *N*-acetyllactosamine, i.e., Galβ1→4GlcNAc, which is modified in some LOS by sialic acid. Given the importance of sialic acid in many biological systems (19), this acidic sugar is likely to play an important role in the pathology of *H. ducreyi* infection.

In this study, we have investigated the presence or absence of *N*-acetyllactosamine and sialic acid in the LOS from *H. ducreyi* 188, NYC23, 233, and ATCC 33921 as well as one pyocin variant of strain 188 (2). Since *N*-acetyllactosamine is thought to be the acceptor for sialic acid (14), two intriguing questions were asked: do strains whose LOS terminate in *N*-acetyllactosamine also contain sialic acid, and do those strains whose LOS do not contain this disaccharide also lack sialic acid?

**Materials.** Glucose (Glc), galactose (Gal), glucosamine (GlcN), galactosamine, 3-deoxy-D-manno-octulosonic acid (Kdo), neuraminidase (from *Clostridium perfringens*), and anhydrous hydrazine were all obtained from Sigma (St. Louis, Mo.). Aqueous HF (48%) was purchased from Mallinckrodt (Muskegon, Mich.), and sodium borodeuteride (98% deuterium) was obtained from Aldrich. Acetonitrile, water, and methanol were obtained from Burdick and Jackson (Muskegon, Mich.). Acetic anhydride was purchased from Supelco (Bellefonte, Pa.); methyliodide was purchased from Fluka (Buchs, Switzerland).

**Methods.** The LOS from *H. ducreyi* 35000, 188, 233, NYC23, 188-2, and 33921 were isolated by using a modified phenol-water extraction procedure (22). LOS samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on a 14% polyacrylamide gel as previously described (14). Approximately 5 to 10 μg of LOS was loaded into each well, and the LOS in even-numbered lanes were preincubated with Newcastle disease virus sialidase (neuraminidase).

For mass spectrometric and composition analyses, 1 to 2 mg of LOS from each strain was O deacylated by treatment with anhydrous hydrazine for 20 min at 37°C (7). To prepare free oligosaccharides, LOS was hydrolyzed with 1% acetic acid (2 mg/ml) at 100°C for 2 h and purified by Bio-Gel P-4 chromatography (1.6 by 80 cm, <400 mesh, 30°C) or by high-pressure liquid chromatography (Bio-Sil TSK-125). To remove phosphate and phosphoesters, ≈100 μg of oligosaccharide was treated with cold 48% aqueous HF (10-μg/μl solution) for 16 h at 4°C. Details of these procedures can be found elsewhere (13, 17).

Saccharide composition analysis of oligosaccharides was performed by using high-pH anion-exchange chromatography (HPAEC) with pulsed amperometric detection after hydrolysis: 2 M trifluoroacetic acid for 3 h at 100°C. Sialic acid was determined by treatment with *C. perfringens* neuraminidase as described by Yamasaki et al. (23). For strains 35000 and 188, the weight purity of the LOS samples was determined by assaying for GlcN content. Weighed quantities of LOS (≈300 μg) were hydrolyzed in 400 μl of 6 N HCl for 4 h, and the resulting GlcN was analyzed by HPAEC as previously described (17).

Linkage analysis was performed on purified oligosaccharides by using a modification of the microscale method of Levery and Hakomori (10). The resulting partially methylated alditol acetates were analyzed by gas chromatography-mass spectrometry, using a VG70SE mass spectrometer equipped with a Hewlett Packard 5890 gas chromatograph and on-column injector (J&W Scientific).

For molecular mass and partial sequence determination, oligosaccharides were directly analyzed by liquid secondary ion mass spectrometry (LSIMS) as described elsewhere (17). To determine LOS heterogeneity and to accurately assign molecular weight and compositions, O-deacylated LOS were analyzed by negative-ion electrospray ionization mass spectrometry (ESI-MS) on a VG/Fison Platform quadrupole mass spectrometer. O-deacylated LOS were dissolved in water and then injected (3-μl aliquot, ≈5 μg) into a stream of $H_2O$-acetonitrile (3:1, vol/vol) containing 1% acetic acid running at 3 to 4 μl/min. Details of these latter methods have been reported in earlier publications (7, 14).

**Heterogeneity of *H. ducreyi* LOS by SDS-PAGE.** To assess LOS heterogeneity and determine the presence of sialic acid, LOS from five strains of *H. ducreyi* were subjected to SDS-

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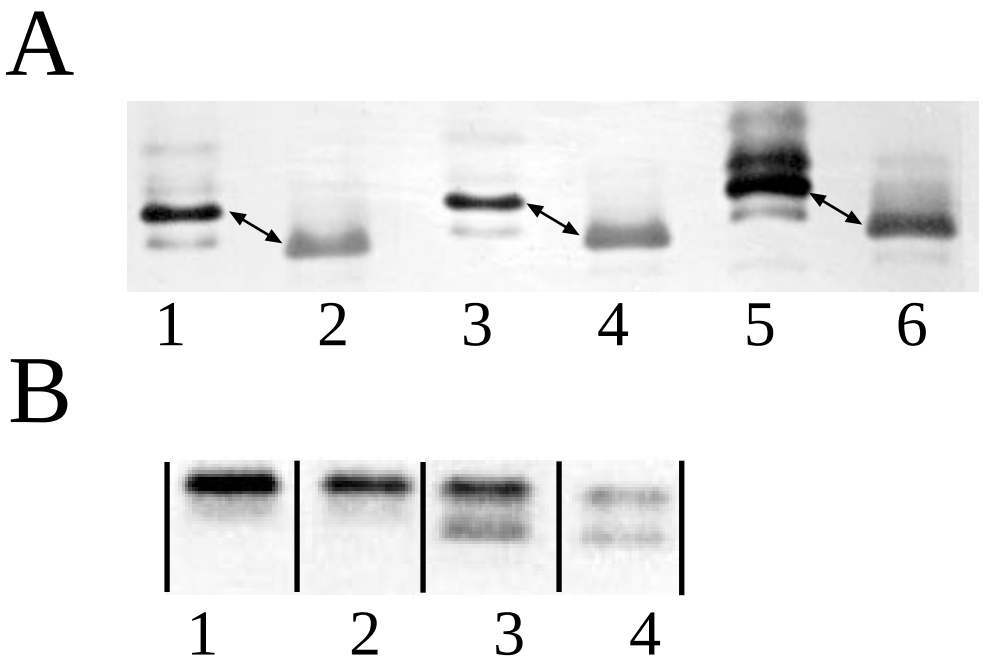


FIG. 1. Composite scan of two SDS-polyacrylamide gels illustrating the LOS patterns from five different strains of *H. ducreyi*. The LOS in even-numbered lanes were preincubated with neuraminidase (sialidase), while the LOS in odd-numbered lanes were not. (A) LOS from strains 35000 (lanes 1 and 2), NYC23 (lanes 3 and 4), and 188 (lanes 5 and 6). (B) LOS from strain ATCC 33921 (lanes 1 and 2) and the pyocin-variant strain 188-2 (lanes 3 and 4).

PAGE analysis before and after treatment with neuraminidase. The composite gel shown in Fig. 1 shows the LOS patterns from the five strains. Figure 1A shows the LOS from *H. ducreyi* 35000, NYC23, and 188 that express the epitope defined by monoclonal antibody (MAb) 3F11, which recognizes the terminal portion of paragloboside, Galβ1→4GlcNAcβ1→3Galβ1→4Glc (3). Preincubation of LOS with neuraminidase caused a downward shift in a prominent LOS band. This electrophoretic shift is consistent with the release of sialic acid. The gel lanes in Fig. 1B show LOS from *H. ducreyi* 33921 and 188-2 that do not contain the MAb 3F11 epitope, and preincubation of these LOS with neuraminidase (lanes 2 and 4) does not cause any shift in the LOS bands. These data suggest that the wild-type strains 35000, NYC23, and 188 all contain LOS species with terminal *N*-acetyllactosamine and sialyl-*N*-acetyllactosamine, while strain 33921 and the pyocin-variant strain do not.

**Mass spectrometric and composition analyses of LOS.** The structure of the major oligosaccharide from strain 35000 (14) and a preliminary set of structures for the LOS of strain 188 and its pyocin variant 188-2 have been previously reported (2). Data from these studies are included here. With the exception of the African strain 33921, LSIMS analysis revealed a major oligosaccharide species for each wild-type strain with a deprotonated molecular ion, $(M - H)^-$, at *m/z* 1,676 ($M_r$ = 1,677). When these oligosaccharides were treated with aqueous HF and reanalyzed by LSIMS, no shift in the mass was observed, demonstrating the absence of phosphate and phosphoethanolamine (PEA). Some glycosidic bond fragments (sequence ions) were observed in these LSIMS spectra (data not shown) but were essentially identical to those reported for the major oligosaccharide of strain 35000 (13). These sequence ions were seen at *m/z* 1,514 and 1,311, which corresponded to losses of hexose (Hex) (−162 Da) and Hex-HexNAc (−365 Da), re-

TABLE 1. Oligosaccharide composition and methylation analysis

| Strain | Composition analysis[a] (mole ratio relative to glucose) | | | | | Methylation analysis[b] (relative peak area) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glc | Glu | Hep | Heptose[c] | $GlcNH_2$ | t-Gal | 3-Gal | 6-Glc | t-Hep | 2-Hep | 3,4-Hep | 4-Hep | 4-GlcNAc | 4-Glc | t-GlcNAc |
| 35000 | 2.5 | 1.0 | 2.7 | 0.8 | 1.1 | 1.0 | 1.5 | 1.7 | 0.7 | 0.9 | 0.7 | 0.6 | 1.8 | | |
| NYC23 | 2.6 | 1.0 | 2.7 | 0.8 | 1.1 | 1.0 | 0.7 | 1.7 | 0.5 | 0.6 | 0.4 | 0.7 | 1.0 | | |
| 233 | 2.0 | 1.0 | 3.3 | —[d] | 0.7 | 1.0 | 0.3 | 1.7 | 0.5 | 0.5 | 0.2 | 0.6 | 1.0 | | |
| 188 | 1.5 | 1.0 | 2.0 | 1.1 | 0.8 | 1.0 | 0.6 | 1.8 | 0.5 | 0.7 | 0.3 | 0.6 | 1.1 | | |
| 188-2 | 0.8 | 1.0 | 2.8 | 0.1 | | 1.0 | | 1.5 | 0.3/1.3[e] | 1.3 | 0.4 | | | | |
| 33921 | 1.3 | 1.0 | 2.9 | | 1.0 | | 0.7 | | 0.5 | 0.7 | 0.5 | | | 1.0 | 0.9 |

[a] Kdo was not detected in the hydrolyzed oligosaccharide fraction because of its conversion to *anhydro*Kdo forms during acetic acid hydrolysis of LOS.
[b] Partially methylated alditol acetates are abbreviated according to their substitution pattern as follows: t-Gal is 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 3-Gal is 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol, etc.
[c] This second heptose has been tentatively identified as D-*glycero*-D-*manno*-heptose on the basis of earlier data (14), as opposed to the major core heptoses, which are L-*glycero*-D-*manno*-heptoses.
[d] The unusual D-*glycero*-D-*manno*-heptose was not found in the compositional analysis even though it was clearly present in the methylation analysis data.
[e] Two terminal heptoses were found; the ratio stated is the unusual D-*glycero*-D-*manno*-heptose to the expected terminal core L-*glycero*-D-*manno*-heptose.

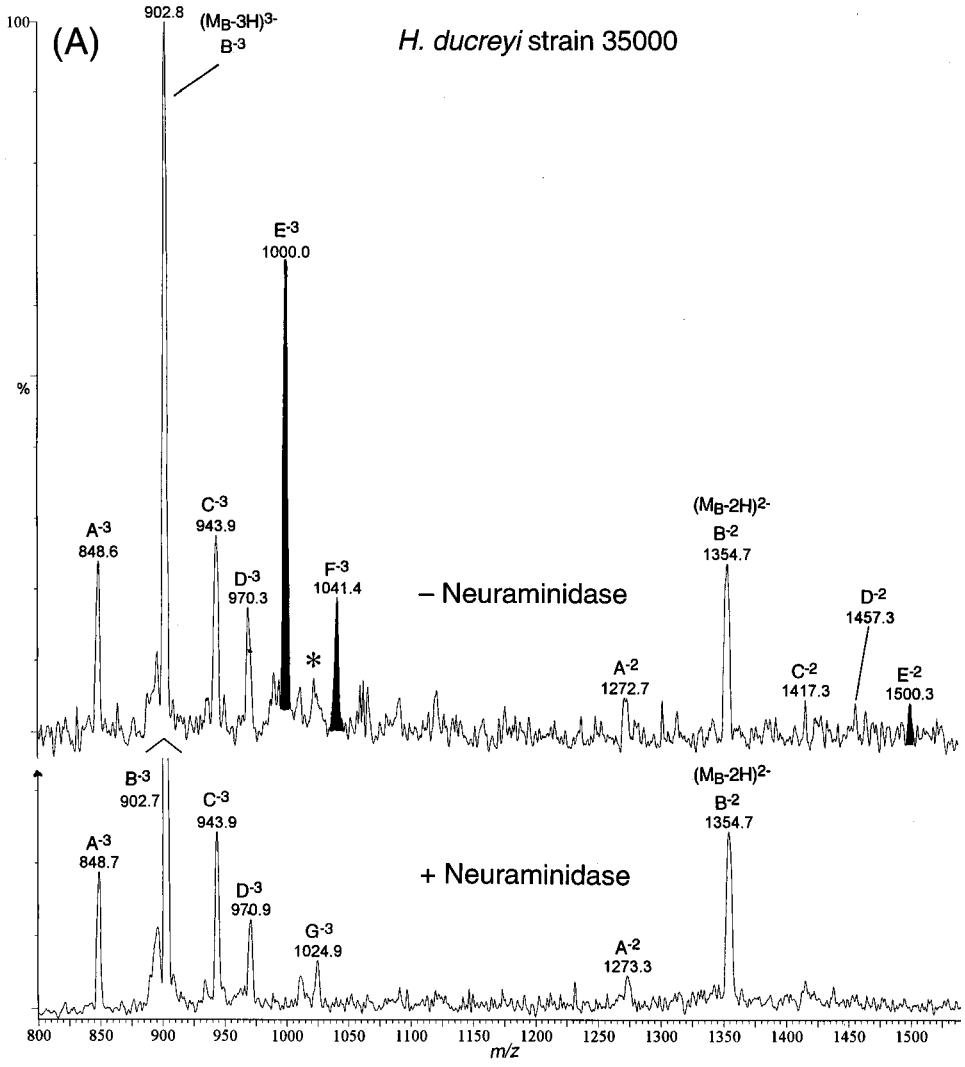


FIG. 2. Partial negative-ion electrospray ionization mass spectrum of O-deacylated LOS from *H. ducreyi* 35000 (A) and 233 (B) before (top) and after (bottom) neuraminidase treatment. LOS-A to LOS-F correspond to the same *m/z* peaks seen for both strains. Shaded peaks at *m/z* 1,000 and 1,041 correspond to LOS species thought to contain sialic acid which disappear after neuraminidase treatment (bottom spectra). See Table 4 for the assignments of LOS compositions.

spectively. The oligosaccharides from strains 35000 and 188 showed additional sequence ions corresponding to further losses of Hex-HexNAc-Hex (*m/z* 1,149) and Hex-HexNAc-Hex-Hep (*m/z* 957) and, from a second branch, losses of L-*glycero*-D-*manno*-heptose (Hep) (*m/z* 1,484) and Hep-Hep (*m/z* 1,292).

The LSIMS spectrum of the oligosaccharide from the LOS of the African strain 33921 had an $(M - H)^-$ at *m/z* 1,322 ($M_r$ = 1,323), representing a much smaller oligosaccharide than in the other strains. There were also sequence ions present corresponding to losses of HexNAc (*m/z* 1,119), HexNAc-Hex (*m/z* 957), and HexNAc-Hex-Hex (*m/z* 795), indicative of a trisaccharide branch off a Hep$_3$*anhydro*Kdo core, $M_r$ (calculated) = 1,323. The LSIMS spectrum of the oligosaccharide from the pyocin-variant strain 188-2 also showed a smaller oligosaccharide with an $(M - H)^-$ at *m/z* 1,434. Treatment of this oligosaccharide with HF to remove phosphoesters shifted the mass to *m/z* 1,311, indicating that PEA ($\Delta m$ = 123 Da) had been removed. Previous sequence analysis (2) had suggested this oligosaccharide contained a Hex-Hep-Hex trisaccharide off a Hep$_3$(PEA)*anhydro*Kdo core, which is consistent with the current LSIMS data.

Composition analysis of the oligosaccharides from each strain by using HPEAC identified Glc, Gal, GlcN, and Hep in the molar ratios shown in Table 1. In the HPAEC profile of strains 35000, NYC23, 188, and 188-2, a peak was observed for D-*glycero*-D-*manno*-heptose (13). Free Kdo was not seen in the composition analysis.

To identify the monosaccharides and their linkages, linkage analysis of the major oligosaccharide from each strain was performed. For strains 35000, NYC23, 233, and 188, eight major sugars eluted in the following order: terminal Gal, 3-Gal, 6-Glc, terminal Hep, 4-Hep, 2-Hep, 3,4-Hep, and 4-GlcNAc (Table 1). Methylation analysis of strain 188-2 shows that this pyocin variant lacks the 3-linked Gal and 4-linked GlcNAc seen in the wild-type strains. Strain 33921 has the same inner core sugars as the other strains and an outer core branch consisting of 4-linked Glc, 3-linked Gal, and a terminal GlcNAc.

Given the similarities in the LSIMS, compositional, and

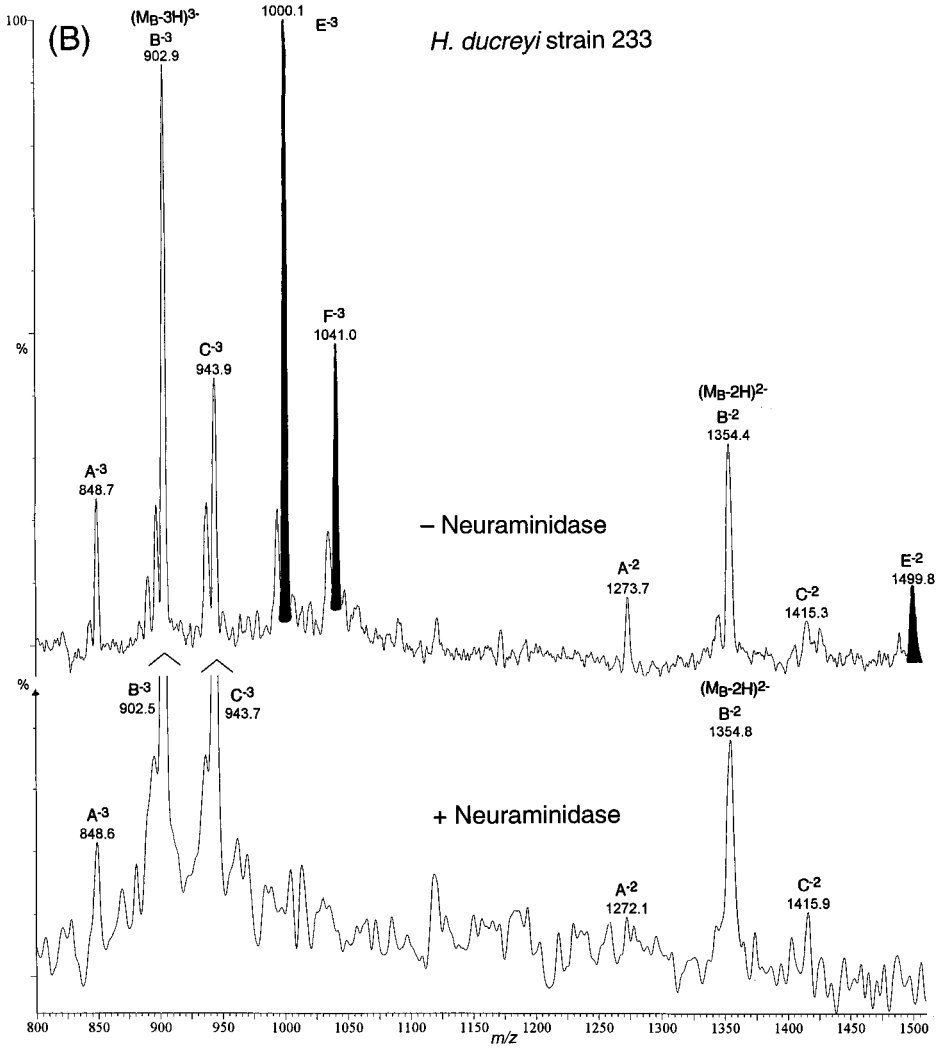


FIG. 2—*Continued.*

linkage data for strains 35000, 233, 188, and NYC23, it appears that all four strains contain an oligosaccharide identical (or highly similar) to that reported for strain 35000 (see below) (14). The oligosaccharide of strain 188-2, which is a pyocin variant of strain 188, lacks the terminal *N*-acetyllactosamine, Galβ1→4GlcNAc, present in the structure shown below and contains a PEA most likely located in the core heptose region (2):

Galβ1→4GlcNAcβ1→3Galβ1→4Hepα1→6Glcβ1→4Hepα1→*anhydro*Kdo
3
↑
Hepα1→2Hepα1

The Kenyan strain 33921, however, appears to possess the same core ($Hep_3$*anhydro*Kdo) but differs in the carbohydrate branch by containing only three sugars, a 4-linked Glc, a 3-linked Gal, and a terminal GlcNAc. This strain, therefore, lacks the terminal *N*-acetyllactosamine and the 4-linked Hep evident in the other wild-type strains. On the basis of immunochemical data that show strong binding to MAb 4C8 specific for *N. gonorrhoeae* 1291a LOS (9), the terminal trisaccharide in this strain is most probably GlcNAcβ1→3Galβ1→4Glcβ1.

**Molecular weight analysis of LOS by ESI-MS.** To determine what additional LOS structures are present and what relationship they bear to the major oligosaccharides, ESI-MS was used to assess LOS heterogeneity. LOS were first converted to water-soluble forms by O deacylation with hydrazine, which forms primarily doubly and/or triply charged deprotonated species, $(M - 2H)^{2-}$ and $(M - 3H)^{3-}$, under ESI-MS conditions, from which the mass (molecular weight) can be readily assigned (7).

The top part of Fig. 2A shows the ESI-MS spectrum of O-deacylated LOS from strain 35000 before neuraminidase treatment. The most abundant ion is the triply charged species $(M - 3H)^{3-}$ at *m/z* 902.8, which corresponds to an average $M_r$ of 2711.4 (LOS-B). The molecular weight of this O-deacylated LOS species is consistent with the attachment of an O-deacylated lipid A moiety to the major oligosaccharide terminating in *N*-acetyllactosamine (but still containing the phosphate on the Kdo) as previously described (13, 14):

Galβ1→4GlcNAcβ1→3Galβ1→4Hepα1→6Glcβ1→4Hepα1→Kdo(P)-*O*-deacyl-lipid A
3
↑
Hep1α→2Hepα1

TABLE 2. ESI-MS analyses of O-deacylated LOS from *H. ducreyi* 35000, 188, 233, and NYC23

| $M_r$ calculated | $M_r$ observed (relative % abundance)[a,b] | | | | Proposed composition[c] |
|---|---|---|---|---|---|
| | 35000 | 188 | 233 | NYC23 | |
| 1,829.7 | | 1,830.3 (13) | | | $Hep_3$ Kdo(P) lipid A[d] |
| 1,952.7 | | 1,953.6 (37) | | | $Hep_3$ PEA Kdo(P) lipid A |
| 1,991.8 | | 1,991.1 (5) | | | Hex $Hep_3$ Kdo(P) lipid A |
| 2,114.8 | | 2,115.0 (4) | | | Hex $Hep_3$ PEA Kdo(P) lipid A |
| 2,184.0 | | 2,181.6 (2) | | | Hex $Hep_4$ Kdo(P) lipid A |
| 2,307.0 | | 2,305.8 (18) | | | Hex $Hep_4$ PEA Kdo(P) lipid A |
| 2,346.1 | | 2,346.0 (26) | | 2,342.7 (10) | $Hex_2$ $Hep_4$ Kdo(P) lipid A |
| 2,469.2 | | 2,469.0 (13) | | 2,470.2 (9) | $Hex_2$ $Hep_4$ PEA Kdo(P) lipid A |
| 2,549.3 | 2,548.8 (25) | 2,549.1 (41) | 2,549.1 (24) | 2,548.2 (60) | $Hex_2$ HexNAc $Hep_4$ Kdo(P) lipid A |
| 2,672.4 | | 2,673.0 (49) | | 2,670.0 (44) | $Hex_2$ $Hep_4$ PEA HexNAc Kdo(P) lipid A |
| 2,711.5 | 2,711.4 (100) | 2,713.8 (46) | 2,711.7 (92) | 2,711.4 (58) | (Hex-HexNAc) $Hex_2$ $Hep_4$ Kdo(P) lipid A |
| 2,834.5 | 2,834.7 (27) | 2,833.8 (98) | 2,834.7 (41) | 2,835.3 (57) | (Hex-HexNAc)$Hex_2$ $Hep_4$ PEA Kdo(P) lipid A |
| 2,914.7 | 2,913.9 (18) | | | | $Hex_3$ $HexNAc_2$ $Hep_4$ Kdo(P) lipid A |
| 3,002.7 | 3,003.0 (66) | 3,002.4 (46) | 3,003.3 (100) | 3,001.5 (72) | (Neu5Ac-Hex-HexNAc)$Hex_2$ $Hep_4$ Kdo(P) lipid A |
| 3,125.7 | 3,127.2 (20) | 3,127.8 (100) | 3,126.0 (47) | 3,125.7 (100) | (Neu5Ac-Hex-HexNAc)$Hex_2$ $Hep_4$ PEA Kdo(P) lipid A |

[a] Reported as average mass value based on the triply charged ions.
[b] Peaks originating from loss of water have been omitted for purposes of simplicity.
[c] Sugars in parentheses are likely present as N = acetyllactosamine and N = acetylsialyllactosamine (see text).
[d] After O deacylation, the lipid A moiety is converted into diphosphoryl diacyl lipid A containing two N-linked β-hydroxymyristic acid chains with an average $M_r$ of 953.0089.

The triply charged ions at *m/z* 848.6, 943.9, and 970.3 represent major oligosaccharide species which are either truncated (−Hex, LOS-A) or extended and/or phosphorylated LOS glycoforms (+PEA, LOS-C; +HexNAc, LOS-D; +Hex-HexNAc, LOS-G) of the major LOS-B species. The average $M_r$'s and compositions of these species are listed in Table 2. However, three of these peaks at *m/z* 1,000.0, 1,041.4, and 1,500.3 correspond to two additional LOS analogs whose masses suggest the addition of sialic acid (LOS-E, $M_r$ = 3,003) and sialic acid plus PEA (LOS-F, $M_r$ = 3,127). In support of these latter LOS assignments, the shaded peaks representing the triply and doubly charged ions of these two sialylated glycoforms disappear after neuraminidase treatment (Fig. 2A, bottom).

The spectrum of O-deacylated LOS from *H. ducreyi* 233 before neuraminidase treatment is similar to that of strain 35000 except that the LOS-D glycoform is absent and the base peak is now the sialylated analog of the major species (Fig. 2B, top). Peaks representing triply charged molecular ions of the sialylated LOS analogs at *m/z* 1,000.1 and 1,041.0 are again shaded and, like the sialylated LOS glycoforms in strain 35000, are quantitatively removed after treatment with neuraminidase (Fig. 2B, bottom).

The ESI-MS spectra of O-deacylated LOS from *H. ducreyi* 188 (2) and NYC23 were found to be similar to those of strains 35000 and 233 with the difference that the latter strains are more heterogeneous and exhibit a greater degree of phosphorylation. The relative abundances and composition assignments are summarized in Table 2.

The ESI-MS spectrum of O-deacylated LOS from *H. ducreyi* 33921 (spectrum not shown; see Table 3) is markedly unlike those of the previous wild-type strains. The major ion in this spectrum is the triply charged ion at *m/z* 825.8, corresponding to a species containing a HexNAc-$Hex_2$ oligosaccharide branch off a $Hep_3$(PEA)Kdo(P)-lipid A core ($M_r$ = 2,480). A peak at *m/z* 784.8 was assigned as a triply charged LOS missing the PEA, and the peak at *m/z* 866.6 as a triply charged LOS containing an extra PEA. There is no evidence for the presence of sialic acid or *N*-acetyllactosamine.

The spectrum of O-deacylated LOS from *H. ducreyi* 188-2 was previously found to contain a triply charged base peak at *m/z* 822.3 ($M_r$ = 2,469.5), a truncated version of the same major structure seen in the other strains lacking N-acetyllactosamine. No sialylation of the major oligosaccharide species was evident (2), and no change was observed after neuraminidase treatment. The observed masses and proposed compositions of the LOS glycoforms for this pyocin-variant strain and for strain ATCC 33921 are listed in Table 3.

**Sialic acid content.** To independently identify the presence

TABLE 3. ESI-MS analyses of O-deacylated LOS from *H. ducreyi* ATCC 33921 and 188-2

| Strain | $M_r$[a] | | Relative % abundance | Proposed composition |
|---|---|---|---|---|
| | Observed | Calculated | | |
| 188-2 | 2,276.8 | 2,277.0 | 10 | $Hex_2$ $Hep_3$ PEA Kdo(P) lipid A[b] |
| | 2,307.3 | 2,307.0 | 29 | Hex $Hep_4$ PEA Kdo(P) lipid A |
| | 2,469.5 | 2,469.2 | 100 | $Hex_2$ $Hep_4$ PEA Kdo(P) lipid A |
| | 2,592.0 | 2,592.2 | 10 | $Hex_2$ $Hep_4$ $PEA_2$ Kdo(P) lipid A |
| | 2,712.7 | 2,711.4 | 4 | $Hex_3$ HexNAc $Hep_4$ Kdo(P) lipid A |
| 33921 | 2,357.4 | 2,357.1 | 72 | $Hex_2$ HexNAc $Hep_3$ Kdo(P) lipid A |
| | 2,480.4 | 2,480.2 | 100 | $Hex_2$ HexNAc $Hep_3$ PEA Kdo(P) lipid A |
| | 2,602.8 | 2,603.3 | 49 | $Hex_2$ HexNAc $Hep_3$ $PEA_2$ Kdo(P) lipid A |

[a] Reported as average mass value based on the triply charged ions.
[b] After O deacylation, the lipid A moiety is converted into diphosphoryl diacyl lipid A containing two N-linked β-hydroxymyristic acid chains with an average $M_r$ of 953.0089.

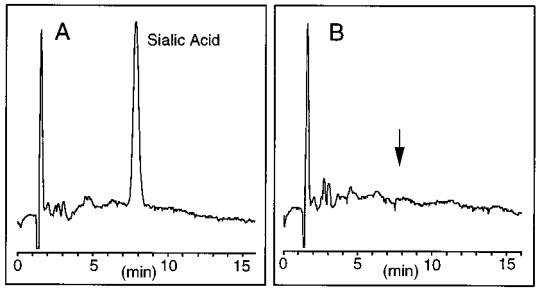


FIG. 3. High-pH anion-exchange chromatogram of precipitated LOS fraction of *H. ducreyi* 35000 incubated with neuraminidase (A) and heat-inactivated neuraminidase (B). Chromatograms for other strains found to contain sialic acid (233, 188, and NYC23) were similar.

of sialic acid in the LOS of these strains, LOS samples were treated with neuraminidase and the released sialic acid was measured by HPAEC. Figure 3 shows the chromatogram of the LOS from *H. ducreyi* 35000 incubated with neuraminidase and heat-inactivated neuraminidase. Sialyllactose was used as a control, and sialic acid eluted at 7.8 min. The chromatograms of the LOS from strains 233, NYC23, and 188 looked similar, while those of strains 188-2 and 33921 contained no detectable levels of sialic acid. Since we would like to know the relative molar proportions of LOS species containing sialic acid (i.e., mole percent of LOS), the purity of the LOS was independently determined by assessing glucosamine content in two LOS preparations in which sufficient materials were available. We assumed that the best estimate of the weight purity of LOS is 3 mol of glucosamine (2 mol from the lipid A and 1 mol from the oligosaccharide) per mol of LOS. Once the weight purities were determined, the sialic acid content was assigned as 21 mol% for strain 35000 and 54 mol% for strain 188 (Table 4). These values were in rough agreement with those estimated from relative peak abundance of sialylated LOS in the ESI-MS spectra. The LOS of strains NYC23 and 233 also contained sialic acid at greater than 10 mol% (39 to 42% by the ESI-MS estimates), but insufficient material was available to accurately assess weight or purity.

**Discussion.** Data obtained on the oligosaccharides of strains 233, 188, and NYC23 suggest that the major LOS glycoforms containing *N*-acetyllactosamine and sialyl-*N*-acetyllactosamine, as previously determined for strain 35000 (14), are also present in these wild-type strains. MAb 3F11, which recognizes the terminal region of paragloboside, also binds strongly to the LOS of these wild-type strains. In contrast, the African strain ATCC 33921 and the variant strain 188-2 have lost the terminal LOS epitope defined by MAb 3F11 (2) as well as the ability to add sialic acid to their LOS. The presence of sialic acid in the LOS of all but one wild-type strain, suggests that *N*-acetyllactosamine is the acceptor for sialic acid, as does the fact that the strains that do not contain this disaccharide (188-2 and 33921) show no evidence of sialylation. Although the precise roles of LOS containing *N*-acetyllactosamine and sialyl-*N*-acetyllactosamine are not known, adherence of the African strain 33921 to cultured human keratinocytes is greatly diminished compared with adherence of all other *H. ducreyi* wild-type strains tested (1a).

TABLE 4. Sialic acid content of LOS from various strains of *H. ducreyi*

| Bacterial strain | Mol% sialic acid | Sialic acid content (%) calculated from ESI-MS data[a] |
|---|---|---|
| 35000 | 21 | 31 |
| 188 | 54 | 47 |
| 188-2 | ND[b] | 0 |
| 233[c] | + | 39 |
| NYC23[c] | + | 42 |
| 33921 | ND | 0 |

[a] Calculated by taking the ratio of the sum of the heights of the sialylated peaks to the sum of the heights of all the peaks in the electrospray spectra.
[b] ND, none detected.
[c] Presence of sialic acid was verified in the LOS of this strain. However, although the sialic acid content appeared to be greater than 10 mol% in each case, the extent of sialylation could not be reproducibly quantified because of small sample amount.

Studies of *Neisseria* spp. have shown that sialyltransferases are primarily limited to pathogenic species, such as *N. gonorrhoeae* and *N. meningitidis* (11). In *N. meningitidis*, the addition of sialic acid to LOS has been shown to be linked, like that in *H. ducreyi*, to terminal *N*-acetyllactosamine (23). Moreover, the sialylation of neisserial LOS can result in the conversion from serum sensitivity to serum resistance in some strains (15) and a marked decrease in susceptibility to killing by human neutrophils (6, 18). There is mounting evidence that sialic acid can prevent the recognition of antigenic sites by components of the immune defense system (19). It may be that large, negatively charged sialic acid residues on the branched oligosaccharide chains of *H. ducreyi* LOS mask antigenic recognition sites on the surface of the bacterial cell, enabling the organism to escape the host defenses. The negative charge of sialic acid can have a significant influence on conformations of complex carbohydrates, as reduction of the carboxyl groups in the terminal sialic acids in *Streptococcus* and *Neisseria* capsular polysaccharides drastically alters their immunological properties (8). Although it is not yet known how or whether sialic acid biosynthesis is regulated in vivo, the mole percent values for LOS species containing sialic acid are very similar (≈30 to 50 mol% in four of five strains) and considerably higher than that reported for *H. influenzae* LOS (12, 16). Indeed, the relatively high level of sialic acid present on the terminal structures of *H. ducreyi* LOS is reminiscent of the group B streptococcal polysaccharides, for which high levels of sialic acid were found to be important in defining immunodeterminants and preventing activation of the alternative complement pathway (5, 20).

Sialylation of LOS could also modulate the cytotoxicity of the lipid A. We have recently constructed an assay using cultured human keratinocytes for assessing LOS adhesion (1) and have now modified this procedure to obtain preliminary results on LOS cytotoxicity. These preliminary data have suggested that the LOS from the pyocin-variant strain 188-2, which lacks both *N*-acetyllactosamine and sialic acid, is less toxic than strains 188 and 35000. Given the complexities of LOS structures, additional biological studies will be needed to determine the precise roles of the LOS glycoforms containing *N*-acetyllactosamine and sialic acid in the pathogenic mechanisms of *H. ducreyi.*

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