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# Biochemical Properties of *Neisseria gonorrhoeae* LgtE

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**A fragment of chromosomal DNA encoding the *lgtE* gene of *Neisseria gonorrhoeae* strain F62 was amplified by PCR and cloned into the expression vector pET15b. Functional LgtE was purified and its biochemical properties were determined. The purified enzyme was maximally active in buffer containing manganese; minimal activity was obtained in buffer containing other divalent cations. LgtE was only able to mediate the addition of UDP-galactose into neisserial lipooligosaccharides (LOSs). We used a variety of genetically defined and chemically verified LOS structures to determine acceptor specificity. LgtE was able to mediate the addition of galactose into a variety of LOS structures, indicating the this enzyme possesses broad acceptor specificity. Furthermore, it was able to add multiple galactose residues onto LOS. We also determined that this enzyme was capable of adding galactose onto both the α and β chains of neisserial LOS.**

Lipooligosaccharide (LOS) is an important virulence determinant of the pathogenic neisseriae (12, 16). It consists of an oligosaccharide component that is attached to lipid A via a Kdo (3-deoxy-D-manno-octulosonic acid) linkage. The genes involved in the synthesis of the oligosaccharide portion of this molecule from a variety of species have been identified and characterized (1, 2, 11, 13, 17, 28). A common feature of LOS expression in all of these species is the expression of multiple phase-variable LOS structures. Most of this variability is attributed to changes in the carbohydrate composition of the molecule. The genetic basis for this variation has been well characterized. Key genes in the biosynthetic pathway contain homopolymeric runs of guanine (2, 11). Changes in the number of guanines result in reading frame shifts, with the end result being the truncation or elongation of a particular LOS molecule, depending on the nature of the starting reading frame (4, 5, 37).

The data presented in Fig. 1 summarize the genetic potential and reported carbohydrate structures that have been identified in *Neisseria gonorrhoeae* (7, 9, 10, 15, 18, 34–36). The genes responsible for the addition of most of these sugars have been defined genetically; loss of gene function results in the truncation of an LOS structure. Biochemical characterization of several of these gene products has been performed by measuring the incorporation of sugars from various UDP-sugars into a variety of synthetic carbohydrates. LgtA possessed broad substrate specificity towards α and β galactosides. Depending on the acceptor, this enzyme could mediate the transfer of GlcNAc from UDP-GlcNAc and GalNAc from UDP-GalNAc (3). However, this broad specificity was not seen in vivo (27). The biochemical properties of LgtB and LgtC have been examined similarly (21, 30, 31). Both enzyme possessed the predicted galactosyltransferase activities.

Genetic evidence supporting the function of *lgtE* as encoding a glycosyl transferase responsible for the addition of galactose β-1,4 to glucose has been reported in a variety of publications (6, 11, 14, 26). However, purified LgtE was unable to mediate the transfer of galactose to synthetic LOS biosynthesis intermediates (30). Erwin et al. (6) showed that when *lgtE* was nonfunctional, galactose was not added to the β chain. However, no direct biochemical evidence was presented to implicate LgtE directly in this addition.

We purified functional LgtE from *Escherichia coli* strains containing recombinant plasmids expressing LgtE and then used this recombinant enzyme to demonstrate its ability to modify various neisserial LOSs that possessed defined structures.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, oligonucleotides, and culture conditions.** *N. gonorrhoeae* strain F62 was obtained from P. F. Sparling, University of North Carolina, Chapel Hill. *Neisseria subflava* 44 and F62ΔlgtAΔlgtFG+ have been characterized previously in this laboratory (24, 28). *Escherichia coli* strain DH5αMCR was obtained from Life Technologies (Rockville, Md.). Strain ER2566 [F$^-$ λ$^-$ *fhuA2* (*lon*) *ompT lacZ*::T7 *geneI gal sulA11* Δ(*mcrC-mrr*)*114*::IS*10* *R*(*mcr-73*::mini-Tn*10*)2 *R*(*zgb-210*::Tn*10*)1 (Tet$^s$) *endA1* (*Dcm*)] was obtained from New England Biolabs, Beverly, Mass. Plasmid pET15b was obtained from Novagen (Madison, Wis.).

*Neisseria* strains were grown in standard gonococcal medium (designated GCP if broth and GCK if agar) (Difco laboratories) plus growth supplements (33) and 0.042% sodium bicarbonate if in broth or in a 37°C $CO_2$ incubator. *E. coli* strains were grown on LB plates (23). Ampicillin was used at 50 μg/ml, spectinomycin at 50 μg/ml, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 35 μg/ml when selection or colorimetric detection was applied.

*N. gonorrhoeae* strain F62ΔlgtAΔlgtFΔrfaKlgtG+ was constructed by PCR amplification of the *rfaK* region with primers RFAK147 and RFAK3780 and cloning this fragment in the *Sma*I and *Hin*dIII sites of pK18up (25), giving pRFAK. A 458-bp *Dra*I fragment, located within the coding sequence of *rfaK* was deleted from this plasmid, giving pRFAKΔ2-1. This deletion was introduced into F62ΔlgtAΔlgtFlgtG+, giving rise to F62ΔlgtAΔlgtFΔrfaKlgtG+. All constructs were verified by PCR amplification of the desired region.

**Chemicals, reagents, and enzymes.** Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beverly, Mass.). All chemicals used for this study were reagent grade or better and were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise specified. Tris-Tricine gels (16.5%) and running buffer were obtained from Bio-Rad Laboratories (Richmond, Calif.). The monoclonal antibody 3G9 was graciously provided by Peter Rice, Boston University, Boston, Mass.

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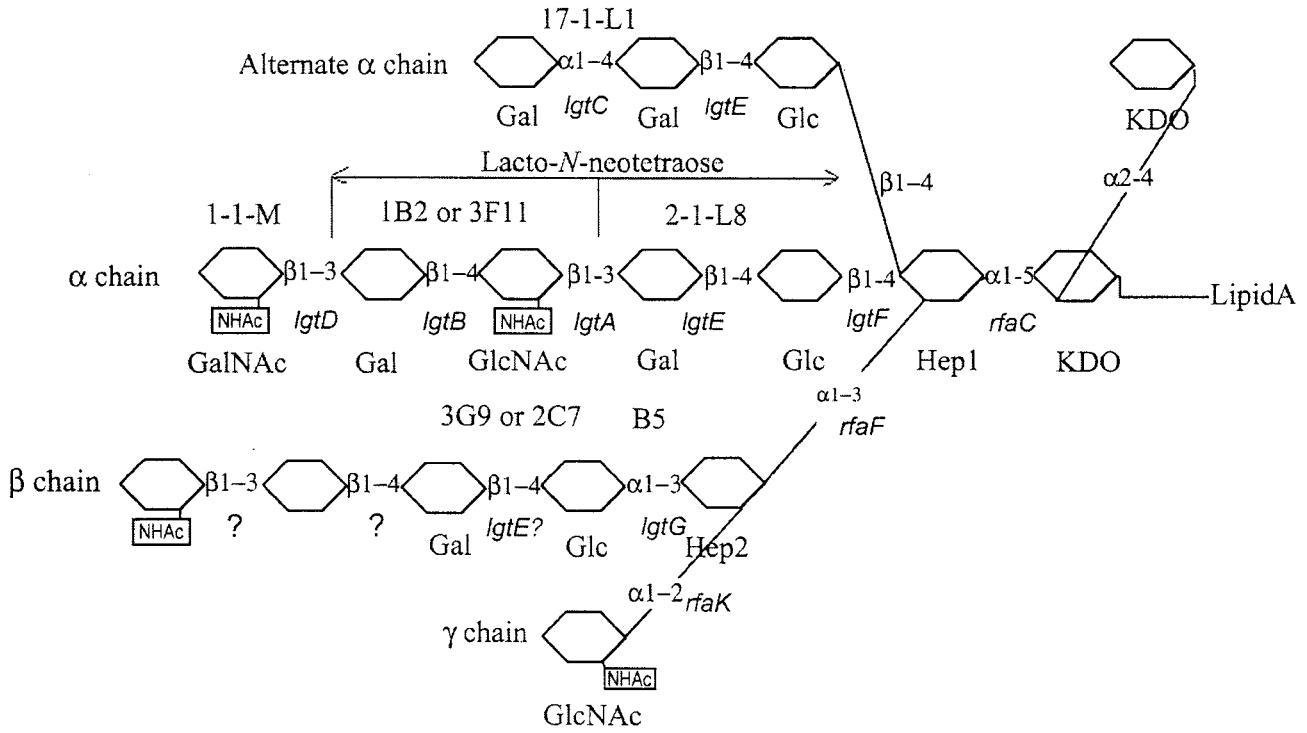


FIG. 1. Possible LOS structures seen in *N. gonorrhoeae*. The data in the figure are a compilation of published LOS structures. The question marks indicate that the structures have been identified, but the gene responsible for the addition and the exact chemical composition of the sugar remain unknown. The labels above the structures indicate various monoclonal antibodies that have been used as markers for the presence of various components that terminate with the indicated sugars. The genes predicted to be responsible for the various sugar additions are indicated in italic.

**LOS purification and analysis.** LOSs were purified from broth-grown cells with acetone-powdered organisms by the hot phenol-water method (32). LOS was extracted with hot phenol-water and concentrated by lyophilization. Extractions were continued until the purified LOS gave a minimal absorbance when measured at 200 nm.

**SDS-PAGE analysis.** Approximately 0.1 μg of LOS was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 16.5% Tris-Tricine gel (from Bio-Rad) in Tris-Tricine running buffer following the protocol suggested by the manufacturer. The gel was fixed overnight in 40% ethanol–5% acetic acid, and the LOS was visualized by silver staining (29).

**Western blot and colony blot analysis.** After SDS-PAGE, LOSs were electrotransferred onto Immobilon-P membrane (Millipore Corp.) in a Tris-Tricine-methanol buffer (10 mM Tris [pH 8.3], 10 mM Tricine, 0.01% SDS, 20% methanol) at a constant voltage of 100 V for 1 h following the protocol provided by Bio-Rad Corp. After air drying for 1 h, the membrane was processed by the procedure described below for colony blot analysis.

For colony blot analysis, overnight colonies were transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, N.H.), incubated in buffer (20 mM Tris, 150 mM NaCl, 2% milk powder) to block all nonspecific binding sites, and screened for reactivity to the appropriate monoclonal antibody. Bound monoclonal antibodies were detected by reacting the nitrocellulose filter with monoclonal antibody and visualizing the bound antibody by reacting the blot with horseradish peroxidase-labeled goat anti-mouse immunoglobulin G.

**Transformation.** Recombinant DNA transformation into *E. coli* was done according to standard protocols (23). Recombinant DNA transformation into *N. gonorrhoeae* was done by resuspending T1 cells to a density of approximately $10^8$ cells/ml in GCP broth containing 1× Kellogg's solution, 0.042% $NaHCO_3$, 10 mM $MgCl_2$, and 1 μg of the DNA of interest. Cells were incubated for about 5 h with shaking at 37°C. Cells were plated onto GCK plates containing spectinomycin.

**PCR.** PCR was used to generate the DNA fragments employed in gene cloning experiments and for mutant *N. gonorrhoeae* strain verification. Primers were made by Bioserve Biotechnologies (Laurel, Md.). DNA amplifications were performed with a PCR supermix kit (Life Technologies, Grand Island, N.Y.) following the procedure provided by the company. Purified chromosome DNA or plasmid DNA was used as the template. For strain construction verification, DNA was isolated directly from colonies by the following procedure. A small colony was added to 5 μl of 0.5 M NaOH, the cell mixture was allowed to incubate at room temperature for 10 min, and the solution was neutralized with 5 μl of 1 M Tris-HCl, pH 7.5. After adding 90 μl of $H_2O$, 3 μl of this solution was used for PCR amplifications.

**Cloning of *lgtE* and purification of LgtE.** A fragment of chromosomal DNA encoding the *lgtE* gene was obtained by PCR amplification of F62 chromosomal DNA with primers LGTE1 (5′-TTCCAACATATGCAAAACCACGTTATCA GC-3′; the *Nde*I site that was used for cloning is underlined) and LGTE2 (5′-ATGCATGGATCCCGCGGGAATGACAGTGTGTCCA-3′; the *Bam*HI site that was used for cloning is underlined). The PCR product was cleaved with *Nde*I and *Bam*HI and ligated into the expression vector pET15b that had been cleaved with the same enzymes. The ligation mixture was used to transform *E. coli* DH5αMCR, and individual transformants were screened for the presence of the appropriate recombinant plasmid.

Plasmid pET15b-lgtE was transformed into *E. coli* strain ER2566, and a single colony was used to inoculate 25 ml of Luria broth containing ampicillin. Cells were incubated with moderate shaking at 37°C until the optical density at 600 nm reached a value of 0.6. Isopropylthiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and incubations were continued at 25°C for 10 h. The cells were collected by centrifugation and resuspended in 1 ml of binding buffer (Novagen). Lysozyme was added to a final concentration of 100 μg/ml, and the mixture was incubated on ice for 60 min. Samples were frozen at −70°C and thawed and sonicated three times, each for 15 s. The cell extract was clarified by centrifugation for 30 min at 15,000 rpm in a Sorvall SS34 rotor. While the majority of LgtE protein was present as an insoluble fraction, soluble proteins were purified on a nickel column according to the protocols of the manufacturers (Novagen), resulting in pure LgtE protein. The enzyme was dialyzed against Tris-HCl (pH 7.5)–20 mM NaCl–1 mM EDTA–50% glycerol and stored at −20°C.

**Galactosyltransferase assays.** The standard reaction volume for galactosyltransferase assays was 30 μl and contained 50 mM MES (morpholineethanesul-

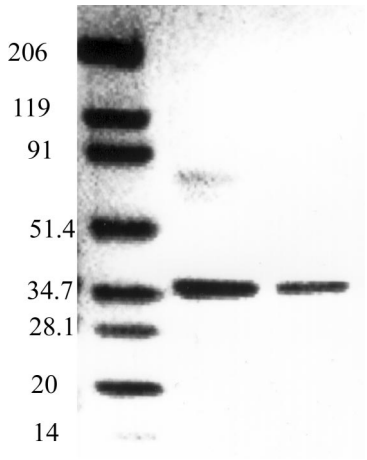


FIG. 2. SDS-PAGE of purified LgtE. *E. coli* strains were grown to mid-log phase, and LgtE expression was induced by the addition of IPTG. Recombinant proteins were purified on a nickel column. Lane 1, molecular size markers; lanes 2 and 3, purified enzyme (two consecutive fractions obtained after elution from the column). The sizes of the molecular mass markers are given in kilodaltons. A single protein of 35 kDa was present in the preparation.

fonic acid) buffer (pH 7.3), 10 mM $MnCl_2$, 10 μg of purified LOS or $5 \times 10^6$ whole cells, and 1.5 μg of purified LgtE protein. When radioactive substrates were employed, 0.2 nmol of UDP-[$^3$H]Gal (17.8 Ci/mmol), UDP-[$^3$H]Glc (25 Ci/mmol), or UDP-[$^{14}$C]GlcNAc (266 Ci/mmol)] was added; when nonradioactive substrates were employed, compounds were added to a final concentration of 1 mM. The reaction mixes were incubated for at least 2 h at 30°C. The reactions were terminated by the addition of 10 μl of 20% SDS and heating at 70°C for 3 min.

For the separation of unincorporated radioactive material from LOS, reaction mixes were loaded onto 3-ml Sephadex G-100 columns (made from a Pasteur pipette and equilibrated with water). The columns were washed with water, and fractions of three drops were collected (the first three fractions contained six drops). LOS eluted at fractions 5 to 12, while the radioactive LOS started at fractions 18 to 20. Samples of fractions 5 to 15 (10 μl) were spotted on filter paper (1 cm by 1 cm) and dried, and the radioactivity was assayed by liquid scintillation counting. The total volume of all fractions containing radioactive LOS was measured and used to calculate the total transfer of radioactive substrate into the acceptor LOS structures.

Galactosyltransferase activity was also determined by autoradiography. LOS samples were radiolabeled and purified as above and then run in parallel on the same SDS-Tricine gel as described above. Half of the gel was stained for visualization of the LOS with a standard silver-staining procedure, while the second half of the gel was soaked in sodium salicylate (1 M, pH 6.0) for 30 min. The gel was placed on 3MM Whatman paper, dried for 10 min on air, covered with Saran paper, and subjected to autoradiography.

## RESULTS

**Cloning and expression of the *N. gonorrhoeae* F62 *lgtE* gene in *E. coli*.** Through the use of PCR amplification, a fragment of chromosomal DNA encoding the *lgtE* gene was obtained and cloned into the expression vector pET15b. While we were able to isolate numerous clones that contained the desired amplicon, most of these clones failed to express LgtE after growth in *E. coli* strain ER2566 and induction with IPTG. Furthermore, in those strains that did express LgtE, the majority of the protein was present as an insoluble fraction. However, we were able to purify the soluble proteins on a nickel column with the protocol of the manufacturer (Novagen); we obtained a significant amount of pure LgtE protein (Fig. 2).

The presence of precipitated proteins both before and after purification prevented us from calculating the specific activity and total yield of the enzyme. The molecular mass of LgtE as determined by SDS-PAGE (35 kDa) agreed with the predicted mass for this protein as translated from the cloned chromosomal fragment encoding this gene. Since *E. coli* strain ER2566 possesses a mutation in the OmpT protease, we did not observe the problem of proteolytic degradation of expressed proteins that was described by Wakarchuk et al. (31).

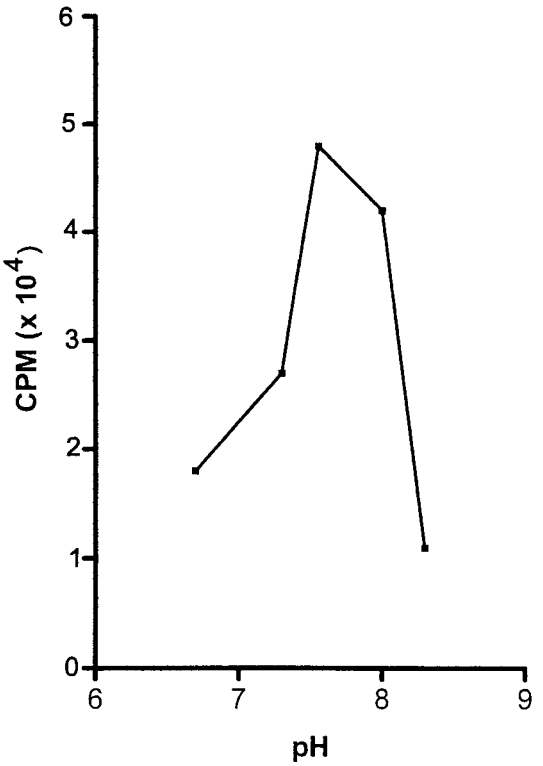


FIG. 3. Effect of pH on activity of recombinant LgtE. The activity was assayed with 1.5 μg of LOS purified from *N. gonorrhoeae* strain F62ΔlgtAΔlgtE as an acceptor under standard incubation conditions except for the variation in buffer pH.

**Biochemical properties of the enzyme.** Optimal conditions for assaying the galactosyltransferase activity of LgtE were determined, with UDP-[$^3$H]galactose as the substrate and purified F62ΔlgtAΔlgtE LOS as the acceptor. Strain F62ΔlgtAΔlgtE expresses a single LOS molecule that possesses a single glucose on the α-chain and a phosphate as the β-chain (28). The purified enzyme showed an absolute dependence on the presence of $Mn^{2+}$. In the presence of $Mg^{2+}$, less then 10% of the maximal activity was observed; in the presence of other divalent ions, minimal activity was observed (activity with no cation or 15 mM $Mg^{2+}$, $Ca^{2+}$, and $Zn^{2+}$ was <1, 8, <1, and <1%, respectively, of that with $Mn^{2+}$). The maximum activity was obtained at pH 7.5 (Fig. 3) and 30°C (data not presented).

Wakarchuk et al. (31) reported that LgtC required free thiols for enzymatic activity, while LgtB did not. We tested our preparation of LgtE to determine if it required free thiols for activity. The data presented in Fig. 4 indicate that purified LgtE showed a linear rate of activity up to 90 min; we concluded that this enzyme did not possess a thiol requirement.

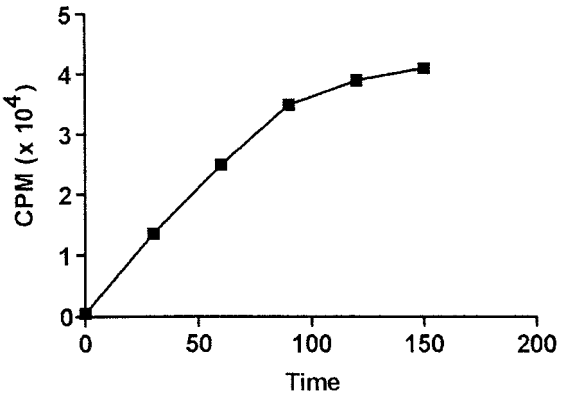


FIG. 4. Linearity over time of the reaction catalyzed by recombinant LgtE. Standard assay conditions were employed with 1.5 μg of LOS purified from *N. gonorrhoeae* strain FA62ΔlgtAΔlgtE.

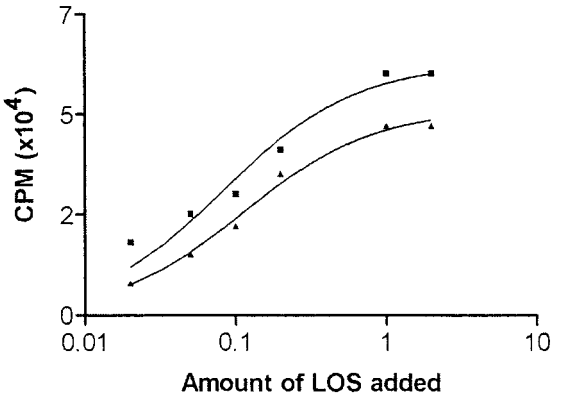


FIG. 5. Dependence of efficiency of transfer of donor UDP-Gal residue to different concentrations of LOS structures isolated from strains F62ΔlgtAΔlgtE and F62. The ability of the LgtE protein to transfer galactose to various concentrations of LOS was determined by incubating different concentrations of LOS in 30 μl of reaction buffer (50 mM MES buffer [pH 7.3], 10 mM $MnCl_2$, and 1 mM UDP-galactose). The reaction mixes were incubated for 2 h at 30°C, and reactions were terminated by the addition of 10 μl of 20% SDS and heating at 70°C for 3 min. Triangles, LOS from F62; squares, LOS from F62ΔlgtAΔlgtE.

**Substrate specificity.** Since some of the other gonococcal glycosyltransferases possessed broad substrate specificity, we used various radiolabeled compounds to test their ability to act as a substrate for LgtE. The data indicate that the enzyme was only able to use UDP-Gal efficiently as a substrate. Incorporation of radioactivity into purified LOS with UDP-GalNAc and UDP-Glc occurred at an efficiency of less then 1% of the level seen for UDP-Gal (data not shown). This is in contrast to LgtA from *N. meningiditis*, which can use both UDP-GlcNAc and UDP-GalNAc as substrates at almost the same efficiency (3).

**Acceptor specificity.** The genetic data indicate that the natural acceptor for LgtE should be the LOS structure present in strain F62ΔlgtAΔlgtE and that the activity of this enzyme should be the addition of a galactose to the α-chain glucose. The LOS structure present in the wild-type strain F62 lacks a free α- or β-chain glucose; hence, it should not be an acceptor for LgtE.

To test this prediction, we used the LOS isolated from several derivatives of *N. gonorrhoeae* strain F62 and from *N. subflava* 44. The data showed that LgtE could mediate the addition of radiolabeled Gal to LOS isolated from a variety of strains in addition to the one that possessed an LOS structure that was predicted to be the biosynthetic intermediate (LOS from strains F62, F62ΔlgtAΔrfaKΔlgtF, F62ΔlgtAΔlgtG, and 44 showed 80, 70, 50, and 50%, respectively, of the strain F62ΔlgtAΔlgtE LOS acceptor specificity). The efficiency of transfer of Gal residues to F62ΔlgtAΔlgtE and F62 showed the same efficiency in the reactions containing decreased concentrations of acceptor LOS structures (Fig. 5).

To prove that the LOS from both strains possessed the same acceptor specificity, an autoradiography experiment was performed. After radiolabeling, LOSs were analyzed by electrophoresis on an SDS-Tris-Tricine gel and then subjected to autoradiography. While the data clearly indicated that radioactivity was incorporated into the LOS samples, the width of the signal on the autoradiogram prevented us from determining which LOS structure was serving as an acceptor (data not shown)

As an alternative approach to demonstrating the addition of galactose to neisserial LOSs, we used unlabeled UDP-galactose as a substrate and various LOS acceptors as recipients. Strain F62ΔlgtAΔlgtE expresses an LOS structure that is the natural acceptor for LgtE. The data presented in Fig. 6A (lanes 4 and 6) indicated that LgtE was able to modify the LOS expressed by F62ΔlgtAΔlgtE. These data indicate that under the experimental conditions that we employed, LgtE appeared to mediate the addition of multiple galactose residues.

The data presented above indicated that strain LOS F62ΔlgtAΔrfaKΔlgtF LOS was able to serve as an acceptor for galactose from UDP-galactose. Since this strain's LOS is truncated and contains only two heptoses, it should not possess the acceptor structure for LgtE. In an effort to visualize this addition, whole cells were incubated with purified LgtE as described above. The data presented in Fig. 6B, lane 2, clearly show that incubation of whole cells with LgtE resulted in the appearance of a new LOS band. Since the mobility of this band was slower than that of LOS isolated from F62ΔlgtA, this suggests that this LOS has had three or more galactose moieties added to it. However, only a small percentage of the molecules were acted upon by LgtE. While the addition of galactose to this acceptor was unexpected, the addition of multiple galactose moieties is consistent with the data in Fig. 6A.

In order to determine if LgtE mediated the addition of galactose to both the α and β chains of gonococcal LOS, we tested whether purified LgtE was able to modify LOS isolated from *N. subflava* strain 44. This strain makes two LOS structures, with the smaller LOS structure possessing a glucose on both the α and β chains (28). LOS isolated from this strain fails to bind monoclonal antibody 3G9; reactivity with this monoclonal antibody requires that the α and β chains consist of lactose (35). The data presented in Fig. 6C indicate that LgtE was able to modify the LOS expressed by *N. subflava* 44.

In order to determine if LgtE added galactose to both chains of the LOS expressed by *N. subflava* 44, we used our in vitro

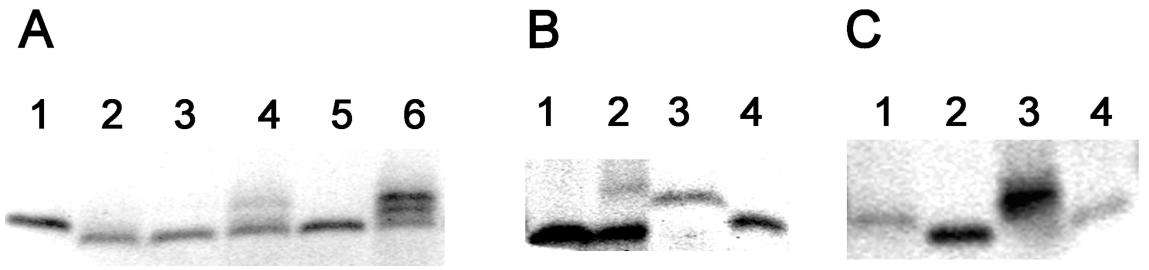


FIG. 6. Transfer of galactose into neisserial LOS. The ability of the LgtE protein to transfer galactose to various LOS structures was determined by incubation in 30 μl of reaction buffer (50 mM MES buffer [pH 7.3], 10 mM $MnCl_2$, $5 \times 10^6$ whole cells, and 1 mM UDP-galactose). The reaction mixes were incubated overnight at 30°C, and reactions were terminated by the addition of 10 μl of 20% SDS and heating at 70°C for 3 min. Then 10 μl of each reaction mix was analyzed on a 16.5% Tris–Tricine gel. (A) The lanes represent LOS isolated from: 1, F62ΔlgtA; 2, F62ΔlgtAΔlgtE; 3, F62ΔlgtAΔlgtE incubated with reagents minus LgtE; 4, F62ΔlgtAΔlgtE plus 1 μl of LgtE preparation; 5, F62ΔlgtAΔlgtE incubated with reagents minus LgtE; 6, F62ΔlgtAΔlgtE plus 7 μl of LgtE preparation. (B) The lanes represent LOS isolated from: 1, F62ΔrfaKA; 2, F62ΔrfaK incubated with reagents plus 7 μl of LgtE preparation; 3, F62ΔlgtA; and 4, F62ΔlgtAΔlgtE. (C) The lanes represent: 1, F62ΔlgtA; 2, *N. subflava* 44; 3, *N. subflava* 44 incubated with LgtE; and 4, F62ΔlgtAΔlgtE.

assay to modify LOS isolated from this strain and then tested the reaction product for its acquisition of reactivity with various monoclonal antibodies. The data presented in Fig. 7A indicate that while F62ΔlgtAΔlgtE LOS could be modified by purified LgtE and that these reaction products now bound monoclonal antibody 2-1-L8, the resulting reactants failed to bind monoclonal antibody 3G9 (Fig. 7B). *N. subflava* 44 LOS, when modified in vitro, clearly acquired the ability to bind both monoclonal antibodies. From these data, we concluded that LgtE is able to mediate the addition of galactose to both the α and β chain glucose moieties.

## DISCUSSION

Many genes that are involved in LOS expression have been identified in *Neisseria* spp. However, few biochemical studies have been performed that demonstrate a clear structure-function relationship between the gene product and its predicted biochemical function. A set of linked genes (*lgtABCDE*) that seem to encode the majority of glycosyltransferases needed to synthesize the carbohydrate portion of the α-chain has been identified (11). The biochemical functions of LgtABCD have been demonstrated and correspond to the functions inferred from analysis of mutations in these genes (3, 30, 31). While there is significant genetic evidence supporting the function of LgtE as the glycosyltransferase responsible for the addition of galactose β-1,4 to glucose (6, 11, 14, 26), biochemical data supporting this assignment have been lacking. Furthermore, since the addition of galactose β-1,4 to glucose can occur on both the α and β chains, it is unclear if LgtE is responsible for both of these biosynthetic processes.

Erwin et al. (6) demonstrated that galactose was not added to either the α or β chains in *lgtE* mutants. While their data clearly indicated that LgtE activity is required for galactose addition, the results could not rule out the possibility that the β-chain addition of galactose was mediated by an additional unlinked enzyme whose activity required before the addition of the α-chain galactose could occur. As a first step in characterizing the *lgtE* gene product, we used a gene cloning strategy to isolate a functional LgtE protein. While we were readily able to isolate seemingly intact DNA fragments into a *lac*-regulated expression vector, most of the recombinant clones failed to express detectable levels of protein, even after induction with IPTG. Furthermore, expressing clones seemed to lose the ability to express the protein after prolonged incubation or storage. While we did not investigate the reason for this instability, we believe that it is probably related to the fact that the LgtE protein is able to modify LPS biosynthesis intermediates in *E. coli* and the accumulation of these modified intermediates is toxic.

Strain F62ΔlgtAΔlgtE produces an LOS that contains a sin-

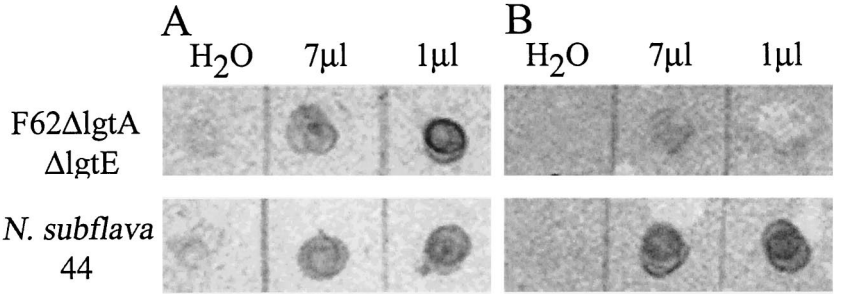


FIG. 7. Reactivity of in vitro-modified LOS with various monoclonal antibodies. Cells were incubated with purified LgtE (1 or 7 μl) plus UDP-galactose in standard reaction buffer, and an aliquot was spotted onto a nitrocellulose filter. Reactivity to various monoclonal antibodies was determined with our colony-blotting procedure. (A) Samples exposed to monoclonal antibody 2-1-L8. (B) Samples exposed to monoclonal antibody 3G9.

gle glucose on the α-chain and a single phosphate as the β-chain (28). As such, this strain produces an LOS with the predicted acceptor structure for LgtE. The data shown above and in Fig. 6 demonstrated that this LOS can serve as an acceptor for galactose from UDP-galactose. Surprisingly, many other LOS structures were also able to serve as acceptor molecules for LgtE. Furthermore, in our in vitro experiments, multiple LOS bands were obtained after incubation of F62ΔlgtAΔlgtE LOS with purified LgtE and UDP-galactose. The SDS-PAGE profile of the elongation product suggests that two galactose residues are added to F62ΔlgtAΔlgtE LOS.

The ability of LgtE to add galactose to a variety of LOS structures was unexpected. Most surprising perhaps was its ability to add sugars to LOS isolated from F62ΔlgtAΔrfaKΔlgtF. The oligosaccharide of this LOS consists of two heptose molecules, and elongation of this LOS by LgtE in vivo has not been reported. Furthermore, when galactose is added to the base oligosaccharide, the resulting band has an SDS-PAGE mobility consistent with the addition of three galactose residues. These data indicate that, depending on the nature of the starting LOS, two or three galactose residues were added.

By varying the amount of LgtE added to the reaction mixture, we could change the relative ratio of the elongation product; more enzyme increased the intensity of the higher-molecular-weight components (data not shown). Since the aberrant additions only occurred during in vitro reactions and were influenced by the amount of exogenous LgtE added, this suggests that, in vivo, the amount of enzyme expressed is quite small. Additional studies from our laboratory indicate that this hypothesis is correct (D. C. Stein, unpublished observations).

Wakarchuk and coworkers demonstrated that purified LgtE was unable to add galactose to synthetic β-Glc acceptors (30). In light of our observation that LgtE is able to mediate addition to a variety of molecules that possess a lipid base, the failure observed by Wakarchuk et al. was most likely due to the inability of the synthetic intermediates to form a stable interaction with LgtE.

Certain LOS immunotypes of *N. meningitidis* possess LOSs with variations in the structure of the α-chain. The observation that an excess amount of LgtE in a reaction results in homopolymer additions suggests a mechanism that can explain how the L5 LOS immunotype might arise. If LgtF were overexpressed, it might result in the addition of a second glucose residue at the base of the α-chain. It is interesting that the LOS isolated from L5 strains possesses a significant amount of a truncated biosynthetic product on SDS-PAGE gels. This may reflect a limitation in the ability of LgtE to add galactose to the diglucosyl structure.

The ability of purified LgtE to modify LOS isolated from *N. subflava* 44 to reactivity with monoclonal antibody 3G9 clearly indicates that LgtE is able to mediate the addition of galactose to the β-chain. Since LgtG-expressing strains that express LgtA (*N. gonorrhoeae* 15253 and F62lgtG+) do not add sugars to the β-chain beyond the galactose, this indicates that LgtA possesses a structural requirement that biases its addition to the α-chain. It is possible that overexpression of LgtA could result in the elongation of both the α and β chains, similar to what we have seen with LgtE. Since the level of expression of the various glycosyltransferases in the gonococcus is quite low (D. C. Stein, unpublished observations), this further suggests an additional mechanism of phenotypic modulation, where subtle changes in the growth rate of the organism would modulate the absolute level of the various proteins, and these results would be translated into small differences in LOS expression. It has been shown by several investigators that alteration in growth conditions affects LOS expression (8, 19, 20, 22), and we believe that this modulation is due to small changes in the levels of the various glycosyltransferases.

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