

Expression Cloning of a *Pseudomonas* Gene Encoding a Hydroxydecanoyl-Acyl Carrier Protein-Dependent UDP-GlcNAc Acyltransferase

GARRY D. DOTSON,¹ IGOR A. KALTASHOV,² ROBERT J. COTTER,² AND CHRISTIAN R. H. RAETZ^{1*}

Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710,¹ and Middle Atlantic Mass Spectrometry Laboratory, Department of Pharmacology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2185²

Received 29 August 1997/Accepted 12 November 1997

UDP-*N*-acetylglucosamine-3-*O*-acyltransferase (UDP-GlcNAc acyltransferase) catalyzes the first step of lipid A biosynthesis (M. S. Anderson and C. R. H. Raetz, *J. Biol. Chem.* 262:5159–5169, 1987). We here report the isolation of the *lpxA* gene of *Pseudomonas aeruginosa* from a library of *Pseudomonas* strain PAO1 expressed in *Escherichia coli* LE392 (J. Lightfoot and J. S. Lam, *J. Bacteriol.* 173:5624–5630, 1991). *Pseudomonas lpxA* encodes a 10-carbon-specific UDP-GlcNAc acyltransferase, whereas the *E. coli* transferase is selective for a 14-carbon acyl chain. Recombinant cosmid 1137 enabled production of a 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase in *E. coli*. It was identified by assaying lysozyme-EDTA lysates of individual members of the library with 3-hydroxydecanoyl-acyl carrier protein (ACP) as the substrate. Cosmid 1137 contained a 20-kb insert of *P. aeruginosa* DNA. The *lpxA* gene region was localized to a 1.3-kb *Sall*-*Pst*I fragment. Sequencing revealed that it contains one complete open reading frame (777 bp) encoding a new *lpxA* homolog. The predicted *Pseudomonas* LpxA is 258 amino acids long and contains 21 complete hexapeptide repeating units, spaced in approximately the same manner as the 24 repeats of *E. coli* LpxA. The *P. aeruginosa* UDP-GlcNAc acyltransferase is 54% identical and 67% similar to the *E. coli* enzyme. A plasmid (pGD3) containing the 1.3-kb *Sall*-*Pst*I fragment complemented *E. coli* RO138, a temperature-sensitive mutant harboring *lpxA2*. LpxA assays of extracts of this construct indicated that it is >1,000-fold more selective for 3-hydroxydecanoyl-ACP than for 3-hydroxymyristoyl-ACP. Mass spectrometry of lipid A isolated from this strain by hydrolysis at pH 4.5 revealed [M-H]⁻ 1,684.5 (versus 1,796.5 for wild-type lipid A), consistent with 3-hydroxydecanoate rather than 3-hydroxymyristate at positions 3 and 3'.

Genetic (13, 15, 30) or pharmacological (22) inhibition of enzymes catalyzing the early steps of lipid A biosynthesis in gram-negative bacteria usually causes cell death. The lipid A pathway is therefore a target for the development of novel antibacterial agents (25). The *lpxA* gene product, UDP-*N*-acetylglucosamine-3-*O*-acyltransferase (UDP-GlcNAc acyltransferase) (2, 3, 10, 11), catalyzes the first reaction of lipid A biosynthesis (Fig. 1). The enzyme transfers an *R*-3-hydroxyacyl group from hydroxyacyl-acyl carrier protein (ACP) to the glucosamine 3-OH of UDP-GlcNAc (Fig. 1) (2, 3). This hydroxyacyl chain eventually resides at both the 3 and 3' positions of the glucosamine disaccharide of mature lipid A (Fig. 1) (24–26).

The fatty acid compositions and structures of lipid A's have been determined for many bacterial species (26, 29). Williamson et al. have shown that the UDP-GlcNAc acyltransferases from several common gram-negative bacteria display high degrees of specificity in measuring the chain lengths of the hydroxy fatty acyl moieties found at the 3 and 3' positions in the lipid A's of these bacteria (33).

To date, only the UDP-GlcNAc acyltransferase from *Escherichia coli*, in which enzymatic specificity is primarily for the transfer of *R*-3-hydroxymyristate, has been isolated and characterized in depth (2, 3). The X-ray crystal structure of the *E. coli* UDP-GlcNAc acyltransferase has been determined to a 2.6-Å resolution (28). The enzyme is composed of an unusual

homotrimer (28). Each monomer of 262 amino acids contains 24 complete, mostly contiguous hexad repeats that fold into a novel secondary structure, termed a left-handed parallel β-helix (28). The crystal structure reveals that several additional hexad-like segments also contribute to the β-helix (28). The location of the active site is not known, but comparison of available LpxA sequences reveals clustering of conserved residues in a basic cleft between the subunits. How the *E. coli* transferase achieves its extraordinary selectivity for 14-carbon hydroxyacyl chains is unclear.

The sequence of a 10-carbon-specific UDP-GlcNAc acyltransferase has not been reported previously. We here present (i) the identification of a 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase gene by assaying crude extracts of individual members of a *Pseudomonas aeruginosa* library expressed in *E. coli* LE392, (ii) the subcloning and sequencing of the *P. aeruginosa lpxA* gene, and (iii) the functional complementation of the temperature-sensitive *E. coli* mutant RO138 (*lpxA2*) by *P. aeruginosa lpxA*. Research on this class of enzymes should provide new opportunities for structure-based drug design and might facilitate the development of novel antibiotics directed against lipid A biosynthesis.

MATERIALS AND METHODS

Materials. UDP-*N*-acetylglucosamine, glucosamine-6-phosphate, inorganic pyrophosphatase, UDP-glucose pyrophosphorylase, ACP, (–)-ephedrine, *RS*-3-hydroxymyristic acid, *RS*-3-hydroxylauric acid, decanoic acid, buffers, and salts were obtained from Sigma. Yeast extract and tryptone were obtained from Difco. Polyethyleneimine-cellulose thin-layer plates and Silica Gel 60 thin-layer plates (thickness, 0.25 mm) were purchased from E. Merck (Darmstadt, Germany). Restriction enzymes were from New England Biolabs, and DNA ligase was from

* Corresponding author. Mailing address: Department of Biochemistry, Duke University Medical Center, Durham, NC 27710. Phone: (919) 684-5326. Fax: (919) 684-8885. E-mail: Raetz@Biochem.Duke.edu.

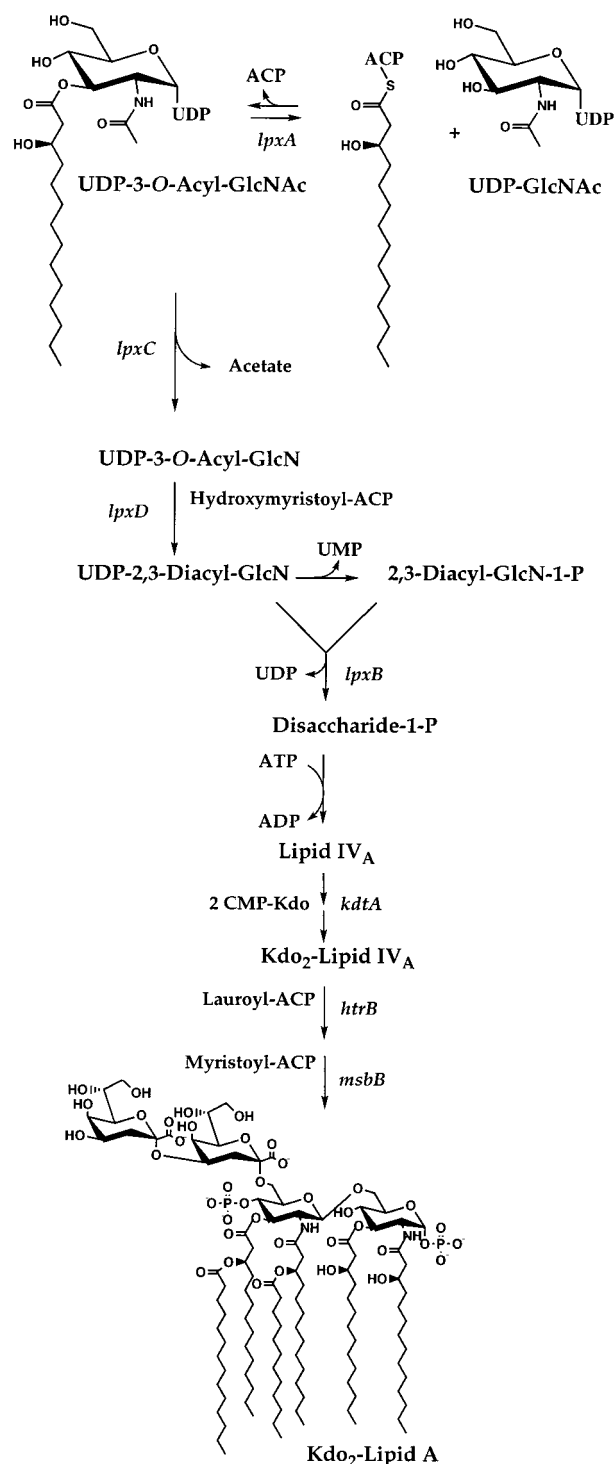


FIG. 1. Role of LpxA in lipid A biosynthesis in *E. coli*. LpxA catalyzes the first step of lipid A biosynthesis (24, 25). The transfer of an *R*-3-hydroxyacyl group from *R*-3-hydroxyacyl-ACP to UDP-GlcNAc is very selective for *R*-3-hydroxymyristoyl-ACP in the case of *E. coli* LpxA (3, 33). UDP-GlcNAc acyl-transferases from other gram-negative bacteria have specificity for ACP thioesters of different acyl chain lengths (33). Biosynthetic intermediates and known genes encoding the enzymes of the rest of the *E. coli* pathway are shown (24, 25). Chemical hydrolysis of lipopolysaccharide or of whole cells at pH 4.5 in the presence of sodium dodecyl sulfate cleaves the 3-deoxy-*D*-manno-octulosonic acid (Kdo)-lipid A linkage without disturbing the phosphates (7, 8). The released lipid product is designated lipid A throughout this paper. The 10-carbon-specific *Pseudomonas* LpxA can replace the 14-carbon-specific *E. coli* enzyme in living cells without interfering with the functioning of the other enzymes of the pathway.

TABLE 1. Plasmids and bacterial strains used in this study

Bacterial strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
<i>P. aeruginosa</i> PAO1	Wild type	G. Pier, Harvard University
<i>E. coli</i>		
LE392	Wild type	17
SURE	<i>lacI</i> ^q ZΔM15, Tc ^r	Stratagene
RO138	<i>lpxA</i> temperature-sensitive mutant, <i>recA</i>	21
Plasmids		
Cosmid 1137	<i>lpxA</i> ⁺ cosmid clone in pCP13, Tc ^r	17, this study
pBluescript KS II	<i>lacZ</i> , Amp ^r	Stratagene
pGD2	3.0-kb <i>Pst</i> I fragment from cosmid 1137 cloned into pBluescript, <i>lpxA</i> ⁺ Amp ^r	This study
pGD3	1.7-kb <i>Sal</i> I deletion from pGD2, <i>lpxA</i> ⁺ Amp ^r	This study

Boehringer Mannheim. A Sequenase version 2.0 DNA sequencing kit and shrimp alkaline phosphatase were from United States Biochemical. *t*-Butylacetate, lithium diisopropylamide, octyl aldehyde, and trifluoroacetic acid (TFA) were obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Anhydrous ethyl ether, chloroform, and methanol were obtained from Mallinckrodt, and glacial acetic acid was from EM Science. [α -³²P]UTP was purchased from DuPont, NEN. α -³⁵S-dATP was obtained from Amersham. [α -³²P]UDP-GlcNAc was prepared and purified as described previously (15). PhosphorImager screens were from Molecular Dynamics, Inc.

Bacterial strains and plasmids. Strains used in this study and their genotypes are listed in Table 1. LCH109/pLCH5/pGP1-2, a T7 promoter-driven overproducer of acyl-ACP synthetase, was obtained from C. O. Rock (St. Jude Hospital) (14). Cultures were grown in Luria broth (LB), consisting of 5 g of NaCl, 5 g of yeast extract, and 10 g of tryptone/liter (4). Antibiotics were added, when required, at 50 μ g/ml for ampicillin, 12 μ g/ml for tetracycline, and 30 μ g/ml for streptomycin.

Recombinant DNA techniques. Plasmid DNAs were isolated with a Wizard Plus Miniprep kit (Promega) or a BIGGERprep kit (5 Prime-3 Prime, Inc.). All other DNA manipulations and cell transformations were done as described previously (4). DNA sequencing was performed by the dideoxy method with a Sequenase version 2.0 DNA sequencing kit with 7-deaza-dGTP (United States Biochemical) and α -³⁵S-dATP.

Synthesis of *RS*-3-hydroxydecanoic acid. The acetate-aldol condensation reaction was used to synthesize *RS*-3-hydroxydecanoic acid in gram quantities. To a solution of *t*-butylacetate (3 ml; 22 mmol) in anhydrous ethyl ether (80 ml) at -78°C, stirred under nitrogen, was added slowly over 5 min 1.1 equivalents of lithium diisopropylamide (12.1 ml; 24.2 mmol). The reaction mixture was stirred for 20 min to allow complete enolization. To this, 1.0 equivalent of octyl aldehyde (3.5 ml; 22 mmol) in 10 ml of anhydrous ethyl ether was added slowly over 10 min. The reaction mixture was stirred for 2 h and then concentrated by rotary evaporation. The *t*-butyl ester was then removed by treating the mixture with TFA (excess) at room temperature for 1 h. The TFA was removed by rotary evaporation, facilitated by three 30-ml additions of chloroform. The residue was redissolved in chloroform, and it was then extracted with 30 ml of 5% aqueous NaOH. The alkaline aqueous layer was acidified by the addition of 1 M HCl until the pH was less than 4, as judged by a reaction on pH paper, and then extracted with 30 ml of chloroform. The chloroform layer was dried over anhydrous sodium sulfate and then concentrated to give a near quantitative yield of *RS*-3-hydroxydecanoic acid (4.1 g). ¹H nuclear magnetic resonance (500 MHz, CDCl₃) δ 4.03 (m, 1H), 2.57 (dd, 1H), 2.48 (dd, 1H), 1.6 to 1.2 (m, 12H), 0.89 (t, 3H).

Synthesis of *RS*-3-hydroxydecanoate (-)-ephedrine salt. *RS*-3-hydroxydecanoic acid (4.1 g) was suspended in anhydrous ethyl ether (230 ml), and (-)-ephedrine (4.47 g) was added. The mixture was warmed slightly by swirling it in a 50°C water bath, and acetonitrile was added until the solution was clear. The water bath was allowed to cool gradually to room temperature, at which time the *RS*-3-hydroxydecanoate ephedrine salt crystallized. Two subsequent recrystallizations resulted in 4.2 g of *RS*-3-hydroxydecanoate ephedrine salt (54% yield).

Preparation of acyl-ACP analogs. The substrate *RS*-3-hydroxydecanoyl-ACP was prepared from purified ACP (Sigma) and synthetic *RS*-3-hydroxydecanoate, with Triton X-100-solubilized LCH109/pLCH5/pGP1-2 membranes being used as the source of acyl-ACP synthetase (9, 14). The enzymatic acylation of ACP

with *RS-3*-hydroxydecanoate was carried out as follows. ACP (1 mg) and 8.6 mM dithiothreitol were incubated in 500 μ l of 40 mM Tris-HCl (pH 8.0) in a sealed tube at 37°C for 1 h. Next, a 320- μ l solution consisting of 0.7 M LiCl, 40 mM MgCl₂, 20 mM ATP (pH 8.0), 750 μ M *RS-3*-hydroxydecanoate ephedrine salt, 0.26% Triton X-100, and 540 mM Tris-HCl (pH 8.0) was added to the tube with the ACP. Last, 400 μ l of 1.25 mg of solubilized LCH109/pLCH5/pGP1-2 membranes per ml, which had been on ice for 15 min in the presence of 2.2 mM 3-decynoyl-*N*-acetylcysteamine (18), was added, and the acylation reaction was allowed to proceed at room temperature for 2 h. The extent of acylation was determined by analyzing 5- μ l portions of the reaction mixture on a urea-polyacrylamide gel (23). The other acyl-ACPs used in this study were made with the appropriate fatty acids as described above with the following modifications: 3-decynoyl-*N*-acetylcysteamine was omitted in the synthesis of *RS-3*-hydroxymyristoyl- and decanoyl-ACP, and a 10-fold-lower concentration of solubilized membranes of LCH109/pLCH5/pGP1-2 was used in the *RS-3*-hydroxymyristoyl-ACP preparation.

To isolate the acyl-ACP product, each reaction mixture was diluted 10-fold with water and loaded onto a 1-ml column of DEAE-Sepharose equilibrated with 10 mM bis-Tris (pH 6.0). The column was washed with 5 bed volumes of 10 mM bis-Tris (pH 6.0), 5 volumes of 10 mM bis-Tris (pH 6.0) containing 50% isopropyl alcohol, and 5 volumes of 10 mM bis-Tris (pH 6.0). The column was eluted with 3 volumes of 10 mM bis-Tris (pH 6.0) containing 0.2 M LiCl and 3 volumes of 10 mM bis-Tris (pH 6.0) containing 0.6 M LiCl. Fractions of 1 ml were collected. The acyl-ACP eluted in the second fraction of 0.6 M LiCl. This fraction was desalted on a P-2 column (10 ml) equilibrated in 50 mM Tris-HCl (pH 7.4) and lyophilized. The lyophilized protein was dissolved in distilled H₂O (1 ml). The acyl-ACPs were ~90% pure, as judged by electrophoresis in the urea-polyacrylamide gel system and staining with Coomassie blue. The concentrations of acyl-ACPs were determined as described previously (3).

Screening of a *P. aeruginosa* PAO1 library for 3-hydroxydecanoyl transferase activity. The *P. aeruginosa* PAO1 library, provided generously by Joseph Lam (University of Guelph), consisted of 10 glycerol stocks, each containing 100 recombinant cosmid clones (17). In a typical round of screening, 100- μ l portions of a 10,000-fold dilution of two glycerol stocks were plated onto LB-tetracycline plates and incubated at 37°C. From each agar plate, representing one glycerol stock, 192 colonies were picked to inoculate two 96-well microtiter dishes (150 μ l of LB with tetracycline per well), and the microtiter dishes were incubated at 37°C with shaking overnight. From these overnight cultures, fresh microtiter dishes (200 μ l of LB-tetracycline per well) were inoculated with a sterile 96-prong transferable solid phase (Nalge Nunc International). These dishes were grown for 6 h at 37°C and were then centrifuged at 3,600 \times g for 20 min at 4°C. The supernatants were decanted, and the pellets were resuspended on ice in 25 μ l of 33 mM Tris-HCl, pH 8.0. To the resuspended cells was added 25 μ l of 33 mM Tris-HCl (pH 8.0) containing 0.2 mg of lysozyme per ml and 5 mM EDTA, and the cells were left on ice for 5 min. The cells were lysed by freezing at -80°C for at least 15 min, followed by thawing at 25°C in a water bath for 3 to 5 min. Portions of the lysates (15 μ l from each well) from four microtiter plates (representative of two of the original glycerol stocks) were combined into 96 pools (four lysates per pool) in the wells of a fresh microtiter plate (60 μ l per well).

The pooled lysates were assayed for 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase activity as follows. A 96-well assay plate was prepared with each well containing 40 mM HEPES (pH 8.0), [α -³²P]UDP-GlcNAc (0.25 μ Ci at a concentration of about 0.1 μ M), 20 μ M 3-hydroxydecanoyl-ACP, 10 mM MgCl₂, and 6 μ l of lysate (added last to give a final volume of 10 μ l). The plate was incubated at 30°C for 15 min, and a portion of each reaction mixture (3 μ l) was spotted onto silica gel thin-layer chromatography plates. The plates were developed in chloroform-methanol-water-acetic acid (25:15:4:2, vol/vol), and the extent of acylation was determined by PhosphorImager analysis.

Preparation of RO138/pGD3 cell extracts. A single colony of RO138/pGD3 was used to inoculate 5 ml of LB and grown overnight at 42°C. A larger volume of LB (500 ml) was inoculated by 100-fold dilution of the overnight culture and incubated at 42°C with vigorous shaking (240 rpm) until the *A*₆₀₀ reached 0.8. The cells were harvested by centrifugation (5,000 \times g) at 2°C and washed once with 20 ml of 10 mM sodium phosphate buffer, pH 7.2. The washed cell pellet was resuspended in 10 mM sodium phosphate buffer, pH 7.2 (5 ml per g of cell pellet), and the cells were disrupted by passage through an ice-cold French pressure cell (SLM Instruments, Urbana, Ill.) at 20,000 lb/in². The broken cell suspension was centrifuged at 100,000 \times g for 75 min to prepare a membrane-free cytosolic extract. The latter was stored in 200- μ l aliquots at -80°C. Protein concentrations were determined by using the Bio-Rad (Richmond, Calif.) protein assay with bovine serum albumin as the standard.

UDP-GlcNAc acyltransferase assay. UDP-GlcNAc acyltransferase assays were performed as described previously (2, 3) with some minor modifications. Assay mixtures contained 100 μ M [α -³²P]UDP-GlcNAc (10³ cpm/nmol), 20 μ M *RS-3*-hydroxydecanoyl-ACP, 10 mM MgCl₂, 40 mM HEPES (pH 8.0), and 10 to 100 μ g of cell extract (added last) per ml in a final volume of 20 μ l. The reaction mixture was incubated at 30°C, and portions (3 μ l) were removed and spotted after 2 and 5 min onto silica gel thin-layer chromatography plates. The plates were developed in chloroform-methanol-water-acetic acid (25:15:4:2, vol/vol), and the extent of acylation was determined by PhosphorImager analysis.

Preparation of lipid A from *E. coli* RO138/pGD3. Lipid A (containing both the 1 and 4' phosphates) was prepared as previously described (21). Briefly, *E. coli*

RO138/pGD3 cells, obtained from a 250-ml culture grown on LB in the presence of ampicillin at 42°C to saturation (*A*₆₀₀ = 1.6), were suspended in 20 ml of 10 mM phosphate buffer, pH 7.2. To the cell suspension was added 300 ml of chloroform-methanol (1:2, vol/vol), and the resulting mixture was allowed to remain at room temperature for 60 min before it was centrifuged (4,000 \times g for 15 min). The pellet was washed with 250 ml of chloroform-methanol-water (1:2:0.8, vol/vol), and the suspension was centrifuged as described above. The washed pellet was resuspended, with sonic irradiation, in an aqueous solution (80 ml) consisting of 12 mM sodium acetate (pH 4.5) and 1% sodium dodecyl sulfate, and this suspension was then placed in a boiling water bath for 30 min (7, 8). The insoluble material was once again removed by centrifugation (4,000 \times g for 20 min) and discarded. The resulting supernatant (80 ml) was mixed with 178 ml of chloroform-methanol (1:1, vol/vol), and the emulsion was centrifuged at 4,000 \times g for 10 min to separate the two phases. The lower phase was saved, and the solvent was removed by rotary evaporation.

The dried material was dissolved in 10 ml of chloroform-methanol-water (2:3:1, vol/vol), and then half of the material (5 ml) was loaded onto a 1-ml DEAE cellulose (Whatman DE52) column, equilibrated as the acetate form in the same solvent. The column was washed with 4 ml of chloroform-methanol-water (2:3:1, vol/vol), and the lipid A was eluted by the stepwise inclusion of increasing amounts of ammonium acetate (60, 120, 240, and 480 mM) as the aqueous component of the solvent. Each step of the elution consisted of four column volumes. The lipid A emerged with the 240 mM ammonium acetate step. The 4 ml of the 240 mM ammonium acetate eluate was mixed with 0.67 ml of chloroform and 1.13 ml of water, and the phases were allowed to separate. The lower phase was removed and dried under nitrogen to yield ~0.5 mg of lipid A.

Mass spectrometry. Negative-ion liquid secondary-ion mass spectra were acquired with a Concept IH (Kratos Analytical, Manchester, United Kingdom) two-sector mass spectrometer at a resolution of 1,000. A 1- μ l portion of sample solution in methanol-chloroform (1:2, vol/vol) was mixed with mono-thioglycerol or triethanolamine (Aldrich Chemical Co.) on the tip of the probe. Analyte ions were desorbed from the matrix by an 8 keV Cs⁺ primary ion beam. Mass spectra were acquired by scanning the magnet in the 100- to 2,500-amu range at a scan rate of 10 s/decade. Typically 10 to 20 scans were signal averaged for each spectrum.

RESULTS

Screening of a *P. aeruginosa* PAO1 library for a 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase. Multiple attempts to clone the *Pseudomonas lpxA* gene by PCR to amplify conserved sequences present in known *lpxA* genes or to detect *Pseudomonas* DNA restriction fragments by low-stringency hybridization with *E. coli lpxA* as the probe were unsuccessful. An expression cloning strategy was therefore developed. Lysozyme-EDTA lysates were made from a total of 1,152 individual clones of *E. coli* LE392 bearing *P. aeruginosa* PAO1 DNA inserts. The 1,152 lysates were assayed in 288 pools of four lysates for their ability to transfer a 3-hydroxydecanoyl group to [α -³²P]UDP-GlcNAc. Interfering background activity attributable to the *E. coli* chromosomal UDP-GlcNAc acyltransferase is nonexistent because of the selectivity of UDP-GlcNAc acyltransferase for the 3-hydroxymyristoyl rather than the 3-hydroxydecanoyl moiety (33).

Of the 288 pools assayed, only one was positive for the expression of a 3-hydroxydecanoyl-ACP-dependent UDP-GlcNAc acyltransferase (Fig. 2). This pool contained lysates from clones 849, 945, 1041, and 1137. These lysates were assayed individually. Only the one derived from clone 1137 displayed the desired activity (Fig. 3). The cosmid from clone 1137 was isolated and found to contain an insert of about 20 kb by restriction enzyme analysis (data not shown).

Subcloning of cosmid 1137. Cosmid 1137, isolated from library clone 1137, was digested in two separate incubations with either *Cla*I or *Pst*I. Fragments from each digest were ligated into pBluescript KS II and used to transform *E. coli* SURE cells. The transformed bacteria were plated on LB agar containing ampicillin, IPTG (isopropyl- β -D-thiogalactopyranoside), and X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) (for selection of blue- or white-stained colonies) and grown overnight at 37°C. White colonies, 36 from the *Cla*I-derived transformants and 60 from the *Pst*I-derived transformants, were used to inoculate a microtiter plate containing 150

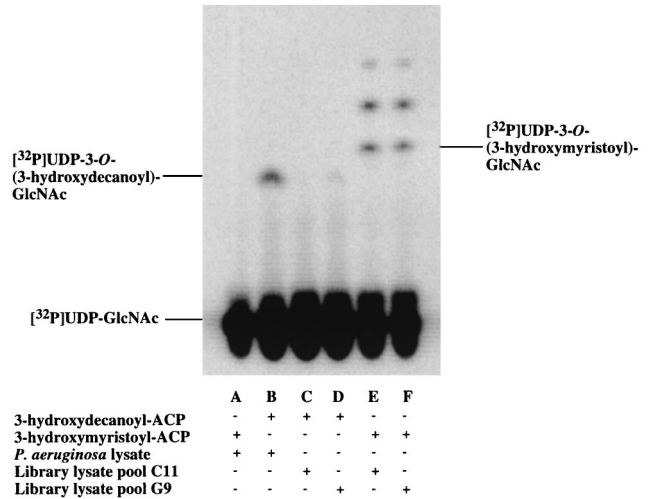


FIG. 2. Expression cloning of a 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase from a *P. aeruginosa* PAO1 DNA library in *E. coli* LE392 assayed in pools of four. When [α - 32 P]UDP-GlcNAc is acylated, it migrates off the origin in this thin-layer system, as indicated. Acylation with 3-hydroxymyristate results in a product that migrates slightly faster than does the product of acylation with 3-hydroxydecanoate. Extracts of wild-type *P. aeruginosa* acylate [α - 32 P]UDP-GlcNAc efficiently in the presence of 3-hydroxydecanoyl-ACP (lane B) but not at all in the presence of 3-hydroxymyristoyl-ACP (lane A). Conversely, strains of *E. coli* that do not harbor the *P. aeruginosa* *lpxA* gene acylate [α - 32 P]UDP-GlcNAc in the presence of 3-hydroxymyristoyl-ACP (lane E) but not in the presence of 3-hydroxydecanoyl-ACP (lane C). The more rapidly migrating compounds in lanes E and F represent further metabolites of the lipid A pathway that are derived in *E. coli* extracts from acylated-UDP-GlcNAc (1, 3). Lanes C to F show the results obtained with pools of four lysates (prepared as described in Materials and Methods), each derived from distinct *E. coli* colonies harboring different *P. aeruginosa* DNA inserts. With 3-hydroxydecanoyl-ACP as the substrate, only lysate pool G9 (of the 288 pools assayed) catalyzed measurable acylation of [α - 32 P]UDP-GlcNAc (lane D). With 3-hydroxymyristoyl-ACP as the substrate (lane F), pool G9 was comparable in activity to all other pools in the collection (such as C11 in lane E), indicating that the protein concentrations of G9 and C11 were about the same.

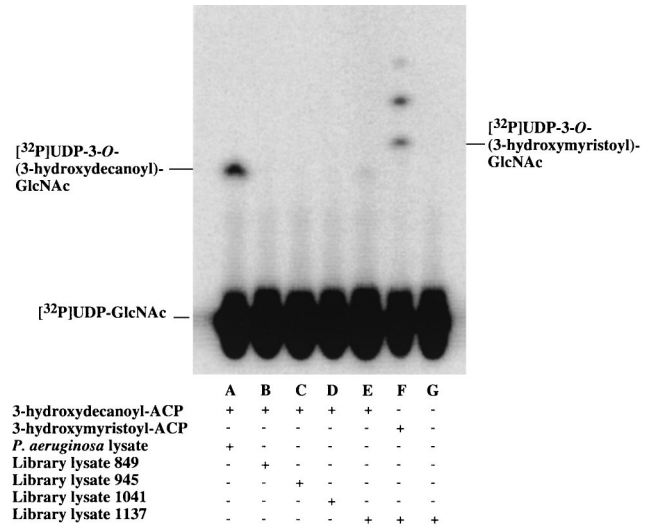


FIG. 3. Identification of a single *P. aeruginosa* library clone in *E. coli* LE392 expressing a 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase. Assays of lysozyme-EDTA lysates of the individual colonies that were used to make pool G9 were carried out as described for Fig. 2. Only extracts of the *E. coli* strain harboring *P. aeruginosa* cosmid 1137 catalyzed 3-hydroxydecanoyl-ACP-dependent acylation of [α - 32 P]UDP-GlcNAc.

μ l of LB with ampicillin per well. The cells were grown to late log phase at 37°C, and cell lysates were assayed for 3-hydroxydecanoyl-ACP-dependent UDP-GlcNAc acyltransferase activity, as described in Materials and Methods for the initial screening of the library. None of the *Clal*-derived transformants expressed the desired activity, but there was one *PstI*-derived transformant that displayed robust 3-hydroxydecanoyl transferase activity (data not shown). The plasmid DNA (pGD2) isolated from this clone contained a 3-kb *PstI* insert. pGD2 was further digested with *SalI* and religated to yield pGD3. When it was used to transform *E. coli* SURE cells, pGD3 directed the expression of 3-hydroxydecanoyl-ACP-dependent UDP-GlcNAc acyltransferase activity (see below). The *P. aeruginosa*-derived *SalI-PstI* DNA fragment in pGD3 was 1.3 kb long, as judged by agarose gel electrophoresis.

DNA sequence analysis of pGD3. The DNA sequence of the *SalI-PstI* insert contained in pGD3 is shown in Fig. 4. The insert is 1,352 bp in length, and it contains one complete open reading frame of 777 bp (nucleotides 54 to 830). This open reading frame is flanked at its 5' end by 53 bp and at its 3' end by 522 bp. The single complete open reading frame encodes a protein of 258 amino acids with a molecular mass of approximately 28 kDa. The predicted protein is 54% identical and 67% similar to the *E. coli* UDP-GlcNAc acyltransferase (LpxA). As has been observed in other UDP-GlcNAc acyltransferases (28, 32), the N-terminal two-thirds of the *P. aeruginosa* LpxA contains more than 20 mostly contiguous hexapep-

tide repeats (Fig. 5). Hexapeptide repeats are indicative of an unusual protein secondary structure, known as a left-handed parallel β -helix (28). The hexapeptide repeats of *Pseudomonas* LpxA are spaced in approximately the same manner as those of *E. coli*, with the exceptions that the first repeat (starting at *E. coli* residue 2) is truncated in *Pseudomonas* and that what

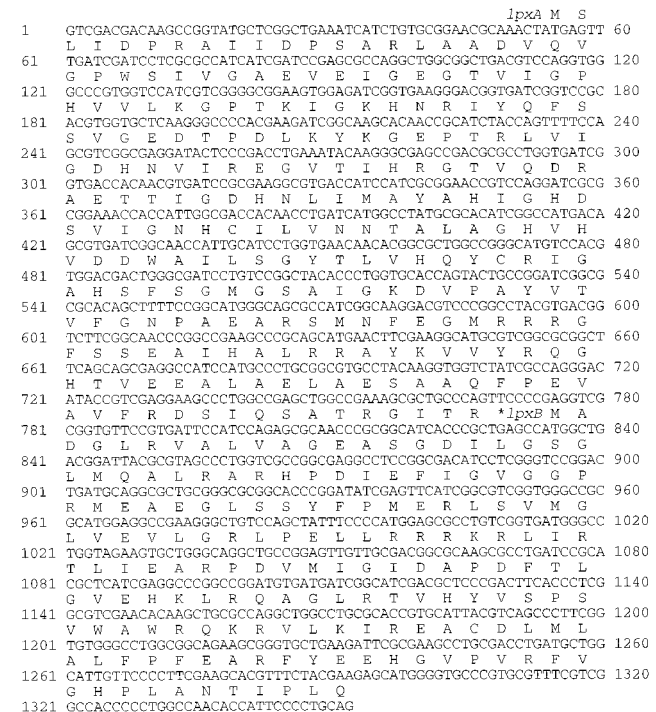


FIG. 4. DNA sequence of the *P. aeruginosa* *lpxA* gene and its flanking regions. The predicted protein sequences of LpxA and of the N-terminal part of LpxB are indicated.

PSEUD	1	17
ECOLI	1	17
PSEUD	18	35
ECOLI	18	35
PSEUD	36	53
ECOLI	36	53
PSEUD	54	71
ECOLI	54	71
PSEUD	84	101
ECOLI	84	101
PSEUD	109	126
ECOLI	109	126
PSEUD	127	144
ECOLI	127	144
PSEUD	145	162
ECOLI	145	162
PSEUD	163	179
ECOLI	163	179
PSEUD	180	220
ECOLI	180	220
PSEUD	221	262
ECOLI	221	262

FIG. 5. Comparison of the amino acid sequences of *P. aeruginosa* and *E. coli* LpxA. The overall sequence identity is 54%, and the sequence similarity is 67%. The aliphatic residues that denote the beginning of each hexapeptide repeat are in bold print. The sequence has been arranged to indicate the stacking of the hexads on top of each other in the formation of the left-handed β -helix seen in the *E. coli* LpxA crystal structure (28). The positions of two loops (residues 72 to 83 and 102 to 108) that can be recognized as interruptions of the contiguous hexad repeats in the primary sequences are located in the same places in both enzymes. One hexad is truncated at the N terminus of *Pseudomonas* LpxA, and valine 171 of the last complete hexad of *E. coli* is replaced by serine (italicized) in *Pseudomonas* LpxA. The significance of these differences to the overall protein fold and acyl chain selectivity of the *Pseudomonas* enzyme remains to be established. PSEUD, *P. aeruginosa*; ECOLI, *E. coli*.

would be the last complete repeat of *E. coli* (starting at *E. coli* residue 171) begins with serine in *Pseudomonas*, rather than with valine, the typical aliphatic residue at the beginning of a hexad (Fig. 5).

Other genes present on pGD2 and pGD3. The downstream partial open reading frame present on the *Pseudomonas* insert of pGD3 (nucleotides 834 to 1352) has a high degree of homology to the *lpxB* gene (Fig. 1) (12). In *E. coli* both *lpxA* and *lpxB* are in a complex operon that begins with *fabZ*, which in turn is downstream of *lpxD* (Fig. 1) (15, 19). Since pGD2 contains 1.7 kb of additional *P. aeruginosa* DNA upstream of *lpxA*, we determined its sequence to establish the presence or absence of *fabZ* and *lpxD*. Both these genes are indeed present on pGD2 (not shown). The full sequences of *Pseudomonas lpxD* and *fabZ* will be reported elsewhere. The termination codon of the *P. aeruginosa fabZ* gene overlaps the initiation codon of *lpxA* (Fig. 4).

Complementation of *E. coli* RO138 with *Pseudomonas lpxA*. *E. coli* RO138 is a *recA* mutant strain with a point mutation in the UDP-GlcNAc acyltransferase gene (*lpxA2*) (13) that imparts a temperature-sensitive growth phenotype and causes inhibition of lipid A biosynthesis at 42°C. In order to determine whether *Pseudomonas lpxA* can correct for this growth phenotype, RO138 was transformed by electroporation with pGD3. A separate derivative of RO138 harboring pBluescript was constructed as a control. Transformation with pGD3, but not with pBluescript, was able to correct the temperature sensitiv-

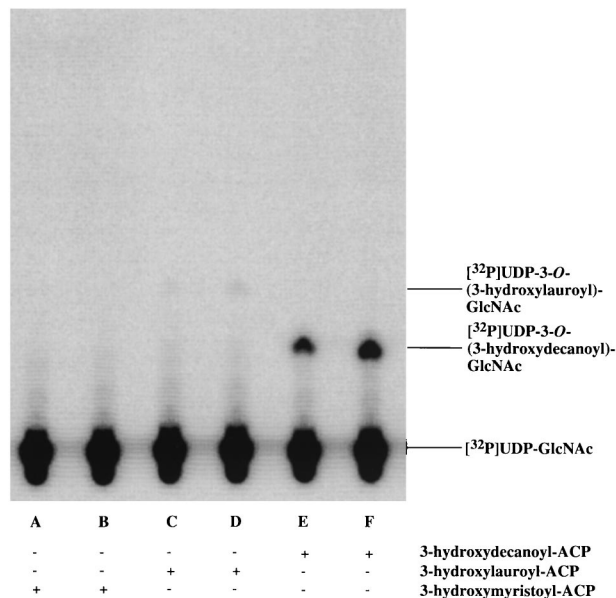


FIG. 6. Acyl-ACP specificity of *Pseudomonas* UDP-GlcNAc acyltransferase determined in cytosolic extracts of RO138/pGD3. Cells were grown to late log phase at 42°C in LB in the presence of ampicillin, and assays of supernatants centrifuged at $100,000 \times g$ were performed at 30°C, as described in the Materials and Methods. The extract concentration was 0.1 mg/ml. Results at the 2- and 5-min time points are shown for each reaction.

ity of RO138, as judged by its ability to grow and form single colonies at 42°C on LB-ampicillin agar (data not shown).

Substrate specificity of *Pseudomonas* UDP-GlcNAc acyltransferase expressed in RO138/pGD3. Extracts prepared from RO138 contain almost no measurable UDP-GlcNAc acyltransferase activity at any assay temperature (13, 21). The substrate specificity of *Pseudomonas* UDP-GlcNAc acyltransferase was therefore determined with a cytosolic fraction isolated by centrifugation at $100,000 \times g$ from RO138/pGD3 grown at 42°C. Figure 6 and Table 2 show the results obtained by assaying this fraction with [α - 32 P]UDP-GlcNAc and either 3-hydroxydecanoyl-, 3-hydroxydecanoyl-, or 3-hydroxymyristoyl-ACP as the acyl donor. The *Pseudomonas* enzyme expressed in *E. coli* RO138 exhibited a 370-fold preference for 3-hydroxydecanoyl- over 3-hydroxydecanoyl-ACP, while no detectable activity was observed with 3-hydroxymyristoyl-ACP. The acyl chain specificity of the cloned *Pseudomonas* LpxA (Table 2) is the opposite of that observed with *E. coli* LpxA, which is over 100-fold more selective for 3-hydroxymyristoyl-ACP than for 3-hydroxydecanoyl-ACP (33).

Composition of lipid A isolated from cells of *E. coli* RO138/pGD3 grown at 42°C. Given the in vitro specificity of *Pseudomonas* LpxA for the transfer of the 3-hydroxydecanoyl moiety (Table 2 and Fig. 6), lipid A (containing both the 1 and the 4'

TABLE 2. Acyl-ACP specificity of *P. aeruginosa* UDP-GlcNAc acyltransferase expressed in *E. coli* RO138

Substrate	Sp act (nmol/min/mg)	% Maximum activity
3-Hydroxydecanoyl-ACP	11.12	100
3-Hydroxydecanoyl-ACP	0.032	0.29
3-Hydroxymyristoyl-ACP	None detected	None detected
Decanoyl-ACP	0.015	0.13

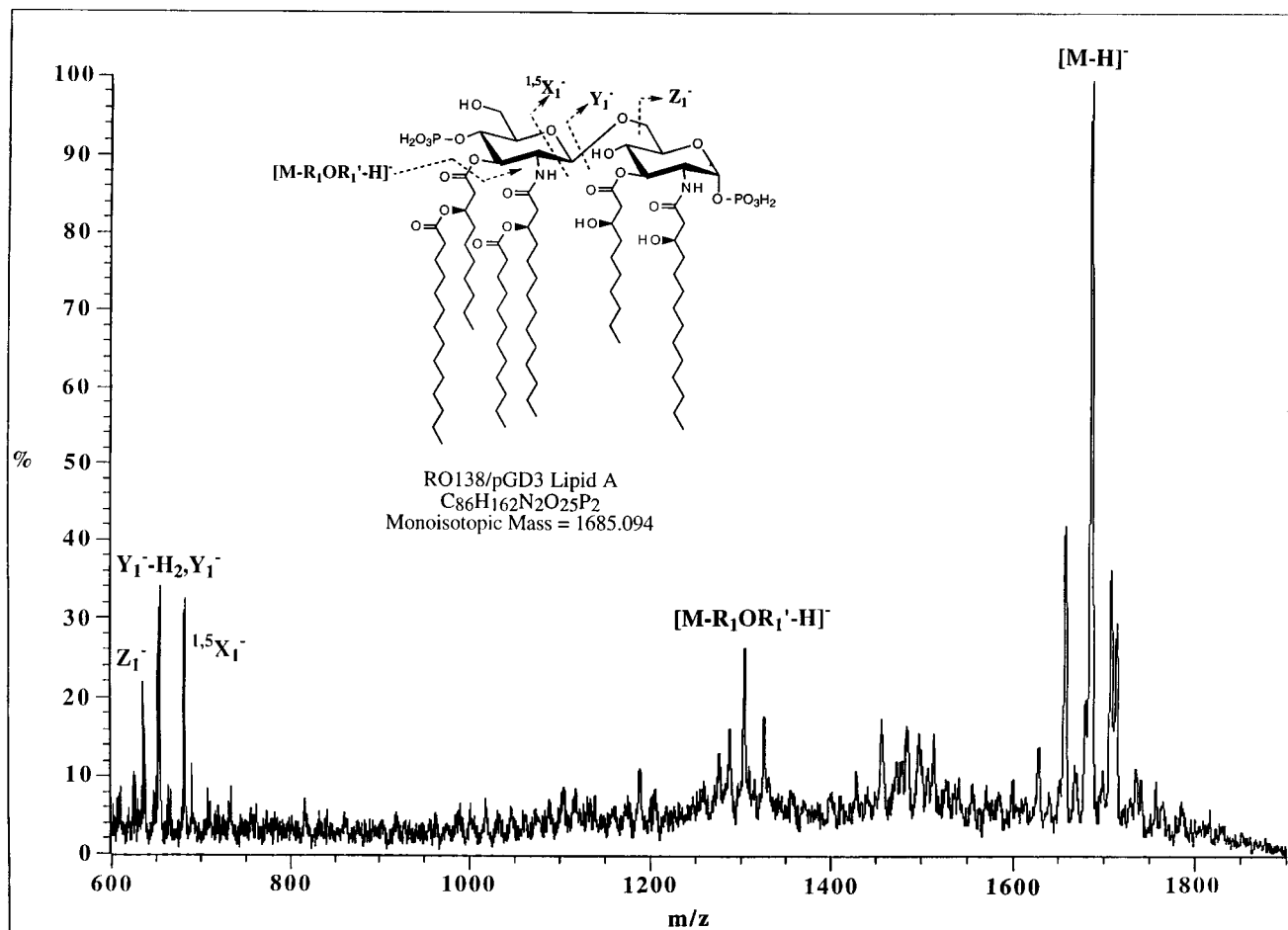


FIG. 7. Mass spectrum of lipid A isolated from strain RO138 complemented at 42°C with pGD3. There is no peak at m/z 1,796 in the lipid A isolated from RO138/pGD3, demonstrating the absence of residual *E. coli* LpxA function in living cells under these conditions.

phosphates) from RO138/pGD3 was isolated by pH 4.5 hydrolysis in the presence of sodium dodecyl sulfate and analyzed by mass spectrometry. Figure 7 shows the mass spectrum obtained by liquid secondary-ion mass spectrometry of the isolated lipid A taken in the negative-ion mode. The spectrum clearly shows the major molecular ion peak $[M-H]^-$ at m/z 1,684.5, which corresponds exactly to the mass predicted from replacement of the two hydroxymyristoyl moieties in wild-type *E. coli* lipid A (21) with two hydroxydecanoyl groups in RO138/pGD3. The fragmentation peak at m/z 1,303.9 arises from the loss of an acyloxyacyl group from the 3'-hydroxyl position of the glucosamine disaccharide, and it is also consistent with the presence of a hydroxydecanoyl rather than a hydroxymyristoyl group within the lost acyloxyacyl fragment. Fragmentation of the glycosidic bond (fragment ion Y_1^- in Fig. 7) gives rise to a peak at m/z 654.1, corresponding to the diacylated glucosamine bearing the anomeric phosphate group. The observed mass is 56 mass units less than that expected for the cleavage of lipid A of wild-type *E. coli* (21) and corresponds to a difference of four CH_2 groups. Consequently, the mass spectrum of RO138/pGD3 lipid A is exactly what is expected if R-3-hydroxydecanoate moieties are incorporated at positions 3 and 3' of lipid A by *Pseudomonas* LpxA. The origin of the small peak at m/z 1,456.2 is uncertain, but it might arise by the loss of a myristoyl side chain from lipid A, with the subsequent loss of one water molecule.

DISCUSSION

Using an expression cloning approach, we have identified *Pseudomonas lpxA*, the structural gene for the first enzyme of lipid A biosynthesis (24, 25, 27). Although several other *lpxA* genes, like that of *E. coli* (10), have been sequenced previously, *Pseudomonas lpxA* represents the first example of a homolog encoding a 10-carbon-specific UDP-GlcNAc acyltransferase (33). A gene encoding a 12-carbon-selective UDP-GlcNAc acyltransferase from *Neisseria meningitidis* has also been reported recently (21), but all other known LpxA sequences are thought to represent 14-carbon-specific acyltransferases. Sixteen- or 18-carbon-specific UDP-GlcNAc acyltransferases probably remain to be discovered, given the compositions of lipid A variants from strains of *Rhizobium* (6) and *Helicobacter* (20).

The sequence of *Pseudomonas* LpxA displays 54% identity and 67% similarity to its *E. coli* counterpart (Fig. 5). Of particular interest is the presence of the hexapeptide repeat motifs, which extend over the N-terminal two-thirds of both UDP-GlcNAc acyltransferases. This motif has been shown to specify a unique left-handed parallel β -helix domain in the X-ray crystal structures of the *E. coli* UDP-GlcNAc acyltransferase (28) and of two other enzymes that possess such hexad repeats (5, 16). Many of the enzymes that contain hexapeptide repeat motifs are hydroxyacyl-, succinyl-, or acetyltransferases that

utilize either ACP or coenzyme A thioester as a substrate. The *E. coli* genome contains as many as 15 hexapeptide-repeat proteins.

Although it is very similar in sequence to *E. coli* LpxA, the *P. aeruginosa* LpxA, expressed in RO138, has a greater than 1,000-fold preference for 3-hydroxydecanoyl-ACP over 3-hydroxymyristoyl-ACP (Fig. 6; Table 2). How fatty acyl chain length is recognized and measured by these acyltransferases is not known. The X-ray crystal structure of the *E. coli* enzyme, which has recently been solved at a 2.6-Å resolution (28), provides a unique opportunity to investigate the chain length issue. To date, no other acyltransferase has had its crystal structure determined.

The active site of *E. coli* LpxA has not been identified conclusively. The available model of the native LpxA homotrimer (28) suggests that the acyl-ACP substrate may dock in a large basic cleft situated between the subunits. Without the structures of both the *E. coli* and the *Pseudomonas* enzymes in the presence of an acyl-ACP, it will be difficult to determine exactly how the acyl chain measuring process works. Given the related sequences and the nearly identical spacings of the hexad repeats (Fig. 5), we expect that the overall folds of the *E. coli* and the *Pseudomonas* enzymes will be quite similar. Only two hexads are missing or are significantly altered in *Pseudomonas* LpxA (Fig. 5). These are the N-terminal hexad (which is not likely to be near the active site) and the hexad that begins at residue 171 in *E. coli* LpxA. In *Pseudomonas* LpxA, this residue is serine, not one of the expected aliphatic amino acids (Fig. 5). Interestingly, *N. meningitidis* LpxA has a threonine at this location (31). It is tempting to speculate that a hydroxylated side chain at this site might alter the protein fold sufficiently to account for the short chain selectivities of the *Pseudomonas* and *N. meningitidis* LpxA enzymes (21, 33).

Even without additional crystal structures, it may be possible to obtain information about key residues involved in determining acyl chain selectivity. One could begin by constructing *E. coli*-*Pseudomonas* LpxA hybrids. A short peptide segment or even a single key side chain that plays a role in establishing selectivity might be identified in this manner. Probing chain length specificity by alteration of small peptide segments has been successful with plant acyl-ACP thioesterase. With these thioesterases, it has been shown that two or three amino acid substitutions can alter the chain length specificity from 12 to 14 carbons (34). However, X-ray structures have not been reported for these thioesterases.

In addition to providing insights into the molecular mechanisms of acyltransferases, the LpxA system offers a new opportunity to modify the structure of lipid A in living cells (Fig. 7) and to study the effects of such modifications on cell growth and outer membrane assembly. Although RO138 can grow at 42°C with *Pseudomonas* LpxA complementing the chromosomal *lpxA2* mutation, the cells are likely to be antibiotic hypersensitive (21). A search for ancillary phenotypes associated with modifications of the lipid A structure is worthwhile, as new insights into the biological functions of lipid A might emerge.

ACKNOWLEDGMENTS

We thank Joseph Lam for providing us with the *Pseudomonas* DNA library and John Cronan and V. Jo Davidson for providing the 3-decyl-*N*-acetyl cysteamine.

This research was supported by NIH grants GM-51310 to C. R. H. Raetz and GM-33967 to R. J. Cotter.

REFERENCES

- Anderson, M. S., C. E. Bulawa, and C. R. H. Raetz. 1985. The biosynthesis of gram-negative endotoxin: formation of lipid A precursors from UDP-

- GlcNAc in extracts of *Escherichia coli*. *J. Biol. Chem.* **260**:15536–15541.
- Anderson, M. S., H. S. Bull, S. M. Galloway, T. M. Kelly, S. Mohan, K. Radika, and C. R. H. Raetz. 1993. UDP-*N*-acetylglucosamine acyltransferase of *Escherichia coli*: the first step of endotoxin biosynthesis is thermodynamically unfavorable. *J. Biol. Chem.* **268**:19858–19865.
- Anderson, M. S., and C. R. H. Raetz. 1987. Biosynthesis of lipid A precursors in *Escherichia coli*: a cytoplasmic acyltransferase that converts UDP-*N*-acetylglucosamine to UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine. *J. Biol. Chem.* **262**:5159–5169.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1989. Current protocols in molecular biology. John Wiley & Sons, New York, N.Y.
- Beaman, T. W., D. A. Binder, J. S. Blanchard, and S. L. Roderick. 1997. Three-dimensional structure of tetrahydrodipicolinate *N*-succinyltransferase. *Biochemistry* **36**:489–494.
- Bhat, U. R., L. S. Forsberg, and R. W. Carlson. 1994. The structure of the lipid A component of *Rhizobium leguminosarum* bv. *phaseoli* lipopolysaccharide. A unique, non-phosphorylated lipid A containing 2-amino-2-deoxyglucuronate, galacturonate, and glucosamine. *J. Biol. Chem.* **269**:14402–14410.
- Caroff, M., C. Deprun, D. Karibian, and L. Szabó. 1991. Analysis of unmodified endotoxin preparations by ²⁵²Cf plasma desorption mass spectrometry. *J. Biol. Chem.* **266**:18543–18549.
- Caroff, M., A. Tacken, and L. Szabó. 1988. Detergent-accelerated hydrolysis of bacterial endotoxins and determination of the anomeric configuration of the glycosyl phosphate present in the "isolated lipid A" fragment of the *B. pertussis* endotoxin. *Carbohydr. Res.* **175**:273–282.
- Clementz, T., J. J. Bednarski, and C. R. H. Raetz. 1996. Function of the *htrB* high temperature requirement gene of *Escherichia coli* in the acylation of lipid A. *HtrB* catalyzed incorporation of laurate. *J. Biol. Chem.* **271**:12095–12102.
- Coleman, J., and C. R. H. Raetz. 1988. First committed step of lipid A biosynthesis in *Escherichia coli*: sequence of the *lpxA* gene. *J. Bacteriol.* **170**:1268–1274.
- Crowell, D. N., M. S. Anderson, and C. R. H. Raetz. 1986. Molecular cloning of the genes for lipid A disaccharide synthase and UDP-*N*-acetylglucosamine acyltransferase in *Escherichia coli*. *J. Bacteriol.* **168**:152–159.
- Crowell, D. N., W. S. Reznikoff, and C. R. H. Raetz. 1987. Nucleotide sequence of the *Escherichia coli* gene for lipid A disaccharide synthase. *J. Bacteriol.* **169**:5727–5734.
- Galloway, S. M., and C. R. H. Raetz. 1990. A mutant of *Escherichia coli* defective in the first step of endotoxin biosynthesis. *J. Biol. Chem.* **265**:6394–6402.
- Jackowski, S., P. D. Jackson, and C. O. Rock. 1994. Sequence and function of the *aas* gene in *Escherichia coli*. *J. Biol. Chem.* **269**:2921–2928.
- Kelly, T. M., S. A. Stachula, C. R. H. Raetz, and M. S. Anderson. 1993. The *firA* gene of *Escherichia coli* encodes UDP-3-*O*-(*R*-3-hydroxymyristoyl)- α -*D*-glucosamine *N*-acyltransferase: the third step of endotoxin biosynthesis. *J. Biol. Chem.* **268**:19866–19874.
- Kisker, C., H. Schindelin, B. E. Alber, J. G. Ferry, and D. C. Rees. 1996. A left-hand beta-helix revealed by the crystal structure of a carbonic anhydrase from the archaeon *Methanosarcina thermophila*. *EMBO J.* **15**:2323–2330.
- Lightfoot, J., and J. S. Lam. 1991. Molecular cloning of genes involved with expression of A-band lipopolysaccharide, an antigenically conserved form, in *Pseudomonas aeruginosa*. *J. Bacteriol.* **173**:5624–5630.
- Magnuson, K., S. Jackowski, C. O. Rock, and J. E. Cronan, Jr. 1993. Regulation of fatty acid biosynthesis in *Escherichia coli*. *Microbiol. Rev.* **57**:522–542.
- Mohan, S., T. M. Kelly, S. S. Eveland, C. R. H. Raetz, and M. S. Anderson. 1994. An *Escherichia coli* gene (*fabZ*) encoding *R*-3-hydroxymyristoyl acyl carrier protein dehydrase. Relation to *fabA* and suppression of mutations in lipid A biosynthesis. *J. Biol. Chem.* **269**:32896–32903.
- Moran, A. P., I. M. Helander, and T. U. Kosunen. 1992. Compositional analysis of *Helicobacter pylori* rough-form lipopolysaccharides. *J. Bacteriol.* **174**:1370–1377.
- Odegaard, T. J., I. A. Kaltashov, R. J. Cotter, L. Steeghs, P. van der Ley, S. Khan, D. J. Maskell, and C. R. H. Raetz. 1997. Shortened hydroxyacyl chains on lipid A of *Escherichia coli* cells expressing a foreign UDP-*N*-acetylglucosamine *O*-acyltransferase. *J. Biol. Chem.* **272**:19688–19696.
- Onishi, H. R., B. A. Pelak, L. S. Gerckens, L. L. Silver, F. M. Kahan, M. H. Chen, A. A. Patchett, S. M. Galloway, S. A. Hyland, M. S. Anderson, and C. R. H. Raetz. 1996. Antibacterial agents that inhibit lipid A biosynthesis. *Science* **274**:980–982.
- Post-Beittenmiller, D., J. G. Jaworski, and J. B. Ohlrogge. 1991. The in vivo pools of free and acylated acyl carrier proteins in spinach. Evidence of sites of regulation of fatty acid biosynthesis. *J. Biol. Chem.* **266**:1858–1865.
- Raetz, C. R. H. 1993. Bacterial endotoxins: extraordinary lipids that activate eucaryotic signal transduction. *J. Bacteriol.* **175**:5745–5753.
- Raetz, C. R. H. 1996. Bacterial lipopolysaccharides: a remarkable family of bioactive macroamphiphiles, p. 1035–1063. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Sal-*

- monella*: cellular and molecular biology, 2nd ed., vol. 1. American Society for Microbiology, Washington, D.C.
26. **Raetz, C. R. H.** 1990. Biochemistry of endotoxins. *Annu. Rev. Biochem.* **59**:129–170.
 27. **Raetz, C. R. H., and W. Dowhan.** 1990. Biosynthesis and function of phospholipids in *Escherichia coli*. *J. Biol. Chem.* **265**:1235–1238.
 28. **Raetz, C. R. H., and S. L. Roderick.** 1995. A left-handed parallel β helix in the structure of UDP-*N*-acetylglucosamine acyltransferase. *Science* **270**:997–1000.
 29. **Rietschel, E. T., L. Brade, B. Lindner, and U. Zähringer.** 1992. Biochemistry of lipopolysaccharides, p. 3–41. *In* D. C. Morrison and J. L. Ryan (ed.), *Bacterial endotoxic lipopolysaccharides*, vol. I. Molecular biochemistry and cellular biology. CRC Press, Boca Raton, Fla.
 30. **Sorensen, P. G., J. Lutkenhaus, K. Young, S. S. Eveland, M. S. Anderson, and C. R. H. Raetz.** 1996. Regulation of UDP-3-*O*-[*R*-3-hydroxymyristoyl]-*N*-acetyl-glucosamine deacetylase in *Escherichia coli*. The second enzymatic step of lipid A biosynthesis. *J. Biol. Chem.* **271**:25898–25905.
 31. **Steeghs, L., M. P. Jennings, J. T. Poolman, and P. van der Ley.** 1997. Isolation and characterization of the *Neisseria meningitidis* *lpxD-fabZ-lpxA* gene cluster involved in lipid A biosynthesis. *Gene* **190**:263–270.
 32. **Vaara, M.** 1992. Eight bacterial proteins, including UDP-*N*-acetylglucosamine acyltransferase (LpxA) and three other transferases of *Escherichia coli*, consist of a six-residue periodicity theme. *FEMS Microbiol. Lett.* **97**:249–254.
 33. **Williamson, J. M., M. S. Anderson, and C. R. H. Raetz.** 1991. Acyl-acyl carrier protein specificity of UDP-GlcNAc acyltransferases from gram-negative bacteria: relationship to lipid A structure. *J. Bacteriol.* **173**:3591–3596.
 34. **Yuan, L., T. A. Voelker, and D. J. Hawkins.** 1995. Modification of the substrate specificity of an acyl-acyl carrier protein thioesterase by protein engineering. *Proc. Natl. Acad. Sci. USA* **92**:10639–10643.