The lpcA locus has been identified in Escherichia coli K12 novobiocin-supersensitive mutants that produce a short lipopolysaccharide (LPS) core which lacks glyceromannoheptose and terminal hexoses. We have characterized lpcA as a single gene mapping around 5.3 min (246 kilobases) on the E. coli K12 chromosome and encoding a 22.6-kDa cytosolic protein. Recombinant plasmids containing only lpcA restored a complete core LPS in the E. coli strain 711E. We show that this strain has an IS5-mediated chromosomal deletion of 35 kilobases that eliminates the lpcA. The LpcA protein showed discrete similarities with a family of aldose/ketose isomerases and other proteins of unknown function. The isomerization of sedoheptulose 7-phosphate, into a phosphosugar presumed to be di-glycerol-o-mannoheptose 7-phosphate, was detected in enzyme reactions with cell extracts of E. coli lpcA+ and of lpcA mutants containing the recombinant lpcA gene. We concluded that LpcA is the phosphoheptose isomerase used in the first step of glyceromannoheptose synthesis. We also demonstrated that lpcA is conserved among enteric bacteria, all of which contain glyceromannoheptose in the inner core LPS, indicating that LpcA is an essential component in a conserved biosynthetic pathway of inner core LPS.

LPS, an integral component of the outer membrane of Gram-negative bacteria, consists of lipid A attached to a core oligosaccharide, and in some microorganisms, contains an O-specific surface polysaccharide which is subsequently attached to the terminal residues of the core (1-2). The core oligosaccharide has an inner domain made of 3-deoxy-D-manno-octulosonic acid and l-glycerol-o-mannoheptose, and an outer domain composed of hexoses and N-acetylgalactosamine. The structure of the inner core is relatively highly conserved among enteric (3) and non-enteric bacteria (4). Most of the genes involved in the biosynthesis and assembly of the core oligosaccharide are located within the rfa cluster, at about 81 min on the chromosome in Escherichia coli K12 and 79 min in Salmonella enterica LT2 (1). However, the genes involved in the early steps of the synthesis of l-glycerol-o-mannoheptose are not located in this region and they have not been characterized as yet.

LPS plays an important role in maintaining the structural integrity of the outer membrane by interacting with other components of the outer membrane and providing a physical barrier against the entry of deleterious compounds and some bacteriophages (3). E. coli LPS mutants with defects in the inner core display a dramatic reduction in porin proteins (5) and are unable to grow in media containing detergents, bile salts, or hydrophobic antibiotics, all of which normally have a reduced permeability across the outer membrane and are toxic only in high concentrations (6). Since these mutants lack an attachment site for the rest of the core oligosaccharide, they are resistant to LPS core-specific bacteriophages (6) and survive poorly within the host environment (7).

Early work by Tamaki et al. (6) resulted in the isolation of mutations conferring supersensitivity to novobiocin which mapped to two different regions on the E. coli K12 chromosome between ara and lac (1-10 min) next to the proAB genes, and between 55 and 60 min; they were designated as lpcA (LPS-core synthesis) and lpcB, respectively (6). Similar mutations were also identified by Havekes et al. (8) as F plasmid conjugation-deficient mutants. The LPS of both lpcA and lpcB mutants lacks heptose (6), suggesting these loci are involved in synthesis of the inner core domain, but since their original discovery, their precise function has not been established.

This study reports the molecular analysis of the lpcA locus, and the biochemical characterization of its gene product. We conclude that LpcA encodes a phosphoheptose isomerase used in the first step of the biosynthesis of the inner core LPS precursor, ADP-l-glycerol-o-mannoheptose. We also demonstrate that lpcA is widely conserved among enteric bacteria, suggesting that its function is part of a conserved pathway for LPS biosynthesis.

**EXPERIMENTAL PROCEDURES**

Bacterial Strains, Plasmids, and Media—Bacteria used in this study include: E. coli K12 strains 705 (F', leu-4, dfrA4, ara-35, T6', chromosomal deletion of the rfa cluster), 711 (F', leu-4, dfrA4, proAB118, StrR, T6', chromosomal deletion of the rfa cluster), Y10 (F', thi-1, rfaD1, supE44), J M109(DE3) (endA1, recA1, s3A6, thi, hisD17), rfaD1, supE44, [lac-proAB], [F', tral36, proAB, lac-proAB], [Z3M15], [DE3), D21e7 (rafl), CS2051 (has a deletion eliminating rfaG, rfaP, rfaM, rfaN, and rfaB), D31m4 (rafl, rfaN), E. coli strains O4, UWO101; Pseudomonas aeruginosa strain AK44, O16; S. enterica strains 10749 serovar New brunswick group E2, 10756 serovar Thomasville group E3, G30 serovar Typhimurium; Proteus mirabilis PMVH146, Proteus vulgaris VHL 453; Entero bacter cloacae clDF13R, clDF13R, clDF13R, clDF13R, and clDF13R.
Identification of a Phosphoheptose Isomerase in E. coli

Enterobacter aerogenes 62-1, Enterobacter agglomerans UW100; Klebsiella pneumoniae VHL-8, Klebsiella spp. Raph 3a, VHL-16, VHL-17; Shigella flexneri FIH01(SF6), and Shigella boydii MV300 type 12. Bacteria were grown in Luria broth. The following compounds were added as appropriate: novobiocin (50 μg/ml), chloramphenicol (30 μg/ ml), ampicillin (100 μg/ml), streptomycin (100 μg/ml), spectinomycin (80 μg/ml), sodium dodecyl sulfate (6 mg/ml), and deoxycholate (10 mg/ml). Cosmid pE4021 was obtained from A. Higashitani and contains EcoRI fragments from the chromosome of E. coli strain W3310, inserted into the EcoRI site of the plasmid vector pHC79. Plasmid pB1 is a deleted derivative of pE4021 containing a single 14-kb EcoRI fragment. Plasmid pB2 and pB6 were constructed by cloning a 3-kb BamHI fragment from pB1 into the BamHI site of pMAV3 (9) and pSP6 (10), respectively. Plasmid pB2-9 through to pB2-34 are unidirectional deletion clones from pB2 (see below). Plasmid pB15 was constructed by deletion of a HindIII fragment from pB2-25. Plasmid pB18 contains an in-frame translational fusion of fpCA with a histidine tag cloned into the expression vector, pQE32 (Qiagen, Chatsworth, CA). PREP4 contains the lac gene encoding the lac repressor (Qiagen). LpcA-labeled SDS-PAGE molecular weight markers were purchased from Amersham Canada, Oakville, Ontario, Canada. Calf alkaline phosphatase was purchased from Boehringer Mannheim, Dorval, Quebec, Canada. HPLC grade acetonitrile was purchased from BDH, Toronto, Ontario, Canada. HPLC grade methanol was purchased from Fisher Scientific, Nepean, Ontario, Canada. All other chemicals and antibiotics were purchased from Sigma.

RESULTS

Identification of a Phosphoheptose Isomerase in E. coli

Gene probes—Small and large scale plasmid DNA extractions and electrophoresis of plasmid DNA were performed as described previously (11). Large scale preparation of RNA from E. coli strain y711 (plB2) was performed using a combination of the methods described by Deuschle et al. (12) and Glisin et al. (13). Colony hybridizations were carried out with the DIG-dUTP-labeled RNA probe (see below) using Zeta Probe membranes (Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) according to the manufacturer’s instructions (Boehringer Mannheim, Dorval, Quebec, Canada). Hybridizations were performed at 37 °C for 21 h, followed by two 15-min washes with 0.1 × SSC (20 × SSC: 300 mM citric acid, 3 M NaCl, pH 7.0) containing 0.1% SDS at 37 °C and development using a chemiluminescent detection system (Boehringer Mannheim, Dorval, Quebec, Canada). Hybridizations were performed at 42°C for 18–20 h for the parental strain and bands developed using a colorimetric detection system as recommended by the manufacturer.

Transcription-translation was performed using the Prokaryotic DNA-directed Translation kit from Amersham with [35S]methionine, as recommended by the manufacturer. Polypeptides were separated by SDS-PAGE (25) followed by treatment with ENHANCE (DuPont NEN). Dried gels were exposed to Kodak X-Omat film at −110 °C for 16–48 h. LPS Analysis—LPS was extracted as described by Mardala et al. (11) and analyzed by Tricine SDS-PAGE as described by Schagger and von Jagow (26). LPS was detected using the silver-staining procedure of Dubray and Bazard (27).

Enzyme Reactions—Cell extracts were prepared from 800 ml of culture grown 3 h at 37 °C. Cells were harvested and resuspended in 2 ml of TDE (50 mM Tris-HCl (pH 7.8), 5 mM dithiothreitol, 1 mM EDTA) (28) and sonicated 2–3 times for 30 s in a Branson Sonic Power sonifier cell disruptor 350 with 1 min cooling between sonications. Cell debris and unbroken cells were sedimented by centrifugation at 27,000 × g at 4 °C for 20 min, and the supernatant was passed through Sephadex G-25 columns using TDE/glycerol (80:20) (v/v) as eluent to remove low molecular weight material. Protein fractions were collected and glycerol added as appropriate: novobiocin (50 μg/ml). Silver-stained 16.4% (w/v) T (total acrylamide), 1.9% (v/v) C (bisacrylamide) Tricine SDS-polyacrylamide gel showing the LPS profiles of E. coli K12 strains. 1, y705; 2, y711; 3, y711(pB2); 4, Y10; 5, D21e7 (rfA-1); 6, CS2051 (has a deletion eliminating rfaR, rfaP, rfaM, rfaN, and rfaB); 7, D31m4 (rfA-299, rfa-230).

addition of 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (14). In vitro transcription-translation was performed using the Prokaryotic DNA-directed Translation kit from Amersham with [35S]methionine, as recommended by the manufacturer. Polypeptides were separated by SDS-PAGE (25) followed by treatment with ENHANCE (DuPont NEN). Dried gels were exposed to Kodak X-Omat film at −110 °C for 16–48 h. LPS Analysis—LPS was extracted as described by Mardala et al. (11) and analyzed by Tricine SDS-PAGE as described by Schagger and von Jagow (26). LPS was detected using the silver-staining procedure of Dubray and Bazard (27).

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HPLC Analysis—ABBE-labeled carbohydrates were separated on a C18 reverse-phase column (Brownlee RP-18, 5 μm resin, 250 × 4.6 mm) run isocratically for 0 to 6 min with acetonitrile/water/dimethylbutane (50:49.05:0.95) by a linear gradient of acetonitrile/water/dimethylbutane (50:49.05:0.95) to acetonitrile/water/dimethylbutane (10:89.05:0.95) over 20 min at a flow rate of 0.5 ml/min at 35 °C.

RESULTS

Characterization of E. coli Strain y711—Curtiss et al. (31) isolated E. coli strain y711 which has been thought to have a chromosomal deletion around the region containing the proline synthesis genes proAB, and is resistant to bacteriophages P1 and T3. Since the lpcA locus has been mapped near the proAB genes (6), we examined strain y711 for characteristics of inner core LPS defects. In contrast to the parental strain y705, E. coli strain y711 did not grow in Luria broth (LB) with SDS, MacConkey agar, LB with novobiocin, and LB with deoxycholate, suggesting a defect in inner core LPS.

Direct evidence of an altered LPS structure was obtained by a comparative analysis of the LPS profiles of strains y711 and y705 (Fig. 1). Strain y705 produces a core digosaccharide identical to that of the prototypic E. coli K12 strain Y10 (Fig. 1, lanes 1 and 4) whereas strain y711 produces a much shorter core (Fig. 1, lane 2). LPS of E. coli strains D21e7, CS2051, and D31m4, containing different mutations in rfa genes, were examined and compared with the y711 core. The LPS core of strains D21e7 and CS2051 was shorter than the wild-type core but still longer than the y711 core (Fig. 1, lanes 5 and 6), whereas the LPS core of D31m4 migrated the same distance in

![Fig. 1. Silver-stained 16.4% (v/v) T (total acrylamide), 1.9% (v/v) C (bisacrylamide) Tricine SDS-polyacrylamide gel showing the LPS profiles of E. coli K12 strains. 1, y705; 2, y711; 3, y711(pB2); 4, Y10; 5, D21e7 (rfA-1); 6, CS2051 (has a deletion eliminating rfaR, rfaP, rfaM, rfaN, and rfaB); 7, D31m4 (rfA-299, rfa-230).](http://www.jbc.org/)
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FIG. 2. Physical map of the lpcA region. Vector sequences are not shown. pE4021 is a cosmid clone containing chromosomal DNA from E. coli W3110, including the region from 4.9 to 5.8 min. RNHP, PEPD, GPTA, PHOE, and PROAB indicate the location of sequenced genes. pJB1 contains a 14-kb EcoRI fragment cloned from pE4021. pJB2 and pJB8 contain a 3-kb BamHI fragment cloned from pJB1 into different vectors. pJB2-9 to pJB2-24 indicate the various deletions of pJB2 spanning the lpcA region. pJB15 indicates the DNA insert used for construction of DIG-labeled riboprobes. ORF1 and ORF2 are two open reading frames found on opposite strands of the DNA. The direction of transcription of lpcA is indicated by the arrow beneath ORF2. The complementation of the novobiocin supersensitivity phenotype by the deletion clones is indicated: R, successful complementation; S, unsuccessful complementation. Restriction enzymes indicated are: A, Aval; B, BamHI; Bs, BstEII; E, EcoRI; Ev, EcoRV; Hc, HindIII; P, PvuI.

FIG. 3. Nucleotide sequence and deduced amino acid sequence of lpcA. The underlined sequence AGGA denotes the possible ribosomal binding site (rbs). The putative -10 consensus sequence is indicated by double underlining. The deletion end points of pJB2-10 and pJB2-25 (Fig. 2) are indicated by arrows followed by the numbers 10 and 25 in parentheses; the direction of the arrows indicates the sequence contained within pJB2-10 and pJB2-25, respectively. A putative transcription termination signal is indicated by arrows beneath the sequence downstream of the termination codon TAA. Lines above the sequence indicate GCCG and CGGC indicate the complementary sequences forming the stem of the hairpin loop structure. Boxed sequences denote the location of the repetitive extragenic palindromic sequence. The gel as the LPS core of χ711 (Fig. 1, lanes 7 and 2). Since strain D31m4 produces a heptoseless LPS made of only 3-deoxy-o-manno-octulosonic acid and lipid A (32) we conclude that χ711 also lacks heptose in its core LPS. Identification of the lpcA Locus—To investigate if sequences near the proAB genes include the gene(s) responsible for the LPS defect of χ711, χ711 cells were transformed with the cosmid pE4021 which contains a DNA segment spanning the proAB region (33) (Fig. 2). Transformants appeared on plates containing novobiocin, indicating that pE4021 could carry the lpcA gene determinant(s). To position the lpcA locus, partial EcoRI digestion and self-ligation of pE4021 was performed, followed by transformation into strain χ711 and selection on plates with novobiocin. The surviving colonies contained plasmids carrying a single 14-kb EcoRI fragment; one of these plasmids was designated pJB1 (Fig. 2) and used to subclone smaller DNA fragments into pMAV3. One of the subclones, pJB2, contained a approximately 3-kb BamHI fragment (Fig. 2) and enabled E. coli χ711 cells to grow in medium containing either novobiocin or SDS. This indicated that the gene(s) of the lpcA locus resides within the 3-kb BamHI fragment of pJB2 (Fig. 2). A comparison of the core LPS profiles of strains χ705 and χ711(pJB2) revealed that this plasmid restored the core LPS defect of E. coli χ711 (Fig. 1, lanes 1 and 3). pJB2, however, failed to complement the core LPS defect in strain D31m4 (data not shown), indicating that the function of the lpcA gene, although associated with a heptoseless core, is different from the functions defined by the mutations rfa-229 and rfa-230 in D31m4. Complementation of the lpcA mutation in χ711 was also achieved with the low copy number construct pJB8 (Fig. 2 and data not shown).

Nucleotide Sequence of the lpcA Locus—Unidirectional deletion derivatives of pJB2 were made as described under "Experimental Procedures." Transformation of these plasmids into E. coli χ711 followed by selection of transformants on medium containing novobiocin, demonstrated that the lpcA locus lies within a 0.826-kb DNA segment located between the deletion end points of plasmids pJB2-9 and pJB2-25 (Fig. 2). DNA sequence data obtained revealed two open reading frames, one on each strand, designated as ORF1 (412 bp) and ORF2 (577 bp) (Fig. 2). To determine which of the ORFs is expressed, we examined the direction of transcription of lpcA using in vitro synthesized DIG-dUTP-labeled riboprobes. Two probes of labeled mRNA independently transcribed from each of the DNA strands spanning the region where the two ORFs overlapped were prepared using either SP6 or T7 RNA polymerase-directed transcription. pJB15 was constructed for this purpose, as a HindIII deletion of pJB2-25 (Fig. 2). pJB15 DNA was cut with EcoRV and HindIII separately to linearize the DNA prior to in vitro transcription in the presence of DIG-dUTP. The RNA probes were then used for hybridization with total RNA obtained from E. coli χ711 containing pJB2. Only the probe synthesized with SP6 RNA polymerase hybridized with cellular RNA, whereas the probe corresponding to the transcript of the opposite strand did not. Therefore, we concluded that ORF2 corresponds to the lpcA gene. We also observed that the deletion plasmid pJB2-25 lacks a small portion of the carboxyl terminus of LpcA from Ile-183 to Lys-192 (Figs. 2 and 3). Since pJB2-25 complements the N5 phenotype of E. coli χ711 (Fig. 2), we conclude that these 10 carboxyl-terminal amino acids are probably not essential for the function of the protein.
The %G + C content of lpcA was 51%, similar to the reported values for %G + C content of the E. coli genome, and the codon usage was typical for E. coli genes. The sequence AGGA found 8 bp upstream of the AUG codon may correspond to the ribosomal binding site (Fig. 3). The sequence TATAAT located 146 bp upstream of the AUG codon may correspond to the ribosome extragenic palindromic sequence and the termination signal were also noted between the repetitive extragenic palindromic sequence and the termination codon (Fig. 3).

The expression of the lpcA gene product in vivo and in vitro identified a 22.6-kDa polypeptide as the LpcA protein (data not shown), which is in agreement with the predicted molecular mass of 20.6 kDa. A hydropathy profile of the LpcA protein (34) deduced from the nucleotide sequence of lpcA, did not reveal any significant regions of hydrophobicity compatible with membrane domains suggesting that LpcA is a cytosolic protein.

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transfersases investigated to date (38); the absence of this region in LpcA suggests that this protein is not an amidotransferase.

To test whether LpcA catalyzes an isomerization reaction, cell extracts of E. coli strain χ711, E. coli strain χ711(pB2), and E. coli strain χ711(pB18, pREP4) were used in an assay containing sedoheptulose 7-phosphate. The reaction products were examined by high performance liquid chromatography (HPLC) after derivatization with aminobenzoic ethyl ester (ABEE) to facilitate their detection with UV light. Chromatograms from reactions with cell extracts of χ711(pB2) and χ711-(pB18, pREP4) revealed the appearance of a new peak with a retention time of 8.2 min (Fig. 5B and data not shown) after 2 min of incubation with the enzyme. After 60 min incubation, the peak corresponding to sedoheptulose 7-phosphate decreased considerably, 39 and 93% of initial amount for χ711(pB2) and χ711(pB18, pREP4), respectively (data not shown), suggesting that the substrate was consumed, whereas the level of sedoheptulose 7-phosphate remained constant for 60 min when incubated with boiled extract (Fig. 5D). Upon incubation of χ711 cell extract with sedoheptulose 7-phosphate (Fig. 5A), a peak with 8.2 min retention as found with χ711(pB2) and χ711(pB18, pREP4) was not apparent, although other peaks with retention times ranging from 8.5 to 10.5 min were observed. These extra peaks could be due to the conversion of sedoheptulose 7-phosphate into fructose 6-phosphate and erythrose 4-phosphate by a transaldolase activity (39) in the extracts, or into D-ribose 5-phosphate and D-xylulose 5-phosphate by a transketolase activity (39), or the conversions of triose phosphates and glucose 6-phosphate which are present as contaminants in the sedoheptulose 7-phosphate preparation.

From this experiment we concluded that the 8.2-min peak corresponded to the reaction product, presumably D-glycero-D-mannoheptose 7-phosphate, however, this could not be verified directly since this phosphosugar is not commercially available. To prove that the product was a phosphorylated form of D-glycero-D-mannoheptose, reaction components were dephosphorylated by treatment with alkaline phosphatase prior to HPLC analysis. A peak with a retention time of 10.5 min corresponding to the retention time of authentic glyceraldehyde phosphate 7-phosphate was detected (Fig. 6). A peak with a retention time of 8.2 min corresponding to the reaction product in the absence of alkaline phosphatase was not detected in this experiment (Fig. 6, arrow). Therefore, we concluded that the product of the reaction in the presence of sedoheptulose 7-phosphate is a phosphorylated form of glyceraldehyde phosphate 7-phosphate. The two peaks in the glyceraldehyde phosphate 7-phosphate standard (Fig. 6) probably indicate the two anomic forms of the sugar.

The HPLC analysis, however, did not allow us to determine if the product seen in the HPLC in the absence of alkaline phosphatase treatment is D-glycero-D-mannoheptose 7-phosphate or D-glycero-D-mannoheptose 1-phosphate. The latter is the product of the second reaction in the biosynthetic pathway of ADP-D-glycero-D-mannoheptose which is catalyzed by phosphomutase (Fig. 7). However, since the phosphomutase reaction takes place after the formation of D-glycero-D-mannoheptose 7-phosphate and a peak corresponding to a phosphorylated D-glycero-D-mannoheptose is not present in the χ711 extract with sedoheptulose 7-phosphate (Fig. 5A) we conclude that it must correspond to D-glycero-D-mannoheptose 7-phosphate and the LpcA protein must be the phosphoheptose isomerase.

**DISCUSSION**

Sequencing and mapping of the cloned LpcA locus revealed a single gene, LpcA, that is located at 5.3 min (246 kb) on the
In this study, we provide genetic and biochemical evidence that LpcA is necessary for heptose biosynthesis of inner core lipopolysaccharide in E. coli. Eides and Osborn (28, 44), proposed a biosynthetic scheme for L-glycero-D-mannoheptose that uses the conversion of sedoheptulose 7-phosphate into ADP-L-glycero-D-mannoheptose. The four reactions needed for the synthesis of ADP-L-glycero-D-mannoheptose shown in Fig. 7, include: 1) conversion of sedoheptulose 7-phosphate to D-glycero-D-mannoheptose 7-phosphate by a phosphoheptose isomerase, 2) conversion of D-glycero-D-mannoheptose 7-phosphate to D-glycero-D-mannoheptose 1-phosphate by a phosphoheptose mutase, 3) conversion of D-glycero-D-mannoheptose 1-phosphate with ATP to ADP-D-glycero-D-mannoheptose and PPi, by an ADP-heptose synthase, and 4) racemization by an epimerase of ADP-D-glycero-D-mannoheptose to the L-isomer. The completed ADP-L-glycero-D-mannoheptose is then used for the transfer of its sugar moiety onto the inner core LPS by a specific transferase. The only genes of this pathway fully characterized to date are rfaD and rfaC, encoding the epimerase (45) and the transferase (46, 47), respectively. Work by Sirisena et al. (47) in Salmonella typhimurium suggests that rfaE encodes the ADP-heptose synthase since addition of ADP-glyceromannoheptose to cell extracts of rfaE mutants restores the synthesis of a complete core LPS, but a similar gene in E. coli has not been identified.

Our data demonstrate that LpcA restores the expression of a complete core LPS by the heptoseless mutant, E. coli strain χ711, and encodes the phosphoheptose isomerase used in the biosynthesis of ADP-L-glycero-D-mannoheptose. Biochemical evidence for a phosphoheptose isomerase includes: 1) appearance of a new product upon incubation with sedoheptulose 7-phosphate with a concomitant reduction of substrate concentration, 2) the new product is a phosphorylated sugar, 3) upon dephosphorylation, the product has the same HPLC retention time as authentic glycero-mannoheptose, 4) the new product does not appear in reactions with cell extracts of the LpcA mutant. In addition, a region of the LpcA protein has amino acid sequence homology with a family of aldo/keto isomerases.

Sedoheptulose 7-phosphate has been isolated from plants such as Sedum spectabile (48), and from animal tissues such as rat liver (49) and chicken muscle (50), as an intermediate in the nonoxidative portion of the pentose phosphate pathway (39). To date there are no reports of an isomerase in plants or animals that uses sedoheptulose 7-phosphate as a substrate. Thus, its conversion into D-glycero-D-mannoheptose 7-phosphate could be interpreted as a specialized branch of the pentose phosphate...
pathway for LPS synthesis. This branch is likely to be present in many if not all Gram-negative bacteria.

The degree of similarity of LpcA with the lincomycin biosynthetic gene product LmbN of S. lincolnensis, suggests that LmbN may encode an isomerase needed for the synthesis of this antibiotic. Chemical synthesis of 8-carbon sugar derivatives as potential intermediates leading to the production of methyl 6-amino-6,8-dideoxy-1-thio glycyryl isomerization step (51).

The fact that glyceromannheptose is a very common component of the inner core LPS of many enteric (52) and non- enteric bacteria (4) and the conservation of LpcA among enteric bacteria suggests that this gene has an essential function in a conserved pathway for ADP-l-glycerol-d-mannohexose synthesis. Although the LpcA homolog was not identified in Pseudomonas, we believe that this function does exist in this genus but lack of hybridization with the probe reflects the high hybridization stringency used in this experiment.

In general, genes necessary for synthesis of the conserved lipid A and inner core components are found scattered in the E. coli K12 chromosome: lipid A synthesis genes, and is close to the average G + C content, of the rfa cluster. The G content of LpcA is similar to the G + C content of the lipid A and 3-deoxy-d-manno- octulosonic acid pathway genes, rfaD and rfaC, and is close to the average G + C content for E. coli and other enteric bacteria. This conservation of G + C supports the suggestion that biosynthesis genes required for lipid A and inner core may have been part of a common enterobacterial genome, and that outer core biosynthesis genes, which have a lower G + C content, evolved later (4).

This study reports the first molecular characterization of a novel phosphoheptose isomerase in prokaryotes that uses sedoheptulose 7-phosphate as a substrate, and it is needed for the first reaction committed to the biosynthesis and assembly of inner core lipopolysaccharide in enteric bacteria. Our findings have relevance to the area of infection since bacterial strains; A. Higashitani forthecosmid pE4021; and B. Gordon, T. Viswanatha, and L. Marrone for assistance with the HPLC analyses.

Acknowledgments—We are grateful to B. Bachmann, D. Colby, R. Curtiss III, I. Lam, H. Lior, K. Sanderson, and G. Reid for the gifts of bacterial strains; A. Higashitani for the cosmid pE4021; and B. Gordon, T. Viswanatha, and L. Marrone for assistance with the HPLC analyses.

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J. Biol. Chem. 1996, 271:3608-3614.
doi: 10.1074/jbc.271.7.3608

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