The *rfaD* Gene Codes for ADP-L-glycero-D-mannoheptose-6-epimerase

AN ENZYME REQUIRED FOR LIPOPOLYSACCHARIDE CORE BIOSYNTHESE=* 

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The *rfaD* gene product, ADP-L-glycero-D-mannoheptose-6-epimerase, is necessary for the conversion of ADP-D-glycero-D-mannoheptose to ADP-L-glycero-D-mannoheptose. The nucleotide ADP-D-glycero-D-mannoheptose accumulates in *rfaD* mutant strains. Two chromic colicin E1 plasmids carrying the coding sequence of the *rfaD* locus have been selected and shown to complement the *rfaD* phenotype. These clones also result in an amplification of ADP-L-glycero-D-mannoheptose-6-epimerase activity.

Lipopolysaccharide is a major component of the outer membrane of Gram-negative bacteria. The outer membrane contains molecules larger than 700 in molecular weight (1) such as oligopeptides (2), hydrophobic antibiotics, and dyes (3). The barrier function can be breached if the lipopolysaccharide core or structure is altered by chemical treatment (4) or by mutation (5), respectively.

The lipopolysaccharide of *Escherichia coli* K12 consists of a core region and a core region. The core region consists of an inner core and outer core. The inner core is composed of 2-keto-3-deoxyoctulosonic acid, L-glycero-D-mannoheptose, phosphate, rhamnose, and ethanolamine. L-Glycero-D-mannoheptose is found to be a typical component of the lipopolysaccharide core region of many Gram-negative bacteria (6). Little is known about the biosynthetic steps leading to the synthesis of L-glycero-D-mannoheptose. Eidels and Osborn (7), using a transketolase mutant of *Salmonella typhimurium*, demonstrated a role for sedoheptulose-7-phosphate in lipopolysaccharide and aldolheptose biosynthesis. These results confirmed a role for sedoheptulose-7-phosphate in aldolheptose biosynthesis, as suggested earlier by Ginsburg et al. (8). Further, Eidels and Osborn (7) postulated a pathway for L-glycero-D-mannoheptose synthesis (Fig. 1) which predicted four enzymatic steps: an isomerase, a mutase, a nucleotide-diphosphate-phenolase, and an epimerase activity. Later, Eidels and Osborn (9) demonstrated the conversion of sedoheptulose-7-phosphate to D-glycero-D-mannoheptose-7-phosphate, thus demonstrating an isomerase activity equivalent to the first enzyme proposed in their biosynthetic scheme.

In a previous study (10) we demonstrated that in *E. coli* K12, a single site mutation, designated *rfaD*, resulted in increased permeability to a large number of hydrophobic drugs and dyes. Further, it was shown that the mutation resulted in an altered lipopolysaccharide that contained primarily an atypical heptose and reduced sugar content. Based on available data, it was concluded that the new heptose was most likely D-glycero-D-mannoheptose. Thus, the *rfaD* gene mutation represented a tool for studying aldolheptose biosynthesis.

Starting with the *rfaD* mutation, the present paper provides evidence in the wild type organism for an epimerase activity necessary for the D to L conversion of the sugar nucleotide derivative of glycero-mannoheptose. This report also demonstrates that in *rfaD* mutants (epimeraseless strains) ADP-D-glycero-D-mannoheptose accumulates. Thus, in the *rfaD* mutant one finds both an abnormal lipopolysaccharide (D-glycero-D-mannoheptose substituted for L-glycero-D-mannoheptose, and a markedly reduced sugar content) and accumulation of ADP-D-glycero-D-mannoheptose.

The above observation that an ADP-nucleotide accumulates in *rfaD* strains was recently and independently buttressed by the isolation of ADP-D-glycero-D-mannoheptose from R mutants of *Shigella sonnei* by Kontrohr and Kocsis (11). They also suggested a role for this nucleotide-diphosphate sugar in aldolheptose biosynthesis.

This paper further reports the identification of colicin E1 hybrid plasmids carrying the coding sequence for ADP-L-glycero-D-mannoheptose-6-epimerase from the Clarke-Carbon bank (12).

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—The *E. coli* K12 strains used in this study are listed in Table I. All cultures were grown aerobically in LB medium (13) or in the minimal media of Davis and Mingioli (14). Carbon sources (glucose or galactose) were added to a final concentration of 0.5%. Solid media were prepared by adding agar (1.5% final concentration) to the media described above. Cells were grown at 30 or 37 °C. Cultures were supplemented, where indicated, with 100 μg of required nutrients, 5 mM CaCl2, 100 μg of streptomycin sulfate, 14 μg of kanamycin sulfate, and 30 or 60 μg of novobiocin per ml. *E. coli* strains carrying hybrid plasmids were grown in the presence of sufficient amounts of colicin E1 to inhibit the growth of sensitive cells.

**Genetic Analysis**—Genetic transfers and miscellaneous techniques were based on procedures described by Miller (15). Transformations were done as described by Cohen et al. (16).

**Plasmid DNA Isolation**—Plasmid DNA was isolated following chloramphenicol amplification (17) using the alkaline extraction procedure described by Birnboim and Doly (18). Additional purification involved phenol extraction and recovery of supercoiled plasmid DNA from agarose gels as described by Tabak and Flavell (19).

**Analytical and Quantitative Techniques**—Agarose gel electrophoresis (0.7%) was carried out in a horizontal slab gel apparatus using a Tris-borate/EDTA buffer system (20). Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis was performed in the presence of urea (4 M) by the method of Laemmli (21). Lipopolysaccharide resolved in 15% sodium dodecyl sulfate-polyacrylamide slab gels was visualized by a silver staining method described by Tsai and Frasch (22). Note that the best resolution of the R mutant lipopolysaccharide samples is obtained using 12% sodium dodecyl sulfate gel and not the 14% sodium dodecyl sulfate gel described by Tsai and Frasch (22). Thin layer chromatography for nucleotides was performed on a Polygram CEL 300 PEI/UV254 anion exchanger. The developing solvent was LiCl (see individual experiments for concentration), and
**L. Glycerol-d-mannohexose-6-epimerase**

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\text{NTP, nucleotide triphosphate; NDP, nucleoside diphosphate.}
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**TABLE I**

| Strains        | Sex       | Genotype/phenotype | Source             |
|----------------|-----------|--------------------|--------------------|
| PL2            | HfrH      | galL28 thi-1 relA1 | M. Gottesman       |
| JCC411         | F         | metB1 lacY1 xyl7   | E. Garcia          |
|                |           | mtl2               |                    |
|                |           | galA hisI tonA2    |                    |
|                |           | tacs-1             |                    |
|                |           | rpsL104 supS9      |                    |
|                |           | lacE21             |                    |
| JM15           | F         | cryE50 tfr-8 thi   | M. Jones-Martiner  |
|                |           |                    | via CGSC*          |
| RHI           | F         | proA2 leuB6 thi-1 lacY1 | Bolivar et al. via E. |
|               |           | rpsL20 ara xyl hsd | Kline             |
|               |           |                     |                    |
| CL27          | F         | galE28 thi-1 relA1 rfaD | Ref. 10          |
|               |           | rpsL λ               |                    |
|               |           | (PStric100KM)        |                    |
| CL29          | F         | tfr-8 rfaD thi 1    | Ref. 10           |
| CL89          | F         | tfr8 rfaD thi-1 rpsL | This paper        |
| CL515         | F         | proA2 leuB6 thi-1 lacY1 | This paper        |
|               |           | rpsL20 ara rpsL hsd |                    |
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* The JM15 strain was obtained from Barbara J. Bachmann of the E. coli Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

**Fractions of 5 ml were collected, and the absorbance at 280 nm was determined. Samples of 0.1 ml were removed from every fifth fraction for radioactivity determination by scintillation counting. Radioactivity (4°C and 4°C) was found between fractions 230 and 259. The ultraviolet absorption spectra of the peak (e.g. 4°C and 4°C) fraction (244) were characteristic of an adenosine derivative. Fractions 230 to 259 were pooled and repeatedly lyophilized to remove ammonium formate. The lyophilized sample was resuspended in 2 ml of deionized H2O, and portions (0.5 ml) were applied onto Whatman No. 3 paper. The chromatograms were developed in 95% ethanol, 1 m ammonium acetate (52.5 v/v). Four UV absorbing spots were detected, two of which were found to be radioactive. The radioactive spots were eluted with deionized H2O, lyophilized, and resuspended in 70% ethanol for further purification (29).

**Preparation of Lipopolysaccharide Acceptors**—Bacterial cultures (100 or 300 ml of rfdD and wild type strains) were grown to midlog phase and harvested by centrifugation. The pellets were resuspended in 0.01 M Tris-HCl buffer (pH 8.0), containing 1 mM EDTA and 5 mM β-mercaptoethanol. These suspensions were passed through a French pressure cell at 12,000 psi and centrifuged at 10,000 x g for 20 min. The centrifugations and all subsequent procedures were performed at 4°C.

**Preparation of Lipopolysaccharide Acceptor Solution**—Several enzymes involved in lipopolysaccharide biosynthesis require a lipopolysaccharide acceptor and a phospholipid cofactor (30). The lipopolysaccharide acceptor was prepared as described by Creeger and Robertson (31) by mixing lipopolysaccharide from the rfdD strain CL29 (10) and crude lipid extract (32) from E. coli K12 strain PL2. This mixture (crude lipid [60 µmol of phosphate in 4 ml of methanol] plus lipopolysaccharide [7.0 µmol of 2-keto-3-deoxyoctulosonic acid]) was added to 20 µmol of EDTA, 4.65 µM of 10 mM Tris-HCl buffer (pH 8.5), and H2O, in a total volume of 20 ml. The mixture was heated for 30 min at 60°C, cooled to room temperature, and sonicated with three 0.5-min bursts at 15°C.

**Epimerase Assay**—The epimerase activity was assayed by the incorporation of [14C]glucose into lipopolysaccharide isolated from the rfdD strain CL29. In other words, enzyme activity was demonstrated by coupling the epimerase activity with subsequent incorporation of heptose and labeled glucose into lipopolysaccharide. The assumption is that the incomplete lipopolysaccharide chain(5) made by the rfaD mutant can serve as an acceptor for such an extension. The assay mixture contained 0.14 µM of the above lipopolysaccharide acceptor solution, 20 µmol of MgCl2, 34 µmol of NAD, 30 nmol of UDP-galactose, 30 nmol of UDP-[14C]glucose (3000 dpm/nmol), and a constant amount of an rfdD aqueous extract, in a total volume of 0.25 ml. The reaction was started by adding various amounts of rfdD to the rfdD strain after 20 min at 37°C with 3 ml of cold 5% trichloroacetic acid containing 1 mg/ml of UDP-glucose. Following filtration on Millipore filters, the radioactivity of scintillation materials was determined by liquid scintillation. [14C]Glucose was not incorporated from UDP-[14C]glucose in control experiments without lipopolysaccharide acceptor solution. Additional controls were performed which excluded rfdD extracts or rfdD extracts, or both, from the reaction mixture, which allowed for corrections for any residual epimerase activity associated with assay components.

**Identification of Plasmids Carrying the rfdD Gene**—The Clarke-Carbon colony bank (12) was screened for plasmids capable of abolishing the rfdD phenotype (e.g. hydrophobic antibiotic hypersensitiv-
ity. Transfer of colicin E1 hybrid plasmids was performed by F-factor-mediated transfer. Donor and recipient cells were mixed in a ratio of 1:3 at 2 x 10^9 cells/ml. After overnight incubation at 37 °C in Falcon plates, samples were transferred to LB plates containing 60 μg/ml of novobiocin and 100 μg/ml of streptomycin. Novobiocin- and streptomycin-resistant colonies resulted from 10 of the mating mixtures. These colonies were tested for colicin E1 resistance. Survivors were checked for the genetic marker of the recipient and an isolatable plasmid capable of complementing a rfaD strain (CL515) in transformation experiments. Two of these isolated plasmids (pLC13-13 and pLC32-45) were selected for further study.

Materials—[2,8,9-H]Adenosine (50 Ci/nmol) and [1,2,3,4,14C]-sedoheptulose-7-phosphate (175 mCi/nmol) were purchased from New England Nuclear. Polygram CEL 300 PEI/UV250 was obtained from Brinkman Instruments. 3% SP-2340 (silicone cyanopropyl) on 100/120 supelcoport was obtained from Supelco. Bio-Gel P2 (200–400 mesh) was obtained from Bio-Rad. Perspectol, L-glycerol-D-mannoheptitol, was obtained from Pfanstiehl. Perspectol was completely acetylated to hepta-O-acetyl-L-glycero-D-mannoheptitol. In this form it was used as the L-glycerol-D-mannoheptose standard. Hepta-O-acetyl-L-glycerol-D-mannoheptose and D-glycero-d-mannoheptose used as the D-glycerol-D-mannoheptose standards were obtained from Dr. M. B. Perry, National Research Council of Canada, Ottawa, Canada. GDP-D-galactose 7-phosphate was obtained from Dr. V. Ginsburg, National Institutes of Health. A filtered supernatant from an overnight mitomycin C (1.2 pg/ml)-induced colicinogenic strain (JC411/ColE1) was used as a source of crude colicin E1.

RESULTS

Previous gas-liquid chromatography and chemical studies of lipopolysaccharide from rfaD mutants demonstrated that the lipopolysaccharide contains primarily the stereoisomer, D-glycero-D-mannoheptose, rather than L-glycero-D-mannoheptose, and very little of the distal sugars (10). The identities of the sugar components were based on gas-liquid chromatographic behavior of alditol acetate derivatives of the lipopolysaccharide components and alditol acetate sugar standards on 5% ethylenesuccinate cyanosylsilicone copolymer (10). The resolution of the heptose components of rfaD mutant lipopolysaccharide by gas-liquid chromatography on a different stationary phase is shown in Fig. 2A (3% SP-2340). The mass spectra of the resolved heptose peaks are shown in Fig. 2B. A comparison of the mass spectrum of the authentic L-glycerol-D-mannoheptose is identical to the mass spectrum of the authentic D-epimer. (Fig. 2C) and the spectra of the resolved heptose peaks (Fig. 2B) confirms the presence of heptose in both peaks. Thus, the presence of both epimers of glycerol-mannoheptose in rfaD lipopolysaccharide was confirmed. The observation that the major heptose epimer was D-glycero-D-mannoheptose is consistent with the premise that d-glycero-D-mannoheptose is the precursor of L-glycero-D-mannoheptose via an epimerase activity (7). Thus, a mutation in the rfaD locus may result in the loss of an epimerase activity. An inversion of the configuration of a carbino group, catalyzed by epimerases, occurs at the sugar nucleotide level (33). In order to test this assumption, cultures of an isogenic rfaD mutant, CL503, were supplemented with [14C]sedoheptulose via an epimerase activity (7). Thus, a mutation in the rfaD locus may result in the loss of an epimerase activity. An inversion of the configuration of a carbino group, catalyzed by epimerases, occurs at the sugar nucleotide level (33).

FIG. 2. Gas-liquid chromatography and mass spectrometry of the alditol acetate derivatives of heptoses from the lipopolysaccharide of rfaD mutant CL29 and authentic L-glycero-D-mannoheptose. Gas chromatography conditions: Perkin-Elmer o 3; column 3% SP-2340; injector temperature 270 °C; column temperature programmed 10 °C/min (200–250 °C); helium flow, 20 ml/min. Mass spectrometer conditions: VG micromass 7070F GC inlet; source temperature 250 °C; electron energy 70 eV. Detector response is expressed in A as total ion current (e.g. over an electronic mass range of 20 to 650) and in B and C as relative intensity (e.g. relative to base fragment).

In 1.6 M LiCl, Acid hydrolysis (0.01 M HCl) of the unknown nucleotide resulted in the release of a nucleotide diphosphate with an Rf value equal to that of ADP (Rf = 0.6). When the UV spot with the Rf value of 0.77 (developed in 1.6 M LiCl) was sprayed with a urea phosphate solution, a heptose-specific reagent (26), a characteristic pink color appeared coincident with the UV spot. The sugar analysis of an acid-hydrolyzed sample (0.1 M HCl) of the unknown nucleotide, using the method of inversion of the configuration of a carbinol group, catalyzed by epimerases, occurs at the sugar nucleotide level (33).
method of Boykin and Liu (25), indicated the presence of ribose and d-glycero-d-mannoheptose. In addition, when this sample was chromatographed on polyethyleneimine plates using 1.6 M LiCl, the UV spot remained at the origin. Similarly, authentic adenine also failed to migrate when the plates were developed using 1.6 M LiCl. These results suggested that the sugar nucleotide was ADP-d-glycero-d-mannoheptose. The identification of the nucleotide was further corroborated by a double-labeling experiment and ultraviolet spectroscopy of the sugar nucleotide. When CL503 (rfaD, Pur-) cultures were subjected to electrophoresis at 20 mA/slab gel, V indicates the direction of sample migration. Sample wells A through C contained 1 μg of lipopolysaccharide, while sample well D contained 0.5 μg of lipopolysaccharide sample. The arrow indicates the position of a lipopolysaccharide band present in the lipopolysaccharide of wild type E. coli K12 strains. A, CL89 (rfaD); B, JM15 (wild type); C, CL89/pLC32-45 (rfaD'); D, CL89/pLC13-13 (rfaD').

Effect of the Hybrid Plasmids on the Lipopolysaccharide of rfaD Strains—Lipopolysaccharide was isolated from rfaD strain CL89 and the rfaD strain CL89 carrying the hybrid plasmid pLC13-13 or pLC32-45. The isolated lipopolysaccharide preparations were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by visualization with silver staining (22). As shown in Fig. 4, the migration of a large fraction of the lipopolysaccharide from CL89 (lane A) was characteristic of a structure smaller than the lipopolysaccharide containing a complete core region, present in lane B, which was isolated from a rfaD' strain, JM15. These results suggested that the major lipopolysaccharide structure present in the mutant strain, CL89, contained few core components. In contrast to CL89, an rfaD mutant strain (CL89) carrying pLC32-45 or pLC13-13 plasmids was found to synthesize a complete lipopolysaccharide structure. As shown in the chromatogram (Fig. 5), mutant lipopolysaccharide (CL89) contained rhamnose, glucose, p-glycero-d-mannoheptose, and a small amount of L-glycero-d-mannoheptose, which was reported previously (10). L-Glycero-d-mannoheptose was the only heptose present in the lipopolysaccharide of rfaD strains carrying the plasmid pLC32-45 or pLC13-13. Galactose was also a component of the lipopolysaccharide isolated from a plasmid-complemented rfaD strain.

FIG. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of lipopolysaccharide from rfaD, wild type, and plasmid-complemented rfaD strains. Lipopolysaccharide was prepared from 200-ml cultures. Samples of lipopolysaccharide were prepared in Laemmli's sample buffer, applied to sample wells, and subjected to electrophoresis at 20 mA/slab gel. V indicates the direction of sample migration. Sample wells A through C contained 1 μg of lipopolysaccharide, while sample well D contained 0.5 μg of lipopolysaccharide sample. The arrow indicates the position of a lipopolysaccharide band present in the lipopolysaccharide of wild type E. coli K12 strains. A, CL89 (rfaD); B, JM15 (wild type); C, CL89/pLC32-45 (rfaD'); D, CL89/pLC13-13 (rfaD').

FIG. 5. An analysis of the sugar content of lipopolysaccharides from rfaD and rfaD plasmid-complemented strains. The lipopolysaccharide samples were prepared from 200-ml LB broth cultures. The lipopolysaccharide samples were hydrolyzed in 0.1 M HCl for 48 h at 100 °C. The hydrolyzed samples were dissolved in 90% ethanol following neutralization and lyophilization. Aliquots (180 μl) were analyzed on the sugar analyzer. x indicates an unknown compound that is consistently observed. The peak labeled y is ribose, resulting from RNA contamination of the lipopolysaccharide samples. The peak labeled z is ribose, resulting from RNA contamination of the lipopolysaccharide samples.
Content of neutral sugar components in the lipopolysaccharides obtained from the rfaD mutant strain and the rfaD plasmid-containing strain

The quantitation of the sugar components was determined by chromatography of known amounts of authentic sugars on the sugar analyzer under the identical conditions used for experimental samples.

| Strain | Rhamnose | Glucose | Galactose | D, D-Hep-tose | L, D-Hep-tose |
|--------|----------|---------|-----------|--------------|--------------|
| CL89   | 24.2     | 77.6    | 0         | 49           | 18.8         |
| CL89/pLC32-45 | 23.8 | 208 | 32.2      | 0            | 97.6         |

a CL89 represents the rfaD mutant strain and CL89/pLC32-45 differs from the CL89 strain in that it contains the plasmid pLC32-45 which abolishes the rfaD phenotype.

The lipopolysaccharide samples from rfaD and rfaD plasmid-complemented strains were subjected to quantitative analyses (Table II). In addition, it was determined that the lipopolysaccharide of the rfaD strain, CL89, had a 2-keto-3-deoxyoctulosonic acid:heptose (i.e., the D and L isomer) ratio of 2:1, respectively. Finally, a higher than expected glucose content (Table II) was observed in lipopolysaccharide samples from rfaD and rfaD+ strains.

Fractionation of the Carbohydrate Moiety of rfaD Lipopolysaccharide by Gel Filtration—Following mild acid hydrolysis of rfaD lipopolysaccharide and removal of the water-insoluble lipid A fraction, the released water-soluble oligosaccharides were characterized by gel permeation on Bio-Gel P2. Four peaks were identified (Fig. 6) as carbohydrate-containing. The peak tube of each carbohydrate fraction was tested for the presence of 2-keto-3-deoxyoctulosonic acid. Peaks II, III, and IV were found to contain 2-keto-3-deoxyoctulosonic acid. Each of the pooled fractions was hydrolyzed and then subjected to sugar analysis on the sugar analyzer. The only sugar detectable in the early eluting carbohydrate peak (I) was glucose.1 Peak II, the major carbohydrate fraction, consisted of glucose, D-glycero-D-mannoheptose, 2-keto-3-deoxyoctulosonic acid, and rhamnose. The third peak contained glucose, D-glycero-D-mannoheptose, and 2-keto-3-deoxyoctulosonic acid. The qualitative analysis of peak IV showed the presence of glucose and 2-keto-3-deoxyoctulosonic acid only.

A fraction containing L-glycero-D-mannoheptose was not observed, although qualitative and quantitative data (Figs. 1 and 2 and Table II) presented above, strongly suggest its presence.

1 The early elution and sugar content of peak I suggest that the lipopolysaccharide samples were contaminated with the membrane-derived oligosaccharide described by Kennedy (34).

Fig. 6. Schematic presentation of the fractionation on Bio-Gel P2 of degraded polysaccharides obtained from the rfaD mutant, CL29. Samples (100 µl) were tested with phenol-sulfuric acid (28) and absorbance was measured at 490 nm.

ADP-L-glycero-D-mannoheptose-6-epimerase Activity—In Fig. 7 a relationship between the rfaD gene product and the activity of ADP-L-glycero-D-mannoheptose-6-epimerase in rfaD+ extracts is suggested. In these experiments the presence
of an epimerase activity was demonstrated by coupling the epimerase activity with subsequent incorporation of heptose and labeled sugars into lipopolysaccharide. Very little epimerase activity was detected in the rfaD mutant strain, CL89. In contrast to the low activity in CL89, significant activity was present in a wild type strain, PL2. Dramatic increases in the epimerase activity were observed in rfaD strains carrying the hybrid plasmid, pLC32-45 or pLC13-13.

The substrate specificity of the epimerase activity was demonstrated by experiments in which ADP-D-glycero-D-mannoheptose or GDP-D-glycero-D-mannoheptose (8) was added as the substrate. As shown in Fig. 8, the addition of increasing amounts of ADP-D-glycero-D-mannoheptose resulted in a stimulation of the incorporation of [14C]glucose into trichloroacetic acid-insoluble material. In contrast, no effect was observed with GDP-D-glycero-D-mannoheptose.

**DISCUSSION**

The biosynthesis of L-glycero-D-mannoheptose in Gram-negative bacteria has been postulated to occur by racemization of D-glycero-D-mannoheptose to the L isomer (7). However, the enzymatic activity responsible for this conversion remained unclear. A possible tool to investigate this process became available when a mutant designated rfaD negative bacteria has been postulated to occur by racemization of D-glycero-D-mannoheptose to L-glycero-D-mannoheptose. It was demonstrated that a mutation in the epimerase gene, L-glycero-D-mannoheptose-6-epimerase (gene product), which is involved in the conversion of D-glycero-D-mannoheptose to L-glycero-D-mannoheptose. It was demonstrated that the enzyme responsible for this conversion became available when a mutant designated rfaD was isolated which contained a defective lipopolysaccharide with an abnormal heptose (10). The present communication presents evidence for the existence of a specific epimerase activity (an rfaD gene product), which is involved in the conversion of D-glycero-D-mannoheptose to L-glycero-D-mannoheptose. It was demonstrated that a mutation in the epimerase gene, i.e., the rfaD mutation, resulted in the loss of the epimerase activity, predominant an incomplete lipopolysaccharide structure which terminates with the heptosyl residue, D-glycero-D-mannoheptose, and the accumulation of the nucleotide, ADP-D-glycero-D-mannoheptose, in the extracts of these mutants. Recently and independently, Kontrohr and Kocsis (11) demonstrated the presence of ADP-D-glycero-D-mannoheptose in a S. sonnei R mutant that incorporates D-glycero-D-mannoheptose in its lipopolysaccharide. They also suggested an intermediate role for this sugar nucleotide in aldehydeptose biosynthesis in Enterobacteria. The identification of ADP-D-glycero-D-mannoheptose and a substrate role for this nucleotide in E. coli lipopolysaccharide core biosynthesis support their contention.

Further, colicin E1 hybrid plasmids were selected which complemented the rfaD phenotype, and amplified the ADP-L-glycero-D-mannoheptose-6-epimerase activity. In addition, only wild type lipopolysaccharide core structure was present in plasmid containing rfaD mutant strains.

The results presented here provide support for the proposed scheme of Eidel and Osborn (7) (i.e. steps 3 and 4 in Fig. 1) for L-glycero-D-mannoheptose biosynthesis (Scheme 1).

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