

Biosynthesis of Membrane-Derived Oligosaccharides: Characterization of *mdoB* Mutants Defective in Phosphoglycerol Transferase I Activity

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Received 5 July 1984/Accepted 30 August 1984

Phosphoglycerol transferase I, an enzyme of the inner, cytoplasmic membrane of *Escherichia coli*, catalyzes the *in vitro* transfer of phosphoglycerol residues from phosphatidylglycerol to membrane-derived oligosaccharides or to the model substrate arbutin (*p*-hydroxyphenyl- β -D-glucoside). The products are a phosphoglycerol diester derivative of membrane-derived oligosaccharides or arbutin, respectively, and *sn*-1,2-diglyceride (B. J. Jackson and E. P. Kennedy, *J. Biol. Chem.* 258:2394-2398, 1983). Because this enzyme has its active site on the outer aspect of the inner membrane, it also catalyzes the transfer of phosphoglycerol residues to arbutin added to the medium (J.-P. Bohin and E. P. Kennedy, *J. Biol. Chem.* 259:8388-8393, 1984). When strains bearing the *dgk* mutation, which are defective in the enzyme diglyceride kinase, are grown in medium containing arbutin, they accumulate large amounts of *sn*-1,2-diglyceride, a product of the phosphoglycerol transferase I reaction. Growth is inhibited under these conditions. A further mutation in such a *dgk* strain, leading to the loss of phosphoglycerol transferase I activity, should result in the phenotype of arbutin resistance. We have exploited this fact to obtain strains with such mutations, designated *mdoB*, that map near min 99. Such mutants lack detectable phosphoglycerol transferase I activity, cannot transfer phosphoglycerol residues to arbutin *in vivo*, and synthesize membrane-derived oligosaccharides devoid of phosphoglycerol residues. These findings offer strong genetic support for the function of phosphoglycerol transferase I in membrane-derived oligosaccharide biosynthesis.

Membrane-derived oligosaccharides (MDO) are periplasmic constituents of *Escherichia coli* and other gram-negative bacteria (16). They are a family of oligosaccharides containing 6 to 10 glucose units joined by β 1 \rightarrow 2 and β 1 \rightarrow 6 linkages and are variously substituted with succinic acid O-ester, phosphoethanolamine, and *sn*-1-phosphoglycerol residues (15, 17). Their synthesis is under strict osmotic regulation, occurring at high rates in media of low osmolarity (6). An understanding of the enzymology of MDO biosynthesis is therefore of interest because of the light it may shed on osmotic adaptation and the possible role of osmotic-signaling substances in *E. coli*.

A tentative working model of MDO biosynthesis is shown in Fig. 1. A glucosyl transferase system described by Weissborn and Kennedy (18) elongates β 1 \rightarrow 2-linked polyglucose chains. It requires UDP-glucose and a heat-stable protein derived from the cytoplasm, as well as a membrane fraction, for activity. Mutants in the *mdoA* locus, mapped by Bohin and Kennedy (1) near min 23 on the *E. coli* chromosome, are defective in the membrane component of this system. Such mutants are also blocked at an early step in the biosynthesis of MDO *in vivo*, offering strong genetic evidence that the glucosyl transferase system catalyzes an essential step in the biosynthesis of MDO.

Phosphoglycerol transferase I, an enzyme of the inner membrane discovered by Jackson and Kennedy (5), catalyzes the transfer *in vitro* of phosphoglycerol residues from phosphatidylglycerol to MDO or to the model substrate arbutin (Fig. 2). The *sn*-1,2-diglyceride that is the product of this reaction is normally phosphorylated by the enzyme

diglyceride kinase in a salvage reaction leading to the formation of phosphatidic acid, which then can be used for the synthesis of cellular phospholipids.

Mutants in the *dgk* gene, described by Raetz and Newman (11), are defective in diglyceride kinase. A study of one such mutant strain, RZ60 *dgk*-6, revealed a greatly reduced ability to grow in media of low osmolarity. Under this condition, MDO synthesis is maximal and large amounts of diglyceride accumulate.

Bohin and Kennedy (2) found that the active site of phosphoglycerol transferase I is on the outer aspect of the inner membrane, as shown by the fact that the enzyme catalyzes the transfer of phosphoglycerol residues to arbutin added to the medium. This β -glucoside is not taken up by the *E. coli* K-12 wild-type at the *bglR* locus (13).

This finding led us to predict that strain RZ60 *dgk*-6 should show the phenotype of arbutin sensitivity because the presence of arbutin in the medium should lead to the formation of large amounts of diglyceride, even in media of high osmolarity. We have found that strain RZ60, generously made available to us by C. R. H. Raetz, is indeed arbutin sensitive and have exploited this fact to isolate arbutin-resistant mutants, defective in a locus we designate *mdoB*. These mutants lack detectable activity of phosphoglycerol transferase I.

In this paper we describe the mapping and biochemical characterization of the *mdoB* mutation.

MATERIALS AND METHODS

Bacteria, bacteriophages, and growth conditions. Table 1 lists the bacteria and bacteriophages used. Unless indicated, bacteria were incubated on rotary shakers at 37°C in LB, M63, or LOS (6) medium. Supplements, except thiamine at 2 μ g/ml, were added as suggested by Curtiss (3). Solid media

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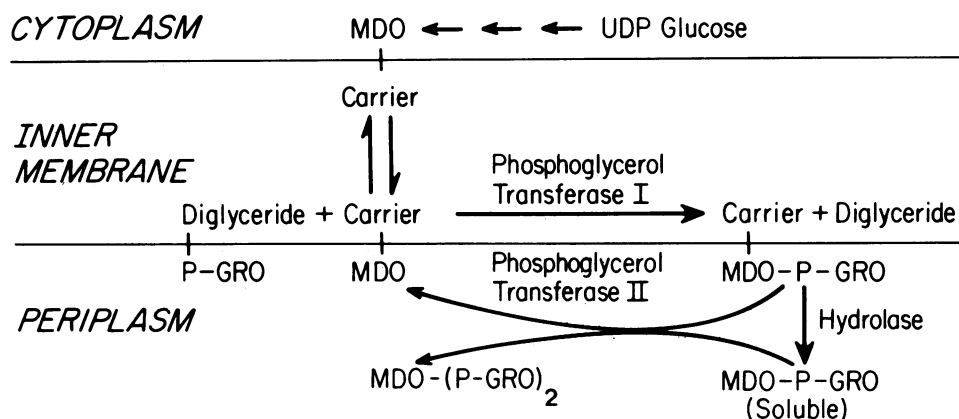


FIG. 1. Working model of MDO biosynthesis. Abbreviation: P-GRO, phosphoglycerol.

contained 15 g of agar per liter. Lysate preparation, P1 *vir* transduction, and bacterial conjugation were done by the method of Miller (10).

Isolation of mutants. Mutagenesis with Tn10 has been described (8). After mutagenesis of strain RZ60 with Tn10 via the λ 440 vector, the cells were concentrated and immediately plated on LB agar containing 20 μ g of tetracycline per ml so that each colony would be derived from a separate insertional event. For mutagenesis with UV light, we grew strain RZ60 to mid-log phase in LOS medium and then harvested the cells and suspended them in M63 salts. The cells were stirred in a 10-cm petri dish and irradiated for 90 s with a 115-V, 5-A Mineralight model R-51 (Ultra-Violet Products) placed 30 cm from the dish. The irradiated cells were transferred to sterile tubes wrapped with foil to prevent exposure of the cells to light and repair of mutations through photoreactivation. After overnight incubation to permit expression of phenotypes, the cells were subjected to three serial transfers into LOS medium containing 100 mM NaCl and 17 mM arbutin. These cultures were incubated at 45°C. Portions were subsequently plated on LOS agar containing 90 mM arbutin and incubated at 42°C. Rapidly growing clones were inoculated by toothpick onto two plates of the same medium and incubated at 30 or 42°C.

Preparation of extracts for phosphoglycerol transferase I assays. Cells were suspended in 0.05 M Tris-hydrochloride buffer (pH 7.8) and either broken at 1,100 atm (1.01×10^5 kPa) in an Aminco French press or sonicated. A 1- to 2-ml sample, cooled in an ice-water bath, was subjected to sonic

irradiation by an MSE ultrasonic disintegrator (100 W) with a 0.75-inch (1.9-cm) probe for a total sonication time of 1 to 2 min. Unbroken cells were removed by centrifugation at $3,200 \times g$ for 10 min. For assays with isolated membranes, the crude sonicated material was centrifuged again, at $100,000 \times g$ for 0.5 to 1 h. The membranes pelleted by this procedure were resuspended in the same buffer with a Dounce homogenizer.

Phosphoglycerol transferase I assay. The *in vitro* assay (5) involves transfer of phosphoglycerol from labeled phosphatidylglycerol to a synthetic β -glucoside. The *in vivo* assay depends on labeling of the phosphatidylglycerol by [$2\text{-}^3\text{H}$]glycerol in intact cells and subsequent transfer of the radiolabel to arbutin. It was performed as described by Bohin and Kennedy (2).

Phosphoglycerol transferase II assay. Phosphoglycerol transferase II was assayed for its hydrolytic activity in liberating phosphoglycerol from MDO as described by Goldberg et al. (4).

Analysis of lipid composition. Cells were grown in LOS medium containing [$2\text{-}^3\text{H}$]glycerol (0.5 mM; 4,200 cpm/nmol) and 35 mM NaCl. At mid-log phase, 5-ml cultures were harvested by centrifugation and suspended in 2 ml of chloroform-methanol-water (1:2:0.8 [vol/vol/vol]) in preparation for extraction and analysis of lipid as previously described (5). Mole percentages of lipid species were calculated from the amount of radioactivity in each lipid on the basis of chromatographic analysis and the known specific activity of the glycerol that was incorporated into these lipids. Diglycer-

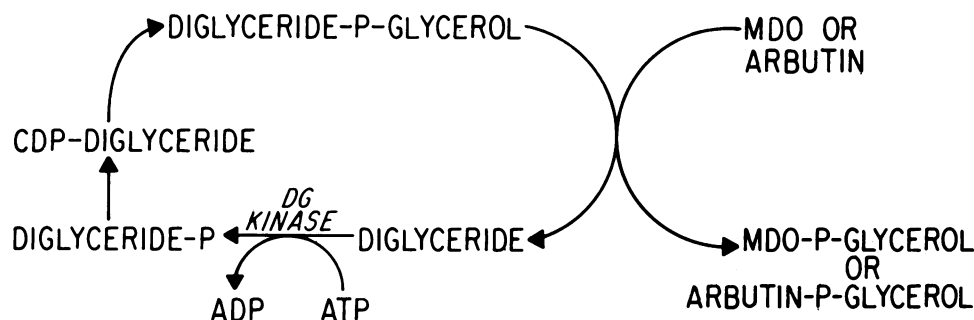


FIG. 2. Diglyceride cycle. From this cycle, one may predict that a *dgk* strain should accumulate large amounts of diglyceride when grown in medium supplemented with arbutin.

TABLE 1. Bacterial strains and bacteriophage stocks

Strain or stock	Relevant marker	Source
Bacterium		
JB1	<i>uxuA1</i>	CGSC ^a
CD4	HfrC	CGSC
KL14	Hfr (point of origin: PO68)	CGSC
PC0950	<i>thr-25 serB28</i>	CGSC
AB1133	F ⁻ <i>thr-1 rpsL31</i>	CGSC
RZ60	HfrH <i>dgk-6</i>	C. Raetz
NFB101	<i>mdoB1</i> derivative of RZ60	This work
NFB106	<i>mdoB6</i> derivative of RZ60	This work
NFB114	<i>mdoB1 dgk+</i> transconjugant of NFB101 × AB113	This work
PT114	<i>mdoB14</i> derivative of RZ60	This work
PT214	<i>mdoB::Tn10</i> derivative of RZ60	This work
PT227	Tet ^r transductant of AB1133 (PT214 donor)	This work
Bacteriophage		
P1 <i>vir</i>		J. Beckwith
λ440	<i>b221 c1857 cIII::Tn10 Oam29</i>	J. Beckwith

^a CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

ide and cardiolipin comigrate in the system used for resolving phospholipids. Therefore, cardiolipin content was calculated after correcting for the percentage of total radioactivity that was accounted for by neutral lipid in the region of the plate containing cardiolipin.

Analysis of MDO. Cells (0.15 to 10 liters) were grown to mid-log phase in LOS medium and harvested by centrifugation. Cell pellets were extracted in a final concentration of 50% (vol/vol) ethanol, taking into account that water makes up 80% of cellular wet weight. The extract was centrifuged at $3,200 \times g$, and the pellet was extracted once more. Pooled supernatants (ca. 100 ml after extraction of 10 liters of culture) were filtered through glass wool and concentrated on a rotary evaporator to ca. 5 ml. This concentrate was centrifuged at $100,000 \times g$ for 1 h to clarify it before applying it to a Bio-Gel P-6 column (37 by 2.8 cm) or a Sephadex G-25 column (1.1 by 50 cm). The column was eluted with 0.05 M ammonium formate in 7% 1-propanol (vol/vol) or with 7% 1-propanol (vol/vol) at a flow rate of 15 ml/h. Fractions (1 to 5 ml) were analyzed for content of sugar (and therefore MDO) colorimetrically, with either the anthrone reagent or the phenol-sulfuric acid procedure. The amount of phosphoglycerol in the MDO was determined enzymatically with glycerokinase (7) after glycerol was liberated from the MDO by hydrolysis with hydrofluoric acid. Samples containing ca. 0.1 mg of anthrone-reactive material or known amounts of glycerol were dried in polyethylene tubes. Samples were suspended in 0.2 ml of concentrated HF (29 N) and held for 16 h at 20°C, after which the HF was removed in an air jet and the samples were analyzed by the glycerokinase method. As expected, free glycerol was not detected in MDO samples that were not treated with HF (data not shown). Glycerol that was added to those MDO samples yielding none upon hydrolysis was readily detected (data not shown). Calculations of the number of moles of phosphoglycerol per mole of MDO were based on a glucose content of 10 residues per mol of MDO.

Protein determination. The protein content was determined by the method of Lowry et al. (9). We used bovine serum albumin as the standard.

RESULTS

Isolation of an *mdoB::Tn10* mutant. Figure 2 outlines the rationale for the prediction that RZ60 *dgk-6* should accumu-

late large amounts of diglyceride in medium supplemented with arbutin and that its growth should therefore be inhibited. We tested this prediction by growing strain RZ60 both in liquid medium and on agar plates containing high concentrations of arbutin (15 mM or higher) and found that the presence of arbutin indeed strongly reduced the rate of growth. Thus, after 2 days of growth on agar plates containing arbutin, clones of strain RZ60 did not exceed 0.2 mm in diameter, whereas derivatives of strain RZ60 which lacked phosphoglycerol transferase I activity formed colonies 1 to 2 mm in diameter.

To search for derivatives of strain RZ60 blocked in phosphoglycerol transferase I, we first mutagenized strain RZ60 with Tn10. Tn10 is an insertional element with genes for tetracycline resistance. We plated portions of the mutagenized culture on LB agar containing 20 µg of tetracycline per ml to permit growth only of clones carrying Tn10 insertions. Next, we replica plated Tet^r colonies onto LOS agar containing 90 mM arbutin to detect clones capable of growing more rapidly than the parental strain in the presence of arbutin. Lysates were made from Tet^r clones which grew rapidly when replica plated onto arbutin-containing medium. These were used to transduce two recipient strains, PC0950 and KL14, to tetracycline resistance. Transductants were then assayed for phosphoglycerol transferase I activity in vivo or in vitro. Whenever strain PT214, an Arb^r Tet^r derivative of strain RZ60, was used as donor, transduction to Tet^r abolished phosphoglycerol transferase I activity as measured in both assays (Table 2). Intact cells whose lipids had been labeled by growth on [2-³H]glycerol no longer transferred radioactivity to arbutin, and isolated membranes from these cells no longer catalyzed transfer of phosphoglycerol from radioactive phosphatidylglycerol to *o*-nitrophenyl-β-D-glucoside in vitro. We conclude that the Tn10 element in strain PT214 has inserted into a locus that affects phosphoglycerol transferase I, and we designate this locus *mdoB*.

Genetic mapping of the *mdoB* locus. Strain PT227 (F⁻ *mdoB::Tn10*) was derived by P1 transduction of strain AB1133 to Tet^r with a lysate of strain PT214. PT227 was then used as the recipient in conjugation experiments with Hfr strains. In such matings, recombination with the donor *mdoB*⁺ locus produces Tet^s recombinants. Such experiments localized *mdoB* to a region of the chromosome between the origins of HfrC and strain KL14. Gradient-of-transmission data obtained from *leu*⁺ Str^r transconjugants of the HfrC × PT227 cross indicated that *mdoB* was located between *thr* and *argE* (data not shown).

TABLE 2. Activity of phosphoglycerol transferase I in *mdoB* mutants^a

Strain	Phosphoglycerol transferase I activity	
	Amt of P-GRO transferred in vivo (cpm)	Amt of P-GRO transferred (nmol/h per mg of protein)
PC0950 <i>dgk+</i> <i>mdoB</i> ⁺	18,000	0.42
KL14 <i>dgk+</i> <i>mdoB</i> ⁺	17,000	ND ^b
RZ60 <i>dgk-6</i> <i>mdoB</i> ⁺	2,500	ND
PT214 <i>dgk-6</i> <i>mdoB::Tn10</i>	0	ND
7 Tet ^r transductants of PC0950	0	<0.047
2 Tet ^r transductants of KL14	0	<0.047

^a Activity of the enzyme in vivo and in vitro was measured as described in the text.

^b ND, Not determined.

TABLE 3. Linkage between Tet^r and markers near min 99 in strain PT214^a

Recipient strain	Selected marker	Unselected marker	Cotransduction frequency
JB1 <i>uxuA1</i> Tet ^s	Tet ^r	<i>uxuA</i> ⁺	0.06
JB1	<i>uxuA</i> ⁺	Tet ^r	0.06
PC0950 <i>thr-25</i> <i>serB28</i> Tet ^s	Tet ^r	<i>thr</i> ⁺	0.36
PC0950	Tet ^r	<i>serB</i> ⁺	0.56
PC0950	<i>thr</i> ⁺	Tet ^r	0.36
PC0950	<i>thr</i> ⁺	<i>serB</i> ⁺	0.82

^a The donor in all transductions was strain PT214 *mdoB::Tn10* *uxuA*⁺ *serB*⁺ *thr*⁺. At least 100 transductants were screened for each cross.

To investigate linkage of *mdoB* to markers near min 99, we transduced various mutations mapping in the interval between min 97 and 100 to the wild type with a P1 lysate of strain PT214 (Table 3). The *mdoB* locus was closely linked to *serB* and less closely linked to *thr*. In three-factor crosses via P1 transduction with the recipient strain PC0950 and the donor strain PT214, the data were consistent with the gene order *mdoB serB thr* in the clockwise direction (Table 4).

Function of phosphoglycerol transferase I in the glycerophosphorylation of MDO in vivo. If phosphoglycerol transferase I does indeed transfer phosphoglycerol residues from phosphatidylglycerol to MDO in living cells, as proposed by Jackson and Kennedy (5), then the *mdoB* mutation should lead to the production of MDO devoid of phosphoglycerol residues. This was found to be true (Table 5) for both the *mdoB::Tn10* mutation and for the *mdoB1* allele, a spontaneous mutation described below.

Other *mdoB* alleles. The selection for arbutin-resistant derivatives of strain RZ60 *dgk-6* is sufficiently powerful that prior treatment with Tn10 or other mutagens is not needed. Several spontaneous *mdoB* mutants were isolated; in each case, the mutant strain simultaneously lost activity in the phosphoglycerol transferase I assay in vitro and lost the ability to transfer phosphoglycerol residues to arbutin in vivo.

The *mdoB-1* allele was transferred from one such spontaneous mutant into the recipient strain AB1133 via a 20-min conjugation, producing strain NFB114 *mdoB1* *dgk*⁺. This construction was useful because phosphoglycerol transferase I activity in *dgk* strains cannot be measured readily by the in vitro assay (see Table 2). Strain NFB114 was tested for the content of phosphoglycerol in its MDO. It was found to contain less than 3% of that of wild type.

TABLE 4. Three-factor analyses of *mdoB::Tn10* *thr-25* *serB28*^a with recipient strain PC0950 and donor strain PT214

Selected marker	Unselected marker	Total no. of each
<i>thr</i> ⁺	<i>serB</i> ⁺ Tet ^r	35
<i>thr</i> ⁺	<i>serB</i> ⁺ Tet ^s	38
<i>thr</i> ⁺	<i>serB28</i> Tet ^r	1
<i>thr</i> ⁺	<i>serB28</i> Tet ^s	26
Tet ^r	<i>thr</i> ⁺ <i>serB</i> ⁺	35
Tet ^r	<i>thr</i> ⁺ <i>serB28</i>	1
Tet ^r	<i>thr-25</i> <i>serB</i> ⁺	21
Tet ^r	<i>thr-25</i> <i>serB28</i>	43

^a A P1 lysate of strain PT214 *mdoB::Tn10* *thr*⁺ *serB*⁺ was used to transduce strain PC0950 *mdoB*⁺ *thr-25* *serB28* Tet^s to *thr*⁺ or Tet^r, and the transductants were scored for inheritance of the unselected markers.

TABLE 5. Effect of *mdoB* mutations on MDO composition^a

Strain	Phosphoglycerol transferase I activity (nmol/h per mg of protein)	mol of P-GRO per mol of MDO
AB1133 <i>mdoB</i> ⁺	6.0	2.0
NFB114 <i>mdoB1</i>	<0.05	0.06
PT227 <i>mdoB::Tn10</i>	<0.09	0.05

^a Assay of phosphoglycerol transferase I and isolation of MDOs was performed as described in the text. The glucose content of MDOs purified by chromatography was determined colorimetrically. Phosphoglycerol content was assayed after treatment of MDO with HF to liberate glycerol, the amount of which was determined by use of glycerokinase and [γ -³²P]ATP.

The map position of an UV-induced mutation in strain PT114 *dgk-6* *mdoB-14* *thr*⁺ (Table 1) was studied. The recipient strain PC0950 *thr*⁺ was transduced with a lysate of PT114, and *thr*⁺ transductants were selected. Crude sonicated material, prepared from cultures of eight *thr*⁺ transductants, was assayed for phosphoglycerol transferase I activity in vitro. No activity was detected in four of the eight transductants. The *mdoB-14* mutation is therefore at a location near the *thr* locus, close to or identical with that of the *mdoB::Tn10* mutation.

Lipid composition of *mdoB* mutants. Strain RZ60 *dgk-6* grows poorly in media of low osmolarity (11). This condition favors maximal rates of MDO synthesis and hence maximal transfer of phosphoglycerol residues to MDO, with concomitant production of diglyceride. It might therefore be supposed that *mdoB* derivatives of strain RZ60, blocked in the transfer of phosphoglycerol, would accumulate less diglyceride. This, however, is not true (Table 6). In the absence of arbutin stress, no striking difference could be detected between the levels of neutral lipid in two *mdoB* strains, NFB101 and NFB106, compared with that in the parental strain RZ60.

Activity of phosphoglycerol transferase II in *mdoB* mutants. Fig. 1 shows the proposed functions of the two phosphoglycerol transferases that are thought to participate in MDO biosynthesis. Since they catalyze related reactions in the same biosynthetic pathway, both enzymes might be affected by the *mdoB* mutation. To test this hypothesis, we assayed the isogenic strains AB1133 *mdoB*⁺ and PT227 *mdoB::Tn10* for phosphoglycerol transferase II. This was done by growing the cells in LOS medium, suspending the harvested cells in 0.01 M Tris-hydrochloride buffer (pH 7.8) containing 4

TABLE 6. Lipid composition of strains RZ60, NFB101, and NFB106^a

Lipid ^b	Amt of lipid (mol%) in strain:		
	RZ60 <i>dgk-6</i> <i>mdoB</i> ⁺	NFB101 <i>dgk-6</i> <i>mdoB1</i>	NFB106 <i>dgk-6</i> <i>mdoB6</i>
Total lipid			
Neutral lipid	7	6	5
Phospholipid	93	94	95
Phospholipid			
PE	78	76	74
PG	17	19	21
CL	5	5	6

^a Cells were grown in LOS medium with [2-³H]glycerol, and their lipids were analyzed as described in the text. Values represent the average of two experiments. The PG value comprises both PG and lyso-PG.

^b Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin.

mM 2-mercaptoethanol, and sonicating them as described above. The activity of phosphoglycerol transferase II was then measured by the hydrolase assay (4). As described above, MDO labeled with [2-³H]glycerol were used as the substrate. The assay revealed that the *mdoB* mutation does not affect phosphoglycerol transferase II. Its specific activity was 0.31 U/mg of protein per h in strain AB1133 and 0.33 U/mg of protein per h in strain PT227.

DISCUSSION

Phosphoglycerol transferase I catalyzes the transfer of phosphoglycerol residues from phosphatidylglycerol to soluble forms of MDO only poorly and at high concentrations of MDO (5). This finding is consistent with the postulate that a carrier-bound form (Fig. 1) of MDO, not soluble forms, is the true physiological substrate. In contrast, model substrates with the β -glucoside structure found in MDO, but with hydrophobic, aromatic aglycones, are effectively utilized by the enzyme. For this reason, studies of the activity of phosphoglycerol transferase I in vivo (2) and in vitro (5) have focused on model substrates, particularly arbutin.

Genetic evidence reported in this paper now strongly suggests that phosphoglycerol transferase I does indeed transfer phosphoglycerol residues to MDO in vivo. Mutants at the *mdoB* locus simultaneously lose enzyme activity as assayed in vitro, the ability to transfer phosphoglycerol to arbutin in vivo, and the ability to glycerophosphorylate MDO.

The soluble, periplasmic phosphoglycerol transferase II described by Goldberg et al. (4) does not utilize phosphatidylglycerol as a phosphoglycerol donor, but it does catalyze the interchange of phosphoglycerol residues among soluble species of MDO. The present study confirms the hypothesis that phosphoglycerol transferase II is not involved in the primary transfer of phosphoglycerol residues from phosphatidylglycerol to MDO. Strains with the *mdoB* mutation contain active phosphoglycerol transferase II, but their MDO do not contain phosphoglycerol residues. It is the loss of phosphoglycerol transferase I that blocks the utilization of phosphatidylglycerol.

As shown in Fig. 1, phosphoglycerol transferase II is postulated to catalyze the transfer of phosphoglycerol residues from carrier-bound MDO to soluble species, giving rise to multiply substituted MDO in the periplasm. If this is true, one would predict that mutants lacking phosphoglycerol transferase II should produce MDO containing a maximum of 1 phosphoglycerol residue per mol.

Rotering et al. (14) have recently described an autoradiographic procedure for isolating mutants lacking phosphoglycerol in their MDO. Although the enzymic lesions in the strains have not yet been identified by these workers, their collection probably includes *mdoB* mutants.

The rationale for our selection of mutants defective in phosphoglycerol transferase I (Fig. 2) is based on the assumption that the transfer of phosphoglycerol residues to arbutin from phosphatidylglycerol should result in the accumulation of diglyceride and inhibition of growth of strain RZ60 *dgk-6*. This indeed proved to be the case. Surprisingly, however, in the absence of arbutin the *mdoB* derivatives of strain RZ60 accumulated about as much diglyceride as the parental strain. Clearly, reactions other than that catalyzed by phosphoglycerol transferase I contribute to the accumulation of diglyceride in the *dgk* strain. If transfer of phosphoethanolamine residues from phosphatidylethanolamine to MDO is occurring, that reaction should also generate diglyceride. We are presently exploring the possibility that

this is a major source of diglyceride in the *mdoB dgk-6* strains. Raetz and Newman (12) introduced the *pgi-2* mutation into a *dgk-6* strain so that it would synthesize MDO only when supplemented with exogenous glucose. Without glucose supplementation, with no MDO synthesis, the cells contained 2.4% neutral lipid; with glucose supplementation they contained 7.0% neutral lipid. These results suggest that the principal, but not the sole, source of diglyceride is the MDO biosynthetic pathway.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants GM18822 and GM22057 from the National Institute of General Medical Sciences and Public Health Service grant GM26625 from the National Institutes of Health. J.-P.B. is Chargé de Recherche at the French Centre National de la Recherche Scientifique. He received partial support from the NATO Science Fellowship Program.

We acknowledge the helpful gifts of bacterial strains from B. Bachmann, J. Beckwith, and C. R. H. Raetz.

LITERATURE CITED

1. Bohin, J.-P., and E. P. Kennedy. 1984. Mapping of a locus (*mdoA*) that affects biosynthesis of membrane-derived oligosaccharides in *Escherichia coli*. J. Bacteriol. 157:956-957.
2. Bohin, J.-P., and E. P. Kennedy. 1984. Regulation of the synthesis of membrane-derived oligosaccharides in *Escherichia coli*. J. Biol. Chem. 259:8388-8393.
3. Curtiss, R., III. 1981. Gene transfer, p. 243-264. In P. Gerhardt (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
4. Goldberg, D. E., M. K. Rumley, and E. P. Kennedy. 1981. Biosynthesis of membrane-derived oligosaccharides: a periplasmic phosphoglycerol transferase. Proc. Natl. Acad. Sci. U.S.A. 78:5513-5517.
5. Jackson, B. J., and E. P. Kennedy. 1983. The biosynthesis of membrane-derived oligosaccharides: a membrane-bound phosphoglycerol transferase. J. Biol. Chem. 258:2394-2398.
6. Kennedy, E. P. 1982. Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 79:1092-1095.
7. Kennedy, E. P., M. K. Rumley, H. Schulman, and L. M. G. van Golde. 1976. Identification of *sn*-glycero-1-phosphate and phosphoethanolamine residues linked to the membrane-derived oligosaccharides of *Escherichia coli*. J. Biol. Chem. 251:4208-4213.
8. Kleckner, N., J. Roth, and D. Botstein. 1977. Genetic engineering in vivo using translocatable elements: new methods in bacterial genetics. J. Mol. Biol. 116:125-159.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
10. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. Raetz, C. R. H., and K. F. Newman. 1978. Neutral lipid accumulation in the membranes of *Escherichia coli* mutants lacking diglyceride kinase. J. Biol. Chem. 253:3882-3887.
12. Raetz, C. R. H., and K. F. Newman. 1979. Diglyceride kinase mutants of *Escherichia coli*: inner membrane association of 1,2-diglyceride and its relation to synthesis of membrane-derived oligosaccharides. J. Bacteriol. 137:860-868.
13. Reynolds, A. E., J. Felton, and A. Wright. 1981. Insertion of DNA activates the cryptic *bgl* operon in *Escherichia coli* K-12. Nature (London) 293:625-629.
14. Rotering, H., W. Fiedler, W. Rollinger, and V. Braun. 1984. Procedure for the identification of *Escherichia coli* mutants affected in components containing glycerol derived from phospholipid turnover: isolation of mutants lacking glycerol in membrane-derived oligosaccharides (MDO). FEMS Microbiol. Lett. 22:61-68.
15. Schneider, J. E., V. Reinhold, M. K. Rumley, and E. P. Kennedy. 1979. Structural studies of the membrane-derived oligosaccharides of *Escherichia coli*. J. Biol. Chem. 254:10135-10138.

16. **Schulman, H., and E. P. Kennedy.** 1979. Localization of membrane-derived oligosaccharides in the outer envelope of *Escherichia coli* and their occurrence in other gram-negative bacteria. *J. Bacteriol.* **137**:686–688.
17. **van Golde, L. M. G., H. Schulman, and E. P. Kennedy.** 1973. Metabolism of membrane phospholipids and its relation to a novel class of oligosaccharides in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **70**:1368–1372.
18. **Weissborn, A. C., and E. P. Kennedy.** 1983. Glycosyl transferase from *Escherichia coli* requiring a membrane fraction, a heat-stable protein, and uridine-5-diphosphoglucose. *Fed. Proc.* **42**:2132.