Metabolism and Function of Bacterial Lipids

II BIOSYNTHESIS OF PHOSPHOLIPIDS IN ESCHERICHIA COLI*

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(Received for publication, December 23, 1963)

In a previous communication (1) we have described some of the general features of the phospholipid metabolism of Escherichia coli B as revealed by experiments in which growing cultures of this organism were labeled with pulses of 32P. In such experiments, phosphatidylglycerol and phosphatidylethanolamine were found to be the principal labeled glycerophosphatides in the fraction of lipids readily extractable with chloroform-methanol. In cultures growing exponentially, 32P-labeled phosphatidylethanolamine is metabolically stable, while the kinetics of labeling of phosphatidylglycerol is consistent with a dynamic function for this phospholipid, requiring turnover or, alternatively, with a role as a metabolic intermediate. Upon very brief exposure of the cells to 32P, labeled phosphatidic acid and phosphatidylglycerol could also be detected among the first lipid products to be labeled.

In this paper, the enzymatic synthesis of phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol in cell-free extracts of E. coli will be described. All of these lipids have been found to be derived from cytidine diphosphate diglyceride. A preliminary report of some of this work has been published (2).

EXPERIMENTAL PROCEDURE

Materials and Methods

Growth of E. coli B — A strain of E. coli B, obtained through the courtesy of Dr. Luigi Gorini, was used throughout these experiments. Cells were grown at 37° with vigorous shaking, on a medium containing Bacto-peptone, glucose, and yeast extract of the following composition (in grams per liter): Bacto-peptone, 15, glucose, 20, and yeast extract, 1.0. Much of the work on the preparation and characterization of the L-serine-CMP phosphatidyltransferase was done with frozen cells of the same strain of E. coli B, grown commercially on a large scale by the Grain Processing Corporation of Muscatine, Iowa. These cells were grown on a medium similar to that described above, except that the Bacto-peptone was replaced by a commercial peptone. The cells were harvested by centrifugation and immediately frozen. The frozen cells appeared to retain the desired enzyme activities during storage at -20° for many months.

(Dipalmitoyl)-L-α-glycerophosphoryl-DL-serine-1-14C was synthesized by a method based on that of Baer and Maurukas (3).

Synthetic (dipalmitoyl)-L-α-glycerophosphoryl-ethanolamine was a product of the Mann Chemical Company. DL-Serine-1-14C and DL-serine-3-14C were products of the Volk Radiochemical Company. L-Serine-3-14C was the generous gift of Dr. Manfred L. Karnovsky.

The nonionic detergent Cutsol (isooctylphenoxypolyoxyethanol) was purchased from the Fisher Chemical Company. CDP-dipalmitin was synthesized from CMP and dipalmitoyl-L-α-glycerophosphosphate and purified by chromatography on silicic acid as described by Paulus and Kennedy (4).

L-Glycerophosphate-1,3-14C was prepared enzymatically from labeled glycerol by phosphorylation with ATP in the presence of glycerokinase (5). The product was isolated by chromatography on Dowex 1-formate.

Buffers containing a mixture of phosphate and Tris were prepared by adjusting 0.50 M solutions of KH2PO4 to the desired pH (measured with a glass electrode on 1:10 dilutions) by the addition of solid Tris.

CDP-diglyceride + L-serine → phosphatidylserine + CMP (1)

Two general methods of assay were used in this work. Method I is based on the measurement of the conversion of radioactive L-serine to a lipid, while in Method II the release of CMP from CDP-diglyceride in the presence of L-serine and enzyme is measured spectrophotometrically. The incubation mixture, which is the same for both assays, contains EDTA, 0.002 M; sodium sulfate, 0.08 M; mercaptoethanol, 0.004 M; CDP-dipalmitin, 0.001 M; DL-serine, 0.006 M; Cutsol, 1 mg per ml; and Tris-phosphate buffer of pH 7.9 (final concentration in the enzyme system, 0.10 M phosphate and 0.14 M Tris). The final volume was 0.25 ml, and the tubes were incubated at 37° for 30 minutes. The omission of EDTA and mercaptoethanol had little effect, but these protective agents were routinely included.

When Method I was used for the assay, radioactive serine was used in the incubation mixture, usually DL-serine-2-14C, although in some experiments DL-serine-1-14C was used. At the end of the incubation period, the reaction was stopped by the addition of methanol (5 ml) containing 10 mg of crude phospholipid from horse liver as carrier. Chloroform (10 ml) was then added, and the solution was thoroughly stirred and then filtered through a plug of glass wool into a vessel of about 50-ml capacity provided with a tightly fitting, standard taper glass stopper. About 30 ml of 2 M KCl were then added, and the tightly stopped vessels were thoroughly shaken. After separation of the phases, the upper aqueous phase was carefully drawn off with a capillary
and the lower chloroform phase was washed twice more in similar fashion with 2 M KCl, and finally with water. Aliquots of the washed chloroform extract were plated and counted in a windowless gas flow counter. Controls, in which the methanol was added immediately after the enzyme, were included in every set of determinations to insure that the removal of labeled serine from the washed lipid extracts was complete. From the known specific activity of the labeled serine, the conversion of serine to phospholipid was calculated. One unit of enzyme is that amount which catalyzes the conversion of 1 millimole of serine to lipid under these conditions.

In Method II, the enzyme incubation mixture was the same as described above, except that nonradioactive L-serine was employed. At the end of the incubation 0.1 ml of 5% (w/v) serum albumin was added, followed immediately by 1 ml of 5% (w/v) perchloric acid. The tubes were chilled in an ice bath for 10 minutes, and the precipitate was removed by centrifugation. CDP-dipalmitin is insoluble under these conditions, and is removed with the precipitate. CMP released during the course of the enzymatic reaction is recovered quantitatively in the supernatant solution. Aliquots of the supernatant solution were suitably diluted with distilled water (usually 3-fold), and the absorbance at 280 mp was measured in a Beckman model DU spectrophotometer. Control vessels, in which serine was omitted from the enzymatic incubation mixture, were carried through the entire procedure in identical fashion. The CMP released was calculated from the difference in absorbance at 280 mp between the complete system and the controls, with a value of 13.6 as the millimolar extinction coefficient. One unit of enzyme is that amount which catalyzes the release of 1 millimole of CMP under these conditions, and is thus identical with 1 unit of enzyme in assay Method I. Crude extracts of E. coli catalyze the release of CMP from CDP-diglyceride in the absence of added serine, apparently as a result of the action of purely hydrolytic enzymes, and the blank value observed with such crude extracts may be appreciable. However, this hydrolytic activity is almost completely removed during the purification procedure described below. With the partially purified enzyme, the release of CMP is linear with added amounts of enzyme up to at least 100 units, and with smaller amounts of enzyme, is linear with time for about 1 hour.

Experiments and Results

Experiments with CDP-ethanolamine—Since phosphatidylethanolamine is the principal glycerophosphatide of E. coli, and CDP-ethanolamine is a precursor of this lipid in animal tissues, attempts were made to demonstrate the enzymatic synthesis of CDP-ethanolamine from CTP and phosphorylethanolamine in crude extracts prepared from frozen bacterial cells after crushing in a Hughes press (6). No evidence for the presence of phosphorylethanolamine cytidylyltranferase could be obtained, nor could the synthesis of labeled phosphorylethanolamine from synthetic CDP-ethanolamine and added d-α,β-diglyceride be detected. These results suggested that the synthesis of phosphatidylethanolamine in E. coli may proceed via phosphatidylserine. Phosphatidylserine has been shown, to give rise to phosphatidylethanolamine by enzymatic deacylation in animal tissues (7).

Preparation of L-Serine-CMP Phosphatidyltransferase—Frozen cells of E. coli B, grown either in this laboratory or commercially on a large scale as described above, were the starting material for the preparation of this enzyme. Active extracts were readily prepared by crushing the frozen cells in a Hughes press. The yield of total protein and of enzyme activity by this method was excellent. However, extraction of the cells after treatment with cold acetone was used for the routine preparation of the enzyme, since this method could more readily be applied to a large quantity of cells. The specific activity (units per mg of protein) of the initial extract of the acetone-treated cells was about the same as in extracts from cells crashed with a Hughes press, but the yield was only about one-third as great.

Frozen cell paste of E. coli B (10 g) was dispersed in 300 ml of ice-cold acetone in a Waring Blendor for 1 minute at full speed. The suspension was immediately filtered with suction on a Buchner funnel in a cold room maintained at 0-5°. The precipitate, still slightly moist with acetone, was weighed, and then extracted with 0.01 M Tris buffer of pH 8.0, containing 0.001 M EDTA and 0.005 M mercaptoethanol. One liter of buffer was used for each 100 g of precipitate, and the suspension was stirred for 45 minutes at 0°. The insoluble material was removed by centrifugation in a refrigerated Servall centrifuge at 10,000 × g for 45 minutes and discarded.

Sufficient solid ammonium sulfate was added slowly with good stirring to bring the supernatant solution to 0.30 saturation. The suspension was stirred for 20 minutes to insure equilibrium. The precipitate was removed by centrifugation. This fraction usually possessed only about 10% of the total activity, and after assay was discarded. To the clarified supernatant solution, sufficient solid ammonium sulfate was added as before to bring the solution to 0.50 saturation. Again the precipitate was collected by centrifugation. Most of the activity of the extract was recovered in this fraction, which was taken up in a small amount of the Tris-EDTA-mercaptoethanol buffer and dialyzed against the same medium. It could be lyophilized and stored as a dry powder at this stage without loss.

The enzyme preparations obtained in this way contained considerable amounts of nucleic acid as revealed by determination of the ultraviolet absorption spectrum according to the method of Warburg and Christian (8). Attempts to remove the nucleic acid by precipitation with protamine, streptomycin, or manganese ions revealed that the enzyme activity was persistently associated with the nucleic acid precipitates. This property was used to achieve a further purification of the enzyme. The lyophilized, 0.30 to 0.50 saturated ammonium sulfate fraction (1.0 g) was dissolved in 100 ml of 0.1 M Tris buffer of pH 8.0 at 0°. To this solution 0.5 M MnCl₂ (10 ml) was added slowly with stirring. After 30 minutes, the precipitate was removed by centrifugation at 10,000 × g for 30 minutes in a refrigerated centrifuge. The supernatant solution was discarded. The precipitate was taken up in 0.1 M glycine buffer of pH 10 containing 0.005 M EDTA, yielding an opalescent solution, which was either stored frozen or lyophilized.

A summary of a typical fractionation is shown in Table I. Although the enzyme at the stage of Fraction 3 has been purified only between 6- and 7-fold, phosphatidylserine decarboxylase and hydrolases attacking CDP-diglyceride have been largely removed.

Net Synthesis of Phosphatidylserine from CDP-diglyceride and L-Serine—Crude extracts of E. coli and partially purified enzyme fractions, prepared as outlined in Table I, catalyze the net synthesis of phosphatidylserine according to the following equation.

\[ \text{CDP-diglyceride} + \text{L-serine} \rightarrow \text{phosphatidylserine} + \text{CMP} \]
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Precipitate with Mn++. 46

1. Extract of acetone-treated frozen cells. 100

2. CDP-dipalmitin omitted. 1.0 ml. Octanol (0.02 ml) was added to each tube just before incubation for 1 hour at 37°. The lipid fraction was extracted, washed, and counted as described in the text. The ninhydrin-reactive lipids were determined by a variation of the method of Cocking and Yemm (9) with serine as standard.

Conversion of \( {\text{L-serine-3-}}^{14}\text{C} \) to lipid by Escherichia coli strain B enzyme system

Each tube contained 0.002 \( \mu \text{mol L-serine-3-}^{14}\text{C} \) (100,000 c.p.m., per \( \mu \text{mole} \)), 0.1 M \( \text{NaSO}_4 \), 0.01 M \( \text{2-mercaptoethanol} \), 0.04 M Tris buffer of pH 8.0, 0.002 M EDTA, and enzyme (approximately 2.5 mg of protein). Cytidine diphosphate dipalmitin (0.001 M) was present in tube 1, but omitted from tube 2. The final volume was 1.0 ml. Octanol (0.02 ml) was added to each tube just before incubation for 1 hour at 37°. The lipid fraction was extracted, washed, and counted as described in the text. The ninhydrin-reactive lipids were determined by a variation of the method of Cocking and Yemm (9) with serine as standard.

Evidence for the net formation of phosphatidylserine is shown in Table II. During the incubation in the presence of CDP-dipalmitin, an increase of 0.67 \( \mu \text{mole} \) of ninhydrin-reactive lipid took place, while measurement of the radioactivity of the newly formed lipid indicated that 0.69 \( \mu \text{mole} \) of labeled serine had been converted to lipid, in agreement with the stoichiometry of Equation 1. The requirement for CDP-diglyceride for the formation of radioactive lipid is absolute.

Isolation and Identification of Enzymatically Synthesized Phosphatidylserine—The experiment shown in Table II was repeated on a scale 12 times larger, with \( \text{L-serine-1-}^{14}\text{C} \) replacing the serine labeled at C 3. The radioactivity of the washed chloroform extract obtained in this experiment indicated the synthesis of 4.1 \( \mu \text{moles} \) of serine-containing lipid. The chloroform extract was taken to dryness under vacuum in a rotary evaporator, and the residue was taken up in a small volume of chloroform and chromatographed on silicic acid with a gradient of methanol in chloroform for elution essentially as previously described (1). Ninety-seven per cent of the radioactivity emerged from the column (Fig. 1) in a single ninhydrin-reactive band in the position to be expected for phosphatidylserine, as established in experiments with the pure synthetic phospholipid. The materials in tubes 17 to 32 were concentrated to a small volume and analyzed for total phosphorus (10) and fatty acid ester bonds (11). The molar ratio found was 1.0:2.04, in close correspondence to theory.

An aliquot of the enzymatically synthesized phospholipid was deacylated by mild alkaline hydrolysis in a variation of the procedure of Dawson (12). The water-soluble products were chromatographed on Whatman No. 1 paper, with phenol saturated with water containing 0.1 % (v/v) of concentrated ammonia as solvent, in the descending technique. A single ninhydrin-reactive spot with an \( R_f \) of 0.17, identical with that of glycerophosphorylserine derived from synthetic phosphatidylserine, contained all of the radioactivity.

The enzymatically synthesized phosphatidylserine was readily decarboxylated by the phosphatidylserine decarboxylase of rat liver (7) or by the bacterial enzyme to be described below, enzymes which are specific for the lipid form of serine.

Stoichiometry of Reaction—Table III shows the results of an experiment in which the conversion of labeled serine to phospholipid (measured by Assay Method I) was compared with the formation of CMP in a pair of identical tubes (measured by Assay Method II). For each mole of CMP released, about 0.80 and 0.85 mole, respectively, of labeled serine was recovered in the lipid fraction. Failure to obtain quantitative recovery of radioactivity in the lipid fraction may be the result of small amounts of enzymatic decarboxylation, since serine-1-\(^{14}\text{C} \) was used in this experiment, or to losses of radioactive lipid in the extraction and repeated washes used in Procedure I.

Identification of CMP—The perchloric acid supernatant solutions obtained in the experiment shown in Table III (tubes 3 and 4) were combined and treated with Norit A charcoal (200 mg). The suspension was stirred at room temperature for 10 minutes, and the charcoal was separated by centrifugation and washed once with water. The charcoal was then extracted with 10 ml

<table>
<thead>
<tr>
<th>Tube</th>
<th>CMP released</th>
<th>L-Serine incorporated into lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table III

Stoichiometry of release of CMP and conversion of labeled serine to lipid

The enzymatic incubation was carried out under the conditions described in "Materials and Methods," except that the final volume was increased to 5.0 ml. Tubes 1 and 2 contained unlabeled serine, and the release of CMP was measured by Assay Method II described above. In tubes 3 and 4, L-serine-1-\(^{14}\text{C} \) was used, and the incorporation of the labeled amino acid into the lipid fraction was measured by Assay Method I.
of 50% (v/v) ethanol containing 1% (v/v) of concentrated aqueous ammonia, by stirring for 10 minutes. The charcoal was removed by centrifugation, and the extract was concentrated to dryness in a conical centrifuge tube in a jet of air. The residue was taken up in about 0.1 ml of water, and an aliquot was spotted on Whatman No. 1 filter paper. Chromatography in the system 1 M ammonium acetate (pH 7)-ethanol (40:60 v/v) revealed that CMP (RF 0.20) was the sole spot detectable under ultraviolet light.

Specificity of L-Serine-CMP Phosphatidyltransferase—The enzyme catalyzing Reaction 1 is highly specific for L-serine; no appreciable activity is noted with the D entantiomorph (2). A number of other substrates were tested for activity, as measured by the release of CMP in Assay Method II. No activity could be detected with L-threonine, ethanolamine, inositol, choline, or glycerol. However, a definite release of CMP could be detected with L-alpha-glycerophosphate, amounting to about 3% of that observed with L-serine. This and other evidence given below leads to the conclusion that phosphatidylglycerol is synthesized in *E. coli* by a CDP-diglyceride-requiring pathway essentially similar to that described in animal tissues (13).

Surface-active Agents and Ionic Strength—A striking property of the L-serine-CMP phosphatidyltransferase is its requirement for both an added surface-active agent and minimum ionic strength for optimal activity. The activity observed in the absence of added surface-active agent varied somewhat from experiment to experiment, but a large effect was nearly always noted, and in many experiments, such as that shown in Table IV, no activity could be detected if octanol (or other surface active agent) was omitted. Table IV also shows the dependence of activity on ionic strength supplied by added sodium sulfate. In the absence of added salt, activity is greatly reduced or absent. Other salts, such as ammonium sulfate or sodium chloride at the same ionic strength, may replace the sodium sulfate.

A number of organic solvents other than octanol have been found to activate the enzyme. Diisobutyl ketone, in saturating amounts, is fully as effective as octanol. In the series of straight chain alcohols, however, a marked specificity is evident as shown in Fig. 2. There is a distinct optimum at octanol, and alcohols of either longer or shorter chain length are considerably less effective. The nonionic detergent Cutscum is also effective, and since it is fully miscible with water at the concentrations used, it has the advantage that a two-phase system is avoided. For this reason, most of the later experiments reported here were carried out in the presence of Cutscum.

Affinity for Substrates—The concentration of L-serine in the enzyme incubation mixture was varied in the experiment shown in Fig. 3. A Michaelis constant of 8 X 10^-4 M was calculated from the Lineweaver-Burk plot. When the concentration of CDP-dialpinatin was varied (at constant L-serine concentration) in similar fashion, the observed results were not those expected of classical Michaelis-Menten kinetics. Instead, the maximum

### Table IV

<table>
<thead>
<tr>
<th>System</th>
<th>Phosphatidylserine synthesized (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>17.3</td>
</tr>
<tr>
<td>Octanol omitted</td>
<td>0</td>
</tr>
<tr>
<td>NaSO₄ omitted</td>
<td>0</td>
</tr>
</tbody>
</table>
rate was observed at about \( 5 \times 10^{-4} \) to \( 10^{-3} \) M CDP-dipalmitin, while higher concentrations were somewhat inhibitory.

**pH Optimum**—When the pH is varied with Tris-phosphate buffers, prepared as described in “Materials and Methods,” the enzyme is active over a broad range of pH between 6.9 and 9.0, with an optimum at about pH 7.9.

**Effects of Inhibitors**—The enzyme is unaffected by iodoacetamide, N-ethylmaleimide, hydrazine, or hydroxylamine when added to the enzyme incubation mixture at a concentration of 0.004 M. (Mercaptoethanol was omitted from the incubation mixture in these experiments.) The enzyme was likewise unaffected by KF at a final concentration of 0.04 M.

**Divalent Cations**—No requirement for divalent cations for the activity of the L-serine-CMP phosphatidytransferase was detected. The enzyme incubation mixture routinely contained EDTA. The addition of calcium, magnesium, or manganese ions did not stimulate the reaction; at high concentrations a distinct inhibition was noted, probably the result of precipitation of the CDP-diglyceride.

**Decarboxylation of Phosphatidylserine**—We have previously reported that phosphatidylethanolamine is the principal glycerophosphatide found in this strain of E. coli. This fact, together with the finding that only traces of phosphatidylserine are found in this strain of E. coli, together with the finding that only traces of phosphatidylserine are found in this strain of E. coli, strongly suggests that phosphatidyldi-}

\[
\text{Phosphatidylserine} + \text{CO}_2 \rightarrow \text{Phosphatidylethanolamine} + \text{serine} \tag{3}
\]

In keeping with this conclusion, we have found an enzyme in E. coli which catalyzes the decarboxylation of phosphatidylserine. The enzyme is specific for the phospholipid form of serine, since \( \text{n-serine} \) itself is not attacked.

Active preparations of phosphatidylserine decarboxylase were prepared from freshly harvested or frozen cells of E. coli in the following manner. Suspensions of cells (1.0 g of cells (wet weight) in 40 ml of 0.1 M phosphate buffer of pH 6.9 containing 0.01 M mercaptoethanol) were subjected to ultrasonic disintegration in a Gilson Medical Electronics apparatus for 10 minutes. The container was immersed in an ice-salt bath during this procedure, during which the temperature of the suspension rose to about 10°C. The suspension was cooled to 0°C and centrifuged at 35,000 \( \times g \) for 40 minutes in a refrigerated centrifuge. The supernatant solution contained little phosphatidylserine decarboxylase activity, and was set aside for the preparation of other enzymes. The precipitate was washed twice with the same buffer used for suspending the cells, and the wash solutions were discarded. The precipitate was then extracted by stirring at 0°C for 30 minutes with buffer containing 5% (w/v) of the nonionic detergent Cutscum, in a volume about one-fifth of that of the original cell suspension. The precipitate was removed by centrifugation at 35,000 \( \times g \) for 40 minutes and was discarded.

Cold ethanol was then added dropwise to the extract with good stirring, in a container immersed in an ice-salt bath, until a final concentration of 30% was reached. The precipitate was removed by centrifugation in a refrigerated centrifuge. The supernatant solution was discarded. The precipitate was dissolved in a small volume of 0.1 M phosphate buffer of pH 6.9 containing 0.02 M EDTA, and was dialyzed against the same buffer. The enzyme could be stored frozen for several weeks with no detectable loss of activity.

The activity of phosphatidylserine decarboxylase can most readily be followed by measuring the release of radioactive CO\(_2\) from phosphatidyl-L-serine \( ^{14} \text{C} \) as was shown in Fig. 4. It is seen that the release of radioactive CO\(_2\) is a linear function of enzyme concentration. Since phosphatidylserine and serine itself are somewhat unstable substances, it is necessary to make sure that nonenzymatic decarboxylation is not taking place under the conditions of the experiment. This is done by appropriate controls from which enzyme is omitted, or in which boiled enzyme is used.

The properties of the phosphatidylserine decarboxylase will be described in greater detail in a later publication.

**Synthesis of Phosphatidylglycerol**—The biosynthesis of phosphatidylglycerol in animal tissues has been shown by work in this laboratory (13) to proceed according to Equations 4 and 5.

\[
\text{CDP-diglyceride} + \text{L-} \alpha\text{-glycerophosphate} \rightarrow \text{Phosphatidylglycerophosphate} + \text{CMP} \tag{4}
\]

\[
\text{Phosphatidylglycerophosphate} \rightarrow \text{Phosphatidylglycerol} + \text{Pi} \tag{5}
\]

Extracts of E. coli catalyze analogous reactions. Reaction 4 was demonstrated by measuring the CDP-diglyceride-requiring conversion of L-\( \alpha\)-glycerophosphate-\( 1,3\)\(^{14} \text{C} \) into lipids (Table V). As mentioned above, Reaction 4 was also detected in enzyme fractions derived from E. coli by spectrophotometric measure-
ment of the release of CMP from CDP-diglyceride in the presence of L-a-glycerophosphate.

When L-a-glycerophosphate-32P was substituted for the 14C-labeled compound, a radioactive lipid (phosphatidylglycerophosphate) was formed in a reaction with the same absolute dependence upon added CDP-diglyceride and Mn++ ion. Phosphatidylglycerophosphate has not been detected in growing cells of E. coli B (1); dephosphorylation via Reaction 5 presumably prevents its accumulation.

Phosphatidylglycerol and phosphatidylglycerophosphate were identified as products of the enzymatic reaction of CDP-diglyceride and L-a-glycerophosphate by essentially the same procedures applied to the study of the enzyme system from liver (13). Chromatography of the labeled lipids on silicic acid yielded two peaks with chromatographic behavior similar to that described for phosphatidylglycerol and phosphatidylglycerophosphate. Hydrolysis of these fractions with mild alkali, or with acetic acid as described by Kiyasu et al. (13), yielded the products to be expected from phosphatidylglycerophosphate and phosphatidylglycerol, respectively.

As in the animal system, no reaction between CDP-diglyceride and free glycerol could be detected in the bacterial extracts. A striking difference, however, is the absolute requirement for Mn++ by the bacterial enzyme; such a requirement has not been detected in the corresponding reaction catalyzed by rat liver mitochondria.

**DISCUSSION**

A scheme summarizing the pathways for the biosynthesis of glycerophosphatides in E. coli is shown in Fig. 5. A salient feature of the scheme is the predominant role of CDP-diglycerides as precursors of phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol. In mammalian liver, the principal reaction for the net synthesis of phosphatidylethanolamine is via CDP-ethanolamine. No enzyme catalyzing a reaction similar to Equation 1 has been detected in liver, although failure to find a given enzyme in a tissue is by no means proof of its absence. Instead, in extracts of animal tissues, an enzymatic exchange of the base moieties of phosphatidylserine and phosphatidylethanolamine has been observed (7), leading to the formation of phosphatidylserine with no net increase in the amount of phospholipid. The experiments of Dawson (15) on the labeling of glycerophosphatides of liver after injection of 32P in vivo are consistent with the view that the phosphorus of phosphatidylethanolamine is the precursor of the phosphorus of phosphatidylserine via the exchange reaction, since the labeling

![Fig. 5. Pathways for the biosynthesis of glycerophosphatides in E. coli B](http://www.jbc.org/content/jbc/249/5/1725/F5)
of phosphatidylserine lags considerably behind that of phosphatidylethanolamine. Liver also contains an active phosphatidylserine decarboxylase (7), and thus an enzymatic cycle (16) of exchange and decarboxylation occurs in this and probably in other animal tissues. In contrast, in E. coli it seems clear that phosphatidylserine is the precursor of phosphatidylethanolamine.

**Summary**

The metabolic pathways for the synthesis of phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol in Escherichia coli have been studied in cell-free enzyme systems. The synthesis of phosphatidylethanolamine takes place via phosphatidylserine according to Reactions 1 and 2.

\[
\text{CDP-diglyceride} + \text{L-serine} \rightarrow \text{phosphatidylserine} + \text{CMP} \quad (1)
\]

\[
\text{Phosphatidylserine} \rightarrow \text{phosphatidylethanolamine} + \text{CO}_2 \quad (2)
\]

The biosynthesis of phosphatidylglycerol occurs by a pathway previously demonstrated in animal tissues.

\[
\text{CDP-diglyceride} + \text{1-cu-glycerophosphate} \rightarrow \text{phosphatidylglycerophosphate} + \text{CMP} \quad (3)
\]

\[
\text{Phosphatidylglycerophosphate} \rightarrow \text{phosphatidylglycerol} + \text{Pi} \quad (4)
\]

Some properties of the enzymes catalyzing these reactions are described.

**Acknowledgments**—The authors are grateful to Mrs. Linda Fielding Halporn and Miss Marilyn Rumley for technical assistance in this work.

**References**
