Synthesis of Phosphatidylserine by *Escherichia coli*

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(Received for publication, October 18, 1961)

Although nearly every other type of biosynthetic process in bacteria has been studied in detail, very little is known about the chemical pathways for the synthesis of phospholipids and other complex lipids in these organisms. Indeed, studies of the chemical composition of bacterial phospholipids by modern methods have only recently been undertaken (1-4).

In this communication, we wish to present evidence for the occurrence of an enzyme in cell-free extracts of *Escherichia coli* B that catalyzes the following reaction:

\[ \text{Cytidine diphosphate diglyceride} + \text{n-serine} \rightarrow \text{phosphatidylserine} + \text{cytidine monophosphate} \]  

Equation 1. The reaction could also be demonstrated by measuring spectrophotometrically the serine-dependent release of cytidine monophosphate from cytidine diphosphate dipalmitin by the method of Paulus and Kennedy (6).

A study of the conversion to lipid of n-serine-Cl4 and n-serine-C14 (100,000 counts per amole); 0.1 M Na2SO4; 0.01 M 2-mercaptoethanol; 0.01 M Tris buffer of pH 8.0; 0.002 M EDTA; and enzyme prepared as described in the text (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 reaction was stopped by the addition of 5.0 ml of methanol followed by 10.0 ml of chloroform. The lipid fraction was extracted, washed, and counted essentially as previously described (7). The ninhydrin-reactive lipids were determined by a variation of the method of Cocking and Yemm (8) with serum as standard.

* This work was supported by grants from the United States Public Health Service, the Life Insurance Medical Research Fund, and the Nutrition Foundation.

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**Table 1**

Conversion of DL-serine-3-C14 to lipid by *Escherichia coli* B enzyme system*

<table>
<thead>
<tr>
<th>System</th>
<th>Analysis of lipid fraction</th>
<th>Radioactivity</th>
<th>Ninhydrin-reactive lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>total counts</td>
<td>total amole</td>
</tr>
<tr>
<td>1. Complete</td>
<td></td>
<td>105,000</td>
<td>0.71</td>
</tr>
<tr>
<td>2. CDP-dipalmitin omitted</td>
<td></td>
<td>0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Each tube contained 0.002 m DL-serine-3-C14 (100,000 counts per amole); 0.1 M Na2SO4; 0.01 M 2-mercaptoethanol; 0.04 M Tris buffer of pH 8.0; 0.002 M EDTA; and enzyme prepared as described in the text (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°.

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carried out on a scale 12 times larger than that described in Table I, with DL-serine-1-C14 replacing the 3-labeled serine. The radioactivity contained in the lipid extract indicated the synthesis of 4.1 amoles of serine-containing lipid. The crude lipid fraction was chromatographed on a column of silicic acid, and eluted with increasing concentrations of methanol in chloroform by gradient elution. Ninety-seven per cent of the total radioactivity was recovered in a single phosphorus-containing, ninhydrin-reactive peak in the position expected for phosphatidylserine.

The material in this band was concentrated to dryness, and upon analysis was found to contain fatty acid ester linkages (9) and organic phosphorus (10) in the proportion of 2.04:1.0. An aliquot of the product was subjected to mild alkaline hydrolysis by the procedure of Dawson (11). Upon paper chromatography of the water soluble products of hydrolysis, a single ninhydrin-reactive spot was found with an Rf identical with that of authentic glycerophosphorylserine derived from synthetic phosphatidylserine. All the radioactivity was associated with this spot.

The enzymatically synthesized phospholipid was found to be readily decarboxylated by the phosphatidylserine decarboxylase of rat liver mitochondria (12), an enzyme that is specific for this phospholipid.

These results, together with the complete requirement for cytidine diphosphate diglyceride, indicate that the enzyme system catalyzes the synthesis of phosphatidylserine according to Equation 1. The reaction could also be demonstrated by measuring spectrophotometrically the serine-dependent release of cytidine monophosphate from cytidine diphosphate dipalmitin by the method of Paulus and Kennedy (6).

A study of the conversion to lipid of D-serine-C14 and L-serine-C14 showed that the L-isomer was preferentially utilized (Table II). The enzyme system shows optimal activity in the presence of moderate salt concentrations (e.g., 0.1 m sodium sulfate) and certain organic solvents, such as octanol.
TABLE II

Activity of D- and L-serine*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Radioactivity of lipid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Serine-3-C14</td>
<td>8380</td>
</tr>
<tr>
<td>D-Serine-3-C14</td>
<td>77</td>
</tr>
</tbody>
</table>

* The conditions of the experiment were similar to those of Table I. The specific activity of both tracers was 135,000 counts per pmole.

A highly active phosphatidylserine decarboxylase is also present in the crude extracts of E. coli but may be almost completely removed from the phosphatidylserine synthetase by the simple fractionation procedure described above. The sequential action of these two enzymes thus accounts for the synthesis of both phosphatidylserine and phosphatidylethanolamine, described by Law (3) and by Kurokawa et al. (4) as the principal glycerophosphatides of E. coli.

A previous report from this laboratory (12) has described the enzymatic formation of phosphatidylserine in mammalian tissues by an exchange of free serine with the ethanolamine moiety of phosphatidylethanolamine. The synthesis de novo of phosphatidylcholamine (and thus indirectly of phosphatidylserine) in these tissues proceeds via cytidine diphosphate ethanolamine and D-α,β-diglyceride (13). The possibility must be considered that Reaction 1 above may also occur in mammalian tissues as an alternative route to the direct, net synthesis of phosphatidylserine.

REFERENCES

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