Phosphatidylserine Synthesis in *Saccharomyces cerevisiae*

**Purification and Characterization of Membrane-Associated Phosphatidylserine Synthase**

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Membrane-associated phosphatidylserine synthase (CDP-diacylglycerol:L-serine O-phosphatidyltransferase, EC 2.7.8.8) was purified from the microsomal fraction of *Saccharomyces cerevisiae* strains S288C and YEPCHO1. VAL2C(YEpCHO1) contains a hybrid plasmid bearing the structural gene for phosphatidylserine synthase and overproduces the enzyme 6-7-fold (Letts, V. A., Klig, L. S., Bae-Lee, M., Carman, G. M., and Henry, S. A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7279–7283) compared to wild-type S288C. The purification procedure included Triton X-100 extraction of the microsomal membranes, CDP-diacylglycerol-Sepharose affinity chromatography, and DE-53 chromatography. The procedure yielded a preparation from each strain containing a major peptide band (Mr = 23,000) upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Phosphatidylserine synthase was dependent on manganese and Triton X-100 for maximum activity at pH 8.0. The apparent Km values for serine and CDP-diacylglycerol were 0.58 mM and 60 μM, respectively. Thioreactive agents inhibited enzyme activity. The enzyme was thermally labile above 40 °C. Results of isotopic exchange reactions between substrates and products suggest that the enzyme catalyzes a sequential Bi Bi reaction.

Phosphatidylserine synthase accounts for approximately 8% of the total membrane phospholipids in *Saccharomyces cerevisiae* and is important for growth (1). Atkinson et al. (1, 2) have shown that cho1 mutants of *S. cerevisiae* are defective in phosphatidylserine synthesis which results in abnormal patterns of phospholipid metabolism and physiological properties. In addition, phosphatidylserine serves as a precursor to the major membrane phospholipids phosphatidylethanolamine and phosphatidylcholine (3). The cho1 mutants however, synthesize phosphatidylethanolamine and phosphatidylcholine by the CDP-choline- and CDP-ethanolamine-based pathways described by Kennedy and Weiss (4) when supplemented with ethanolamine or choline (2). The CHO1 locus has been identified from *Escherichia coli* (9), was purified to homogeneity (10–12), and extensively characterized (10, 13). Unlike the membrane-associated phosphatidylserine synthase from yeast, the enzyme from *E. coli* (14), as well as other Gram-negative bacteria (15), is associated with ribosomes in cell-free extracts.

Phosphatidylserine synthase from yeast is a difficult enzyme to purify due to its membrane-bound nature and low levels of activity. These obstacles have been overcome by the developments of a solubilization procedure to release phosphatidylserine synthase from yeast membranes (5, 6, 16), the application of CDP-diacylglycerol-Sepharose affinity chromatography (17, 18) for the partial purification of the enzyme (7), and the cloning of the CHO1 gene on the YEp13 plasmid which directs the 6-7-fold overproduction of the enzyme (7). In the present paper, we report the purification of phosphatidylserine synthase from *S. cerevisiae* and our initial studies on the characterization of the enzyme. This is the first report of the purification of the membrane-associated form of phosphatidylserine synthase from a eukaryotic organism. The differences in phosphatidylserine synthase between prokaryotic organisms and yeast are discussed.

**Experimental Procedures**

**Materials**

All chemicals were reagent grade. Phospholipids, L-serine, myo-inositol, sn-glycerol 3-phosphate, ethanolamine, cysteine, threonine, CTP, CMP, bovine serum albumin, N-ethylmaleimide, and p-chloromercuriphenylsulfonic acid were purchased from Sigma. Triton X-100 (octylphenoxypolyethoxyethanol) is a product of Rohm and Haas Company. 1-[3-3H]Serine, myo-[2-3H]inositol, sn-[2-3H]glycerol 3-phosphate, and [5-3H]CTP were purchased from New England Nuclear. [5-3H]CMP was obtained from Schwarz/Mann. dCDP-diacylglycerol was purchased from Serdary Research Laboratories, Inc. Sepharose 4B and Sephadex G-25 were products of Pharmacia Fine Chemicals. DE-53 (DEAE-cellulose) was from Whatman. Precoated Silica Gel 60 analytical thin-layer chromatography plates were purchased from E. Merck. Electrophoresis reagents and molecular weight standards were obtained from Bio-Rad Laboratories. Amino acids for yeast growth were from ICN Nutritional Biochemicals. Yeast Nitrogen Base without amino acids, yeast extract, and peptone were purchased from Difco Laboratories.
CDP-diacylglycerol derived from egg lecithin was prepared by the method of Carman and Fischl (19). [5-3H]CDP-diacylglycerol was prepared enzymatically from phosphatidic acid and [5-3H]CTP with yeast mitochondrial CDP-diacylglycerol synthase (20). [3-3H]Phosphatidylserine synthase was prepared from CDP-diacylglycerol and L-[3-3H]serine with purified phosphatidylserine synthase under standard assay conditions. The labeled product was purified by thin-layer chromatography (19) in the solvent system chloroform-methanol-water (65:25:4, v/v).

Preparation of CDP-diacylglycerol-Sepharose

The sodium periodate-oxidized derivative of CDP-diacylglycerol was covalently attached to Sepharose 4B via an acetyl acid dihydrazide spacer arm as described by Larson et al. (18) with the modifications of Fischl and Carman (17).

Yeast Strains and Growth Conditions

Wild-type strain S288C (ural2) was grown in YEPD medium (1% yeast extract, 2% peptone, and 2% glucose). Strain VAL2C(YEpCHO1), which overproduces phosphatidylserine synthase activity (17), was grown in synthetic complete medium (1) without uracil. Cultures were grown at 30 °C to stationary phase, harvested by centrifugation, and stored at -80 °C (17). Strain VAL2C(YEpCHO1) was kindly provided by Susan A. Henry and Verity A. Letts (Albert Einstein College of Medicine, Bronx, NY).

Enzyme Assays

Phosphatidylserine synthase activity was measured at 30 °C for 20 min by following the incorporation of L-[3-3H]serine (10,000 cpm/nmol) into chloroform-soluble material or the release of water-soluble material. A unit of enzymatic activity was defined as the amount of radioactive CMP from [5-3H]CDP-diacylglycerol (400 cpm/nmol) into chloroform-soluble material or the release of water-soluble material or the release of water-soluble material.

Preparation of Substrates

Phosphatidylinositol synthase activity was measured at 30 °C for 20 min by following the incorporation of L-[3-3H]serine (10,000 cpm/nmol) into chloroform-soluble material or the release of water-soluble radioactive CMP from [5-3H]CDP-diacylglycerol (400 cpm/nmol) as described by Carman and Matas (6). The assay mixture contained 50 mM Tris-HCl (pH 8.0), 0.6 mM MnCl₂, 0.5 mM L-serine, 0.2 mM CDP-diacylglycerol, 4 mM Triton X-100, and enzyme protein in a total volume of 0.1 ml. Phosphatidylserine synthase activity was linear with time and protein concentration under assay conditions when measured with either labeled substrate. The products of the reaction, phosphatidylserine and CMP, were identified with standards by thin-layer chromatography and paper chromatography, respectively (6). Phosphatidylinositol CMP, and wild-type S288C were used as the source for phosphatidylserine synthase purification. By using the microsomal fraction of yeast, the major purification of phosphatidylserine synthase activity was extracted from microsomes with Triton X-100. The column was washed with 10 ml of equilibration buffer followed by elution of the enzyme with equilibration buffer containing 1 M NaCl. The column was then saturated with chromatography buffer containing 1 mM CDP-diacylglycerol and 1 M NaCl and incubated for 1 h. Phosphatidylserine synthase was then eluted from the column with this buffer at a flow rate of 1 ml/min. Fractions (3 ml) were assayed for phosphatidylserine synthase activity. Fractions containing activity were pooled and desalted on a Sephadex G-25 column equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 10 mM MgCl₂, 0.3 mM MnCl₂, 20% glycerol, 10 mM 2-mercaptoethanol, and 0.5% Triton X-100.

Step 3. CDP-diacylglycerol-Sepharose Chromatography—A CDP-diacylglycerol-Sepharose column (0.9 x 4 cm) was eluted with 50 ml of chromatography buffer (50 mM Tris-HCl (pH 8.0), 30 mM MgCl₂, 0.6 mM MnCl₂, 20% glycerol, 10 mM 2-mercaptoethanol, and 0.5% Triton X-100). The Triton X-100 extract was applied to the affinity column in 1.5-ml aliquots. Each aliquot was incubated in the column for 10 min before the addition of the next aliquot to effect enzyme binding. After the entire Triton X-100 extract was applied, the column was washed with 30 ml of chromatography buffer containing 1 M NaCl. The column was then saturated with chromatography buffer containing 1 mM CDP-diacylglycerol and 1 M NaCl and incubated for 1 h. Phosphatidylserine synthase was then eluted from the column with this buffer at a flow rate of 1 ml/min. Fractions (3 ml) were assayed for phosphatidylserine synthase activity. Fractions containing activity were pooled and desalted on a Sephadex G-25 column equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 10 mM MgCl₂, 0.3 mM MnCl₂, 20% glycerol, 10 mM 2-mercaptoethanol, and 0.5% Triton X-100.

Step 4. DE-53 Chromatography—A DE-53 column (0.6 x 4 cm) was eluted with 20 ml of 20 mM Tris-HCl buffer (pH 8.0) containing 10 mM MgCl₂, 0.3 mM MnCl₂, 20% glycerol, 10 mM 2-mercaptoethanol, and 0.5% Triton X-100. Desalted enzyme from the previous step was applied to the column at a flow rate of 1 ml/min. The column was washed with 10 ml of equilibration buffer followed by elution of the enzyme with equilibration buffer containing 1 M NaCl. Fractions containing activity were pooled and desalted on a Sephadex G-25 column as described above. The pure enzyme was stable for at least 4 months at -80 °C. After two cycles of freezing and thawing, approximately 50% of the original activity was retained.

RESULTS

Purification of Phosphatidylserine Synthase—The microsomal fraction of S. cerevisiae strains VAL2C (YEpCHO1) and wild-type S288C were used as the source for phosphatidylserine synthase purification. By using the microsomal fraction for enzyme purification, we eliminated the CDP-diacylglycerol-dependent phosphatidylglycerophosphate synthase which is associated with the mitochondrial fraction of S. cerevisiae (8). A summary of the purification for the enzyme from each strain is presented in Table I. Solubilization of phosphatidylserine synthase from microsomes with Triton X-100 was dependent on the presence of manganese ions in the solubilization buffer (6, 17). The major purification of phosphatidylserine synthase from both strains was attained by CDP-diacylglycerol-Sepharose chromatography. Binding of phosphatidylserine synthase to the affinity column was dependent on Triton X-100 and manganese in the chromatography buffer. The elution of phosphatidylserine synthase from the affinity column was dependent on the substrate CDP-diacylglycerol and NaCl in the elution buffer. Further purification by chromatography on DE-53 resulted in only a slight increase in specific activity; however, the enzyme was concentrated by the procedure. This concentrated enzyme contained approximately 0.5 mM CDP-diacylglycerol as determined previously (19). The purification procedure yielded essentially a homogeneous protein preparation from the overproducing strain as evidenced by polyacrylamide gel electrophoresis under non-denaturing conditions (RF of 0.15) and in the pres-
Phosphatidylserine Synthase from S. cerevisiae

Purification of phosphatidylserine synthase from wild-type (S288C) and strain VAL2C(YEpCHO1)

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Wild type (S288C)*</th>
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<tbody>
<tr>
<td></td>
<td>Total units</td>
<td>Protein</td>
<td>Specific activity</td>
<td>Yield</td>
<td>Purification</td>
<td>Total units</td>
<td>Protein</td>
<td>Specific activity</td>
<td>Yield</td>
<td>Purification</td>
<td></td>
<td></td>
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<td>Microsomes</td>
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<td>493</td>
<td>1.2</td>
<td>100</td>
<td>1</td>
<td>3550</td>
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<td>10.2</td>
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<td>1</td>
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<tr>
<td>Triton X-100 extract</td>
<td>579</td>
<td>241</td>
<td>2.4</td>
<td>98</td>
<td>?</td>
<td>2700</td>
<td>151</td>
<td>17.9</td>
<td>76</td>
<td>1.76</td>
<td></td>
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<td>CDP-diacylglycerol-Sepharose DE-53 chromatography</td>
<td>457</td>
<td>0.34</td>
<td>1340</td>
<td>77</td>
<td>1120</td>
<td>1620</td>
<td>0.76</td>
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<td>421</td>
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<td>71</td>
<td>1920</td>
<td>1510</td>
<td>0.40</td>
<td>3760</td>
<td>42</td>
<td>370</td>
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</table>

*Data are based on starting with 75 g (wet weight) of yeast pellet.

Properties of Phosphatidylserine Synthase—The enzymological properties of phosphatidylserine synthase purified from both strains were essentially the same. The pH optimum for the reaction was 8.0 (Fig. 2A). Phosphatidylserine synthase activity was dependent on the addition of either manganese or magnesium ions (Fig. 2B). The maximum activity obtained with manganese (0.6 mM) was 2-fold greater than the maximum activity obtained with magnesium (20 mM). Calcium ions did not serve as a cofactor for the enzyme. The addition of Triton X-100 to the assay mixture stimulated phosphatidylserine synthase activity to a maximum at a concentration of 4 mM, (molar ratio of Triton X-100 to CDP-diacylglycerol of 20:1), followed by an apparent inhibition of activity at higher detergent concentrations (Fig. 2C). These results resemble substrate dilution kinetics (13). Kinetic experiments were conducted using a uniform mixed micelle substrate of Triton X-100 and CDP-diacylglycerol (13) at a molar ratio of 20:1. Normal saturation kinetics were shown by phosphatidylserine synthase when the CDP-diacylglycerol concentration was held constant (1 mM) and L-serine (K_m = 0.58 mM) was varied (Fig. 3A). Saturation kinetics were also shown by the enzyme when the L-serine concentration was held constant (2 mM) and CDP-diacylglycerol (K_m = 60 μM) was varied (Fig. 3B). Phosphatidylserine synthase was inhibited by various thioreactive compounds (Table II). The addition of mercaptoethanol to the assay system reversed this inhibition. The addition of mercaptoethanol to the standard assay mixture slightly stimulated activity. These results suggest that a sulfhydryl group is essential for phosphatidylserine synthase activity. The ability of phosphatidylserine synthase to utilize substrates related to L-serine was tested under...
FIG. 2. Effects of pH, manganese and magnesium, and Triton X-100 on phosphatidylserine (PS) synthase activity. A, phosphatidylserine synthase (0.01 unit) was measured at the indicated pH values with 50 mM Tris-HCl (○) or 2-(N-morpholino)ethanesulfonic acid (MES)-HCl (○). B, phosphatidylserine synthase (0.016 unit) was assayed with the indicated concentrations of MnCl₂ (○) or MgCl₂ (○). C, phosphatidylserine synthase (0.018 unit) was assayed with 0.2 mM CDP-diacylglycerol and the indicated concentrations of Triton X-100. Activity was measured by following the incorporation of L-[³⁻H]serine into chloroform-soluble product as described in the text. Units.

FIG. 3. Dependence of phosphatidylserine synthase activity on the concentration of L-serine and CDP-diacylglycerol. A, the data are plotted as 1/V (nanomoles/min/ml) versus the reciprocal of the L-serine concentration. B, the data are plotted as 1/V (nanomoles/min/ml) versus the reciprocal of the CDP-diacylglycerol concentration. The molar ratio of Triton X-100 to CDP-diacylglycerol was maintained at 20:1. Enzyme activity was measured with L-[³⁻H]serine as described in the text. The curves drawn were a result of a least-squares analysis of the data.

TABLE I
Effect of thioreactive compounds on phosphatidylserine synthase activity

<table>
<thead>
<tr>
<th>Component</th>
<th>Relative activity %</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>+ 1 mM HgCl₂</td>
<td>13</td>
</tr>
<tr>
<td>+ 1 mM p-chloromercuri-phenylsulfonic acid</td>
<td>17</td>
</tr>
<tr>
<td>+ 1 mM N-ethylmaleimide</td>
<td>17</td>
</tr>
<tr>
<td>+ 1 mM p-chloromercuri-phenylsulfonic acid + 10 mM 2-mercaptoethanol</td>
<td>100</td>
</tr>
<tr>
<td>+ 1 mM 2-mercaptoethanol</td>
<td>130</td>
</tr>
</tbody>
</table>

standard assay conditions using 0.2 mM [⁵⁻H]CDP-diacylglycerol as the labeled substrate. At concentrations of 0.5, 1, and 10 mM, the compounds cysteine, ethanolamine, and threonine did not substitute for serine as a phosphatidyl acceptor in the reaction. When measured under standard assay conditions using L-[³⁻H]serine as the labeled substrate, these compounds (10 mM) did not inhibit phosphatidylserine synthase activity. dCDP-diacylglycerol (0.2 mM) could substitute for CDP-diacylglycerol as a phosphatidyl donor for L-[³⁻H]serine under standard assay conditions. Belediuk et al. (20) reported that CDP-diacylglycerol synthase from S. cerevisiae is unable to catalyze the formation of dCDP-diacylglycerol. Phosphatidylserine synthase was examined for its stability to temperatures ranging from 30–60 °C (Fig. 4). The enzyme was stable up to 40 °C. However, about 90% of the activity was lost on heating at 50 °C for 20 min with total inactivation at 60 °C.

Reactions Catalyzed by Phosphatidylserine Synthase—Phosphatidylserine synthase was examined for its ability to catalyze a series of isotopic exchange reactions according to Cleland (27, Table III). Phosphatidylserine synthase did not catalyze an exchange reaction between L-serine and phosphatidylserine (Table III; reactions 1 and 2) nor an exchange reaction between CMP and CDP-diacylglycerol (Table III, reactions 3 and 4). The enzyme did not catalyze the hydrolysis of phosphatidylserine (Table III, reaction 5) nor the hydrolysis of CDP-diacylglycerol (Table III, reaction 6). The inability of phosphatidylserine synthase to catalyze these reactions indicate that the enzyme does not follow a ping-pong reaction mechanism (27). Phosphatidylserine synthase did not cata-
Phosphatidylserine Synthase from S. cerevisiae

Phosphatidylserine synthase activity was measured at 30 °C. Aliquots (10 μl, containing 0.018 unit) of phosphatidylserine synthase were incubated for 20 min at the indicated temperatures in a controlled-temperature water bath. After incubation, the aliquots of enzyme were placed on ice, assay components were added, and phosphatidylserine synthase activity was measured at 30 °C. U, units.

Table III
Reactions catalyzed by phosphatidylserine synthase
Reactions were measured in the standard assay buffer described in the text. The assays were run for 1 h with 1 μg of purified enzyme and the indicated reaction components. Serine-phosphatidylserine exchange reactions were measured using either L-[3-3H]serine (10,000 cpm/nmol) or [3-3H]phosphatidylserine (15,000 cpm/nmol) as substrates (10). CMP-CDP-diacylglycerol exchange reactions were measured using either [5-3H]CMP (10,000 cpm/nmol) or [5-3H]CDP-diacylglycerol (400 cpm/nmol) as substrates (10). The release of water-soluble radioactive serine or CMP was measured after a chloroform-methanol-water phase partition (6). Chloroform-soluble radioactive phosphatidylserine or CDP-diacylglycerol was measured after a chloroform-methanol-water phase partition (6).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Incorporated or released</th>
<th>nmol</th>
</tr>
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<tbody>
<tr>
<td>1. 0.5 mM L-[3-3H]serine + 0.5 mM phosphatidylserine</td>
<td>&lt;0.02</td>
<td></td>
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<tr>
<td>2. 0.5 mM L-serine + 0.5 mM [3-3H]phosphatidylserine</td>
<td>&lt;0.02</td>
<td></td>
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<tr>
<td>3. 0.5 mM [5-3H]CMP + 0.5 mM CDP-diacylglycerol</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>4. 0.5 mM CMP + 0.5 mM [5-3H]CDP-diacylglycerol</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>5. 0.5 mM [3-3H]phosphatidylserine hydrolysis</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>6. 0.5 mM [5-3H]CDP-diacylglycerol hydrolysis</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>7. 5.0 μM CMP + 0.5 mM [5-3H]CDP-diacylglycerol</td>
<td>&lt;0.02</td>
<td></td>
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<tr>
<td>8. 0.5 mM [5-3H]CMP + 0.5 mM CDP-diacylglycerol + 0.5 mM L-serine</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>9. 0.5 mM L-serine + 0.5 mM [3-3H]phosphatidylserine + 0.5 mM CDP-diacylglycerol</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>10. 0.5 mM L-[3-3H]serine + 0.5 mM phosphatidylserine + 0.5 mM CDP-diacylglycerol</td>
<td>3.90</td>
<td></td>
</tr>
<tr>
<td>11. 0.5 mM CDP-diacylglycerol + 0.5 mM L-[3-3H]serine</td>
<td>42.30</td>
<td></td>
</tr>
<tr>
<td>12. 0.5 mM CDP-diacylglycerol + 0.5 mM L-[3-3H]serine + 0.5 mM phosphatidylserine</td>
<td>43.90</td>
<td></td>
</tr>
<tr>
<td>13. 5.0 μM CMP + 0.5 mM [3-3H]phosphatidylserine</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>14. 5.0 μM CMP + 0.5 mM [3-3H]phosphatidylserine + 0.5 mM L-serine</td>
<td>4.49</td>
<td></td>
</tr>
<tr>
<td>15. 5.0 μM [5-3H]CMP + 0.5 mM phosphatidylserine + 0.5 mM CDP-diacylglycerol</td>
<td>&lt;0.02</td>
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</table>

FIG. 4. Thermal stability of phosphatidylserine synthase activity. Aliquots (10 μl, containing 0.018 unit) of phosphatidylserine synthase were incubated for 20 min at the indicated temperatures in a controlled-temperature water bath. After incubation, the aliquots of enzyme were placed on ice, assay components were added, and phosphatidylserine synthase activity was measured at 30 °C. U, units.

DISCUSSION
The study of phosphatidylserine synthase in yeast is important because the enzyme plays a role in the regulation of phospholipid metabolism and yeast growth (1, 2). In this paper, we describe the purification and preliminary characterization of phosphatidylserine synthase from wild-type strain S288C and from strain VAL2C(YEpCHO1) which contains the structural gene for phosphatidylserine synthase on the YEp13 plasmid (7). This is the first report of the purification of the membrane-associated phosphatidylserine synthase from a eukaryotic organism. Successful purification of phosphatidylserine synthase from S. cerevisiae required the solubilization of the enzyme from the microsomal fraction with detergent, CDP-diacylglycerol-Sepharose affinity chromatography, and ion-exchange chromatography with DE-53. The availability of strain VAL2C(YEpCHO1) facilitated the acquisition of larger amounts of pure enzyme. Phosphatidylserine synthase required a 370-fold purification from strain VAL2C(YEpCHO1) compared to approximately a 2000-fold purification from strain S288C to attain similar final specific activities of pure enzyme with a subunit molecular weight of 23,000. These results further support that phosphatidylserine synthase is overproduced, as opposed to being activated, in strain VAL2C(YEpCHO1).

The enzymological properties of phosphatidylserine synthase purified from S. cerevisiae were similar to those reported for pure phosphatidylserine synthase from E. coli with respect to pH optimum, the dependence on Triton X-100 for maximum activity, and kinetic properties (10, 13). On the other hand, there are differences between phosphatidylserine synthase from S. cerevisiae and phosphatidylserine synthase from E. coli. The subunit molecular weight of the yeast enzyme was 23,000 while the subunit molecular weight of the E. coli enzyme is 54,000 (10). At 30 °C, the turnover number (90

lyze exchange between CMP and CDP-diacylglycerol in the presence of either phosphatidylserine (Table III, reaction 7) or L-serine (Table III, reaction 8). On the other hand, phosphatidylserine synthase did catalyze exchange between L-serine and phosphatidylserine in the presence of either CDP-diacylglycerol (Table III, reaction 9) or CMP (Table III, reaction 10). In addition, phosphatidylserine synthase stimulated the incorporation of labeled L-serine into phosphatidylserine in the forward reaction (Table III, reaction 12) and L-serine stimulated the release of labeled L-serine from phosphatidylserine in the reverse reaction (Table III, reaction 14). According to Cleland (27), the results in Table III would suggest that phosphatidylserine synthase in S. cerevisiae catalyzes a sequential reaction mechanism with phosphatidylserine released before CMP in the reaction sequence. In the forward reaction, phosphatidylserine synthase may bind to CDP-diacylglycerol before L-serine in the reaction sequence as evidenced by purification on CDP-diacylglycerol-Sepharose (Table I). The lack of exchange between CMP and CDP-diacylglycerol in the presence of phosphatidylserine (Table III, reaction 7) might indicate that the binding of CDP-diacylglycerol in the forward reaction is ordered. However, this lack of exchange may also be due to the inhibition of the reverse reaction by CDP-diacylglycerol (Table III, reaction 15).

The ability of the yeast phosphatidylserine synthase to catalyze the calcium-dependent phospholipid-base exchange reaction for the synthesis of phosphatidylserine was examined under the optimal assay conditions reported for the mammalian base exchange enzymes (28). Phosphatidylserine synthase did not catalyze the exchange of L-[3-3H]serine for ethanolamine in phosphatidylethanolamine.
min\textsuperscript{−1}) for phosphatidylserine synthase from yeast was about 18-fold lower than the turnover number (1650 min\textsuperscript{−1}) for the enzyme from E. coli (10). Phosphatidylserine synthase purified from yeast required the addition of divalent metal ions for in vitro activity. In contrast, there is no cation requirement for the assay of phosphatidylserine synthase from E. coli (10, 28). There is evidence which indicates a probable difference in the reaction mechanism between phosphatidylserine synthase derived from S. cerevisiae and the enzyme from E. coli. Based on a series of exchange reactions catalyzed by phosphatidylserine synthase from S. cerevisiae, the enzyme appears to follow a sequential Bi Bi reaction mechanism. On the other hand, the enzyme from E. coli follows a ping-pong reaction mechanism (10, 29). An additional indication of differing reaction mechanisms between both enzymes is that phosphatidylserine synthase from S. cerevisiae binds to CDP-diacylglycerol-Sepharose, while the enzyme from E. coli does not bind to the affinity resin (10). Yeast phosphatidylserine synthase is sensitive to thioeactive agents while E. coli phosphatidylserine synthase (partially purified) is not affected by 4 mM N-ethylmaleimide (9). The enzyme from yeast is an integral membrane protein (3, 6) while the enzyme from E. coli is associated with ribosomes in cell extracts (14). Phosphatidylserine synthase from E. coli is not associated with ribosomes during catalysis (30) and is believed to be a peripheral membrane protein (30, 31). The differences between the enzymes from yeast and from E. coli indicate that two distinct forms of phosphatidylserine synthase catalyze the same overall reaction. Furthermore, the cloned phosphatidylserine synthase structural gene from E. coli (11, 12) shows no homology to the cloned CHO1 gene from S. cerevisiae.\textsuperscript{1} Phosphatidylserine synthase is an integral membrane protein in Gram-positive bacteria (32–35) and has been partially purified from Clostridium perfringens (36). The intracellular location and catalytic properties of phosphatidylserine synthase from Gram-positive bacteria more closely resemble those of phosphatidylserine synthase from S. cerevisiae than those of E. coli.

Higher eukaryotic organisms do not have phosphatidylserine synthase activity. Instead they synthesize phosphatidylserine by an exchange reaction between phosphatidylethanolamine and free serine (28). The purified phosphatidylserine synthase from S. cerevisiae did not catalyze this base exchange reaction. The evolutionary significance of the loss of phosphatidylserine synthase in animals and plants is unclear.

In summary, we have purified the membrane-bound form of phosphatidylserine synthase from S. cerevisiae and have found its basic enzymological properties. A liposome system containing CDP-diacylglycerol in the absence of Triton X-100 is being developed to study the kinetic and physical properties of the enzyme.

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\textsuperscript{1} V. A. Letts, personal communication.

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