Phospholipids Chiral at Phosphorus. Steric Course of the Reactions Catalyzed by Phosphatidylserine Synthase from Escherichia coli and Yeast†

Christian R. H. Raetz
Department of Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706

George M. Carman
Department of Food Science, New Jersey Agricultural Experiment Station, Rutgers, The State University, New Brunswick, New Jersey 08903

William Dowhan
Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas 77225

Ru-Tai Jiang, Wieslaw Waszkuc, William Loffredo, and Ming-Daw Tsai*
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

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ABSTRACT: The steric courses of the reactions catalyzed by phosphatidylserine (PS) synthase from Escherichia coli and yeast were elucidated by the following procedure. Rₘ and Sₘ isomers of 1,2-dipalmitoyl-sn-glycero-3-[¹⁰⁵]O,¹⁰⁶]O-phosphoethanolamine ([¹⁰⁵]O,¹⁰⁶]O)DPPE were synthesized with slight modification of the previous procedure [Bruzik, K., & Tsai, M.-D. (1984) J. Am. Chem. Soc. 106, 747–752] and converted to (Rₘ)- and (Sₘ)-1,2-dipalmitoyl-sn-glycero-3-[¹⁰⁵]O,¹⁰⁶]O]phosphoric acid ([¹⁰⁵]O,¹⁰⁶]O)DPPA, respectively, by incubating with phospholipase D. Condensation of [¹⁰⁵]O,¹⁰⁶]O]DPPA with cytidine 5'-monophosphomorpholidate in pyridine gave the desired substrate for PS synthase, [¹⁰⁵]O,¹⁰⁶]O]cytidine 5'-diphospho-1,2-dipalmitoyl-sn-glycerol ([¹⁰⁵]O,¹⁰⁶]O)CDP-EPG, as a mixture of several isotopic and configurational isomers. Incubation of [¹⁰⁵]O,¹⁰⁶]O]CDP-EPG with a mixture of L-serine, PS synthase (which converted [¹⁰⁵]O,¹⁰⁶]O]CDP-EPG to phosphatidylserine), and PS decarboxylase (which catalyzes decarboxylation of phosphatidylserine) gave [¹⁰⁵]O,¹⁰⁶]O]DPPE. The configuration and isotopic enrichments of the starting [¹⁰⁵]O,¹⁰⁶]O]DPPE and the product were analyzed by ³¹P NMR following trimethylsilylation of the DPPE. The results indicate that the reaction of E. coli PS synthase proceeds with retention of configuration at phosphorus, which suggests a two-step mechanism involving a phosphatidyl-enzyme intermediate, while the yeast PS synthase catalyzes the reaction with inversion of configuration, which suggests a single-displacement mechanism. Such results lend strong support to the ping-pong mechanism proposed for the E. coli enzyme and the sequential Bi-Bi mechanism proposed for the yeast enzyme, both based on previous isotopic exchange experiments.

Table I: Properties of PS Synthases from Different Sources

<table>
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<th>Location</th>
<th>Ribosomes</th>
<th>Membrane</th>
<th>Membrane</th>
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</thead>
<tbody>
<tr>
<td>Mol wt (subunit)</td>
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<td>53000</td>
<td>23000</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>None</td>
<td>Mn²⁺</td>
<td>Mn²⁺ or Mg²⁺</td>
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<tr>
<td>Turnover no. (min⁻¹)</td>
<td>3000</td>
<td>4</td>
<td>90</td>
</tr>
</tbody>
</table>


Scheme I: Possible Mechanisms of PS Synthase

A

B

Detailed kinetic analysis is usually difficult for such enzymes requiring detergent in vitro assays, the enzyme-catalyzed
exchanges between isotopically labeled substrates and products (Cleland, 1970) have been investigated and used to suggest a ping-pong mechanism for the E. coli enzyme (Raez & Kennedy, 1974; Larson & Dowhan, 1976) and a sequential Bi-Bi mechanism for the enzyme from B. licheniformis (Dutt & Dowhan, 1985) and yeast (Bac-Lee & Carman, 1984). Such proposals would predict a two-step mechanism involving a phosphatidylinositol intermediate (Scheme 1A) for the E. coli enzyme and a single-displacement reaction (Scheme 1B) in the other two cases.

Stereochemical studies may support or refute the above proposed mechanisms. It has been generally established that, except in special cases involving a 1,2-migration (Buchwald et al., 1984), a single-displacement catalyzed by enzymes will result in inversion of configuration at phosphorus, whereas a double-displacement reaction will result in retention (for reviews, see Eekstein (1985), Gerl et al. (1983), Frey (1982), Tsai (1982), and Knowles (1980)). Such studies, however, require relatively large quantities of enzymes. Since strains overproducing PS synthase have been constructed for E. coli (Raez et al., 1977; Otta et al., 1981; Sparrow & Raetz, 1983) and yeast (Saccharomyces cerevisiae) (Letts et al., 1983), we set forth to elucidate the steric course of the reaction catalyzed by PS synthase from these two sources, using substrates stereospecifically labeled with $^{18}O$ and $^{13}C$.

**Materials and Methods**

**Materials.** $H_2^{31}O$ (51.0 atom % $^{18}O$, 38.6 atom % $^{13}C$) and $H_2^{18}O$ (97.5 atom % $^{18}O$) were purchased from Monsanto. BSA, CMP-morpholidotripeptide, and other biochemicals were obtained from Sigma. Bio-Sil A (200-400 mesh) was purchased from Bio-Rad. Pd/C (10%, type 1, 325 mesh) was obtained from Johnson Matthey Inc. Silicon gel (Kieselgel 60, 230-400 mesh) and precoated TLC plates (0.2-mm aluminum support, silicon gel 60, F-254) were purchased from E. Merck. All other chemicals were of reagent grade or highest purity available commercially.

**Enzymes.** The international unit "U" ($\mu$mol/min) is used to describe the activity of all enzymes used in this paper. The yeast PS synthase was purified from an overproducing strain VAL2C (YEpCHO1) of Saccharomyces cerevisiae (Bac-Lee & Carman, 1984). The purified enzyme (3.7 U/mg of protein) contained some CDP-diacylglycerol derived from egg lecithin (Carman & Fischl, 1980), which was required to stabilize the enzyme. The E. coli PS synthase (35 U/mg) was purified from an overproducing strain ACS/pRS 4017 (Sparrow & Raetz, 1983). PS decarboxylase (49 U/mg) was purified from E. coli strain JA 200/pPS26b (Li et al., 1986), as described in Dowhan et al. (1974). Phospholipase D (from cabbage, 0.3 U/mg of lyophylitate) was purchased from Boehringer.

**Analytical Methods.** Routine $^{1}H$, $^{13}C$, and $^{31}P$ NMR analysis were carried out on a Bruker WP-200 NMR spectrometer. $^{31}P$ NMR analysis of chiral $[1^{18}O]DPPA$ was performed on a Bruker AM-500 NMR spectrometer. Broad-band $^{1}H$ decoupling was used in $^{31}P$ and $^{13}C$ NMR. $^{1}H$ and $^{13}C$ chemical shifts were referenced to internal Me$_2$Si standard, while $^{31}P$ chemical shifts were referenced to external 85% H$_3$PO$_4$.

When unlabeled pyridine was used as solvent, an inner tube containing D$_2$O was used for deuteration lock. The positive sign represents a downfield shift in all cases. The justification of Gaussian multiplication in the quantitative analysis of the $^{31}P$ NMR spectra of $[1^{18}O]^{18}O]DPPA$ was described in Bruzik and Tsai (1984a).

TLC plates were visualized by spraying with a 10% ethanolic solution of phosphomolybdic acid followed by heating. The solvent systems and $R_f$ values are as follows. System A: $\text{CHCl}_3/\text{CH}_2\text{OH}/\text{H}_2\text{O}/\text{HOAc}$ (25/15/4/2); DPPA (0), CMP-morpholidotripeptide (0.21), CDP-DPG (0.6), DPPE (0.8). System B: $\text{CHCl}_3/\text{pyridine}/\text{HOAc}$ (50/20/7); DPPA (0.7).

**Syntheses of $[1^{18}O]^{31}P\text{DPPA}$ (1a and 1b).** This followed essentially the same procedure as described in Bruzik and Tsai (1984a), except the following modifications in the last two steps ($6 \rightarrow 7 \rightarrow 1$ in Scheme IV). To 1.0 g (1.28 mmol) of the dried 6 in 10 mL of freshly distilled (from lithium aluminum hydride) 1,2-dimethoxyethane (DME) was added 0.77 mL of $H_2^{18}O$, followed by dropwise addition of 1 N HCl in DME (dry HCl in dry DME) until the pH turned ca. 2 according to a pH paper. The hydrolysis was complete after stirring for ca. 1 h at room temperature as monitored by TLC (ether, $R_f$ 0.43 and 0 for 6 and 7, respectively) and by the precipitation of 7. The above procedures were performed in a drybox. The thick suspension was then evaporated in vacuo, redisolved in CHCl$_3$, and washed with aqueous NaHCO$_3$ to H$_2$O. After evaporation of the CHCl$_3$ phase the product 7 was obtained in 98% yield as a white powder, which gave $R_f$ 0.55 in EtOH/CHCl$_3$/H$_2$O (50/50/4). $^{1}H$ NMR (not reported previously) (CDCl$_3$, 200 MHz): 3.086 (t, J = 6.7 Hz, 6H, terminal CH$_2$), 1.23 (br s, 8H, long-chain CH$_3$), 1.57 (m, 4H, CH$_2$CH$_2$C=O), 1.71 (d, J = 6.8 Hz, 3H, CH$_3$CH$_2$), 2.26 (t, J = 7.5 Hz, 4H, CH$_2$C=O), 2.9 (m, 2H, OCH$_2$CH$_2$N), 3.97-4.21 (m, 3H, OCH$_2$CH$_2$N and CH$_2$CH$_2$N), 4.0 (dd, J$_{CH_2CH_2}$ = 5.4 Hz, J$_{CH_2CH_2}$ = 7.2 Hz, 1H, CH$_2$CH$_2$O), 4.06 (dd, J$_{CH_2CH_2}$ = 5.4 Hz, J$_{CH_2CH_2}$ = 7.2 Hz, 1H, CH$_2$CH$_2$O), 4.16 (dd, J$_{CH_2CH_2}$ = 6.4 Hz, J$_{H_2O}$ = 12.0 Hz, 1H, CH$_2$CH$_2$O = 0), 4.37 (dd, J$_{CH_2CH_2}$ = 3.5 Hz, J$_{H_2O}$ = 12.0 Hz, 1H, CH$_2$CH$_2$O = 0), 5.22 (m, 1H, CH$_2$CH$_2$O = 0), 7.3-7.5 (5H, phenyl). The assignments were assisted by homonuclear $^1$H decoupling.

In the hydrogenolysis of 7 by Hz over Pd/C, n-BuOH instead of acetic acid was used as solvent. The catalyst was removed by filtration through a short column of Celite 545 and washed with a 1:1 mixture of CHCl$_3$/CH$_2$OH. The product was then further purified by chromatography on silica gel.

$[1^{18}O]^{13}C$[DPPA (2a and 2b). The phospholipase D catalyzed hydrolysis of $[1^{18}O]^{13}C$[DPPA to $[3^{18}O]^{13}C$[DPPA was carried out as described in Bruzik and Tsai (1984b).

Alternatively, 7 was converted to 2 as follows. To a solution of thoroughly dried 7 (0.1 mmol) in 1 mL of freshly distilled CHCl$_3$ was added 70 µL (0.48 mmol) of iodotrimethylsilane. The reaction was complete after incubation at room temperature for ca. 3 h, as monitored by TLC, and by the formation of precipitates of byproducts. The precipitate was then filtered off and washed with cold acetone. The filtrate was evaporated to dryness and the solid residue redissolved in 1.5 mL of 95% MeOH and refluxed for 15 min. After cooling, crystalline

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1 Abbreviations: BSA, bovine serum albumin; CDP-DG, cytidine 5'-diphospho-1,2-diacyl-sn-glycerol; CDP-DPPE, cytidine 5'-diphospho-1,2-diacyl-sn-glycerol; CMP-morpholidotripeptide, cytidine 5'-mono-phosphomorpholidotripeptide; DME, 1,2-dimethoxyethane; DPPA, 1,2-diacyl-sn-glycerol-3-phosphoric acid; DDPE, 1,2-diacyl-diacyl-sn-glycerol-3-phosphoethanolamine; DPPS, 1,2-diacyl-diacyl-sn-glycerol-3-phosphoserine; KP$_2$, potassium phosphate; PS, phosphatidyisoleine; Me$_2$Si, iodotrimethylsilane.

2 Although solvent system A is adequate for identification of CDP-DPG on TLC plates, an attempt to purify CDP-DPG on a silica gel column using solvent system A resulted in decomposition of the compound. The decomposed product, though characterized, gave the same $R_t$ on the TLC system A. Thus, it is possible that the $R_t$ value reported for CDP-DPG is that of the decomposed product. Indeed the spot at $R_t$ 0.6 for CDP-DPG was always accompanied by other spots at the origin and the solvent front.
DPPA formed, in 68% yield after filtration and drying, and was characterized by TLC and $^{1}H$ NMR.

$[^{17}O,^{16}O]^{1}CDP$-DPG (3). The $[^{16}O,^{17}O,^{18}O]$DPPA obtained from the phospholipase D reaction, possibly as a calcium salt, was converted to the acid form as follows: 122 mg of DPPA was dissolved in 2 mL of CHCl$_3$ and added with 3 mL of 6/4 CH$_3$OH/H$_2$O. The upper (aqueous) layer was adjusted to pH 2.5 with 0.1 N HCl and the mixture shaken. The CHCl$_3$ layer was then separated, washed with 3 $\times$ 3 mL of 25/75 CH$_3$OH/H$_2$O, evaporated, and dried in vacuo (0.01 mmHg) over P$_2$O$_5$ to give 109 mg of white powder (one spot on TLC, system B).

The CMP-morpholodite from Sigma gave two spots on TLC and several $^{31}$P NMR signals. It was purified as follows [modified from Turcotte et al. (1980)]: 300 mg was dissolved in 3 mL of CH$_3$OH and mixed with 30 mL of anhydrous ether. The precipitate was centrifuged down and the solvent discarded. The procedure was repeated several times until the precipitate (white powder after drying in vacuo over P$_2$O$_5$) gave a single spot on TLC (system A).

The purified reactants (0.17 mmol each) were placed in a 50-mL flask, further dried for 20 h under 0.01 mmHg, and added with 15 mL of pyridine (distilled over CaH$_2$ in the vacuum line). The flask was then transferred under dry nitrogen to a drybox and stirred at room temperature. The reaction was monitored by TLC and by $^{31}$P NMR (directly in pyridine, δ 2.2 for DPPA, 6.8 for CMP-morpholodite, and -9.8 and -10.1 for CDP-DPG). The conversion was 96% at 24 h, 75% at 54 h, and complete after 5-10 days. In some runs ca. 5-10% excess of CMP-morpholodite was used to ensure full conversion of 2 to 3. After evaporation of pyridine under vacuum, the product was used directly as a substrate of PS synthase without further purification.

**Coupled Reactions of PS Synthase (E. coli) and PS Decarboxylase.** The reaction mixture was prepared by mixing the following solutions: 37 mg of 3 in 15 mL of Triton X-100, 15 mL of 0.5 M potassium phosphate (KP) buffer (pH 7.0), 15 mL of 0.5% BSA (5 mg/mL), 15 mL of 10 mM l-serine (neutral form), 15 mL of H$_2$O, 200 μL of E. coli PS synthase (40 μg/mL, in 0.1 M KP buffer, pH 7.0, containing 1 mg/mL of BSA), and 200 μL of PS decarboxylase (200 μg/mL, in the same buffer as PS synthase). The progress of the reaction was monitored by TLC. After incubation at 25 °C for ca. 40 h, the reaction was largely complete, and the reaction mixture was mixed with 85 mL of CHCl$_3$/CH$_3$OH (1/1). The upper layer (water/methanol) was then adjusted to pH 1.5 with concentrated HCl and shaken again. The organic layer was collected and the aqueous layer further washed with 2 $\times$ 30 mL of CHCl$_3$. The CHCl$_3$ fractions were combined, evaporated in vacuo, and loaded onto a Bio-Sil A column (10-mm diameter, 250-mm height). After Triton-X100 was washed off with ca. 150 mL of CHCl$_3$/CH$_3$OH (95/5), DPPE was eluted off by CHCl$_3$/CH$_3$OH (70/30) in 60% yield and characterized by TLC and $^{1}H$ NMR.

**Coupled Reactions of PS Synthase (Yeast) and PS Decarboxylase (3-5).** A 25-mg portion of 3 was dissolved in 7 mL of 1% Triton X-100 and mixed with 60 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 0.6 mM MnCl$_2$ and 12.5 mg of serine. The reaction was started by addition of 12 mL (in the case of Figure 3a) or 20 mL (in the case of Figure 3b) of PS synthase from yeast (3 μg/mL) in 20 mL Tris-HCl buffer, pH 8.0 (containing 10 mM MgCl$_2$, 0.3 mM MnCl$_2$, 20% glycerol, 1.5 mM CDP-DG, 10 mM 2-mercaptoethanol, and 0.5% Triton X-100), and 100 μL of PS decarboxylase from E. coli. The reaction mixture was incubated at room temperature for 24 h and monitored and worked up as described above. The yield was ca. 60%.

**RESULTS AND DISCUSSION**

*General Approach.* In principle, we would synthesize $[^{17}O,^{18}O]CDP$-DG of known configuration and determine the configuration of the product, as shown in Scheme II. Our strategy is outlined in Scheme III. Since we were able to synthesize $[^{17}O,^{18}O]$DPPE and analyze its configuration (Bruzik & Tsai, 1982, 1984a), our procedure started with $[^{17}O]DPPE$ (1) as a precursor for $[^{17}O,^{18}O]$CDP-DPG (3) (detailed synthetic procedures are described in the next section). Since the procedure involved $[^{16}O,^{17}O,^{18}O]$DPPA (2)
as an intermediate, the resulting $[\text{O}^{15} Tray: the image contains a chemical structure diagram with several labeled components, including various atoms and functional groups, such as $\text{H}_2\text{C} - \text{C} = \text{O}$, $\text{H}_2\text{O}$, and $\text{H}_2\text{Pd} - \text{C}$.

**Figure 1:** $^{31}$P NMR analyses of (a) $(R_p)-[\text{O}^{15} Tray: the image contains a chemical structure diagram with several labeled components, including various atoms and functional groups, such as $\text{H}_2\text{C} - \text{C} = \text{O}$, $\text{H}_2\text{O}$, and $\text{H}_2\text{Pd} - \text{C}$.

**Table II: Summary of Isotopic and Configurational Analyses of Chiral $[\text{O}^{15} Tray: the image contains a chemical structure diagram with several labeled components, including various atoms and functional groups, such as $\text{H}_2\text{C} - \text{C} = \text{O}$, $\text{H}_2\text{O}$, and $\text{H}_2\text{Pd} - \text{C}$.

**Scheme IV:**

- $\text{H}_2\text{C} - \text{C} = \text{O}$
- $\text{H}_2\text{O}$
- $\text{H}_2\text{Pd} - \text{C}$

**Table I:**

<table>
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<th>Isotope</th>
<th>Position</th>
<th>Ratio of Intensity</th>
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<td>$\text{H}_2\text{C} - \text{C} = \text{O}$</td>
<td>A</td>
<td>17</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>B</td>
<td>47</td>
</tr>
<tr>
<td>$\text{H}_2\text{Pd} - \text{C}$</td>
<td>C</td>
<td>95</td>
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**Enzymatic Conversion of $[\text{O}^{15} Tray: the image contains a chemical structure diagram with several labeled components, including various atoms and functional groups, such as $\text{H}_2\text{C} - \text{C} = \text{O}$, $\text{H}_2\text{O}$, and $\text{H}_2\text{Pd} - \text{C}$.

**The PS synthase from yeast requires CDP-DG for stabilization (Bae-Lee & Carman, 1984).** Thus, the enzyme solution contained ca. 1.5 mM of unlabeled CDP-DG derived...
from egg lecithin (Carman & Fischl, 1980). Since the enzyme may be denatured upon removal of CDP-DG, no attempt was made to remove unlabeled CDP-DG. Thus, the product \([^{1}O,^{18}O]DPPE\) was contaminated with a substantial amount of unlabeled phosphatidylethanolamine derived from egg lecithin. The \(^{31}P\) NMR analysis of \([^{1}O,^{18}O]DPPE\) obtained from the reaction catalyzed by PS synthase from yeast is shown in Figure 3.

**Stereochemical Analysis.** The quantitative analysis of the isotopic enrichments and configuration of 1a and 1b has been explained in detail previously (Bruzik & Tsai, 1984a). The method involves silylation of one of the two diasteropic oxygen atoms of the phosphate group to give two sets of species, A and B, as shown in Figure 1. Since all species containing \(^{17}O\) will not give rise to a detectable sharp \(^{31}P\) NMR signal due to the quadrupolar effect of the \(^{17}O\) nucleus (Tsai & Bruzik, 1983), the \(^{31}P\) NMR spectrum consists of eight peaks due to unlabeled species, A and B, and 

\(^{18}O\)-containing species, Ab, An, Abn, Bb, Bn, and Bbn (b and n designate bridging and nonbridging \(^{18}O\), respectively). The \(^{18}O\)-induced isotope shifts in \(^{31}P\) NMR have been reviewed by Cohn (1982). It is important to note that in the \(^{31}P\) NMR analysis of \((R_p)-[^{17}O,^{18}O]DPPE\) (Figure 1a) we are observing predominately \((R_p)-[^{16}O,^{18}O]DPPE\) (Ab and Bn) and \([^{18}O]DPPE\) (Abn and Bbn), while in that of \((S_p)-[^{17}O,^{18}O]DPPE\) (Figure 1b) the predominant species are \((S_p)-[^{16}O,^{18}O]DPPE\) (An and Bb) and \([^{18}O]DPPE\) (Abn and Bbn).

As illustrated in Scheme III, the \([^{17}O,^{18}O]DPPE\) obtained from \((R_p)-[^{17}O,^{18}O]DPPE\) (1a) should consist of ca. 33% each of \((R_p)-[^{17}O,^{18}O]DPPE\) (4a), \((S_p)-[^{18}O]DPPE\) (4b), and \((R_p)-[^{17}O,^{18}O]DPPE\) (4c), if the steric course of PS synthase is retention. In the \(^{31}P\) NMR analysis, \((S_p)-[^{18}O]DPPE\) (4b) is fully observable, whereas species 4a and 4c will each give rise to \(1/3 \times 33\%\) of \((R_p)-[^{17}O,^{18}O]DPPE\) (plus \(^{17}O\)-labeled, double \(^{18}O\)-labeled, and unlabeled species). Thus, the \(^{31}P\) NMR analysis will show an apparent inversion if the reaction catalyzed by PS synthase proceeds with retention of configuration. In other words, \((R_p)-[^{17}O,^{18}O]DPPE\) (1a), which gives Ab > An and Bn > Bb in \(^{31}P\) NMR analysis, will result in an opposite pattern in the product (An > Ab and Bb > Bn) in the case of retention. The relative intensities of the peaks, however, will change substantially in the products of DPPE, due to loss of 33% of both \(^{17}O\) and \(^{18}O\) isotopes during the reaction catalyzed by the PS synthase, as well as the formation of both \(R_p\) and \(S_p\) isomers in the product. The spectra shown in Figure 2 (a and b) indicate that the reaction catalyzed by the PS synthase from E. coli proceeds with retention of configuration at phosphorus. The spectra in Figure 3, though containing strong peaks due to contaminating unlabeled DPPE, clearly indicate that the steric course of the reaction catalyzed by the PS synthase from yeast is inversion, in contrast to that of the enzyme from E. coli. Since this is the first time we analyzed the configuration of \([^{17}O,^{18}O]DPPE\) obtained after random (yet stereospecific) substitution of an oxygen atom of \([^{16}O,^{18}O]DPPE\) (and \([^{18}O]DPPE\)), we confirmed the peak assignments by adding starting \([^{17}O,^{18}O]DPPE\) to samples in Figures 2 and 3, to ensure all the peaks match (spectra not shown).

The quantitative determination of isotopic enrichments at the \(^{17}O\) and the \(^{18}O\) positions, as well as the diastereomeric purity, of 1a and 1b was achieved by varying the three parameters within the proximity of theoretical values to obtain the best fit simulation, as described in Bruzik and Tsai (1984). The calculated spectra are shown on the left side of the observed spectra in Figure 1, and the data are summarized in Table I. From these three parameters, we calculated the "purity", defined as the percentage of the chirally labeled species (i.e., the M + 3 species), and the "chirality", defined as the optical purity of the chirally labeled species (Tsai, 1980; Bruzik & Tsai, 1984a). The data for 1a and 1b were then used to calculate the spectra of the products, by varying the degree of retention or inversion. The best fit spectra thus obtained are shown on the left-hand side of Figures 2 and 3. In all cases the spectra calculated on the basis of 100% ste-
respecificity agree with the observed spectra within experimental errors.

Conclusion. We have developed a procedure to elucidate the steric course of the reaction catalyzed by phosphatidyl-
serine synthase. The method may be applied to other biosynthetic enzymes of phospholipids, such as phosphatidyl-
glycerol phosphatase synthase and phosphatidylinositol synthase. The PS synthases from both E. coli and yeast were found to
involve cleavage of the P-O bond between the β-phosphorus and the αβ-bridging oxygen of CDP-diacylglycerol, with re-
tention and inversion of configuration, respectively. The results suggest the formation of a phosophatidyl-enzyme intermediate
in the E. coli enzyme and a single displacement in the yeast enzyme and support the mechanisms proposed on the basis of
isotope-exchange experiments.

References