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Steric Course of the Reaction Catalyzed by Phosphatidylserine Decarboxylase from *Escherichia coli*

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We have determined the steric course of the reaction catalyzed by the membrane-bound, pyruvoyl-dependent phosphatidylserine decarboxylase. The decarboxylation reaction was carried out in D_2O, and the resulting [^{2}H]phosphatidylethanolamine was degraded by phospholipase D to give 2-amino-[2-$^2H_1$]ethanol. The configuration of the latter was determined by derivatizing with (−)-camphanoyl chloride to give *N,O*-dicamphanoyl-2-amino-[2-$^2H_1$] ethanol, followed by ^{1}H NMR analysis, according to an established procedure. The result indicated the reaction is completely stereospecific, with overall *retention* of configuration. Thus, this membrane-bound decarboxylase shows the same stereochemical property as most soluble decarboxylases.

INTRODUCTION

Phosphatidylserine (PS) decarboxylase from *Escherichia coli* is an integral membrane enzyme which catalyzes the decarboxylation of phosphatidylserine to yield phosphatidylethanolamine (PE) (*1*). PS decarboxylase possesses a covalently bound pyruvoyl cofactor (*2*) which is believed to be involved in a Schiff base intermediate during catalysis in a manner analogous to other pyruvoyl-containing enzymes and similar to the pyridoxal phosphate (PLP)-dependent decarboxylases (*3*).

The decarboxylation catalyzed by PLP-dependent enzymes usually occurs with retention of configuration (*3, 4*), but exceptions have been observed in *meso*-diaminopimelate decarboxylase (*5, 6*) and in aspartate β-decarboxylase (*7*). The steric course of two of the pyruvate-dependent decarboxylases, histidine decarboxylase (*8, 9*) and *S*-adenosylmethionine decarboxylase (*10*), have also been elucidated, both in the retention mode. Since both PS decarboxylase and its substrate are thought to be intimately associated with membranes (*1*), it is interesting to speculate that there may be "mechanistic imperatives" (*11*) associated with this decarboxylase which are not present for the soluble decarboxylases. Since enzymatic stereochemistry is thought to be susceptible to the influences of

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mechanistic imperatives (*12, 13*), we investigated the steric course of PS decarboxylase-catalyzed reaction and report it herein.

EXPERIMENTAL

Materials. Phosphatidylserine (**1**) from bovine brain was obtained from Sigma. (−)-Camphanoyl chloride and D_2O, (99.8 at.% D) were purchased from Aldrich. Silica gel (Kiesegel 60, 230–400 mesh) and precoated thin-layer chromatography (TLC) plates (0.2-mm aluminum support, silica gel 60, F-254) were products of Merck. All other chemicals were of reagent grade. The international unit U (μmol/min) is used to describe the activity of enzymes used in this study. Phospholipase D (from cabbage, 0.3 U/mg of lyophilate) was obtained from Boehringer. PS decarboxylase (49 U/mg) was purified from *E. coli* strain JA 200/pPSD2b (*14*), as described in Dowhan *et al.* (*1*).

Methods. ^{1}H NMR spectra were recorded on Bruker AM-250 and AM-500 NMR spectrometers. Mass spectra were obtained with a VG 70-250S mass spectrometer using double focusing. TLC plates were visualized by spraying with a 10% ethanolic solution of phosphomolybdic acid followed by heating. The solvent systems and R_f values were as follows. *System A:* $CHCl_3/CH_3OH/H_2O$ (100/33/4): PS (0.3), PE (0.6), phosphatidic acid (PA) (0). *System B:* $CHCl_3/CH_3OH$ (20/1): *N,O*-dicamphanoylethanolamine (0.5).

Decarboxylation of PS. The reaction mixture was obtained by mixing the following components: 100 mg (0.127 mmol) of PS, 4 ml of 1% Triton X-100 in D_2O, 4 ml of 0.5 M potassium phosphate (KP_i) buffer (pH 7.4) in D_2O, 4 ml of bovine serum albumin (1 mg/ml) in D_2O, and 4 ml of D_2O. After stirring vigorously for 20 min at 37°C, 200 μl (2.5 U) of PS decarboxylase was added to the reaction mixture, and the mixture was incubated at 37°C without stirring. The progress of the reaction was monitored by TLC (system A). After 20 h, 600 μl (7.5 U) more of PS decarboxylase was added. At 60 h the reaction was observed to be complete. The reaction mixture was then mixed with 25 ml of $CHCl_3/CH_3OH$ (1/1), and the upper layer (D_2O and CH_3OH) was adjusted to pH 1.5 with concentrated HCl. The organic layer was collected and the aqueous layer was washed with 2 × 15 ml of $CHCl_3$. The $CHCl_3$ fractions were combined, evaporated *in vacuo,* and loaded onto a silica gel column (20-mm diameter, 40-cm height). The product [^{2}H]PE · HCl (**2**) was eluted by $CHCl_3/CH_3OH$ (3/1) in 85% yield (84.5 mg) and characterized by TLC and ^{1}H NMR.

*Conversion of **2** to **3** by phospholipase D.* This was performed according to the procedure of Bruzik and Tsai (*15*), except for the isolation of **3**. A mixture of 80 mg (0.1 mmol) of [^{2}H]PE · HCl (**2**) in 1.6 ml of 0.1 M sodium acetate buffer (pH 5.6) containing 0.1 M $CaCl_2$ and 4 ml of ethanol-free diethyl ether was stirred until both phases became apparently clear. Phospholipase D (12 mg) was then added, and the reaction monitored by TLC. The reaction was complete after stirring for 2 days. Diethyl ether was then evaporated and the aqueous layer was adjusted to pH 1.5 by 2 N HCl. One of the products, PA, was removed by extraction of the acidic aqueous solution with chloroform/methanol (1/1, 2 × 2.0 ml). The aqueous

layer was adjusted to pH 12.5 and then sublimed *in vacuo* (0.01 mm Hg) into a chilled receiving flask. The resulting solution was then acidified to pH 1.5 and lyophilized to give a white solid, (2*R*)-2-amino-[2-$^2H_1$]ethanol hydrochloride (**3**) (7.6 mg, yield 76%). ^1H NMR (D_2O, 250 MHz): δ 3.05 (m, 1H, CHDN), 3.73 (d, J = 5.2 Hz, 2H, CH_2OD).

Synthesis of N,O-dicamphanoyl-(2R)-2-amino-[2-$^2H_1$]ethanol (**4**). After dissolution of **3** (3.5 mg, 35 μmol) into 0.14 ml of dry pyridine, (−)-camphanoyl chloride (35.0 mg) and 1.0 ml of dry diethyl ether were added. The solution was stirred vigorously for 2 days at room temperature. Addition of water (1 ml), followed by solvent removal *in vacuo,* gave a residue, which was redissolved in chloroform (1 ml) and washed successively with 2 N HCl (1 ml), saturated sodium bicarbonate solution (2 ml × 2), and water (1 ml × 2). The solution was dried over Na_2SO_4 and filtered, and the solvent was removed to yield the crude product (6.3 mg, 42%). Column chromatography (silica gel, 10-mm diameter, 15-cm height) with $CHCl_3/CH_3OH$ (20/1) gave 5.2 mg (35%) of the product **4**. ^1H NMR ($CDCl_3$, 500 MHz): δ 0.89 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.78 and 1.96 (m, $-CH_2CH_2-$), 3.66 (q, 1H, 2-CHN), 4.33 (m, 2H, 1-CH_2O), 6.66 (br, 1H, NH). m/z 422.24 (calcd for $C_{22}H_{31}NO_5D_1$ 422.22). The nondeuterated *N,O*-dicamphanoylethanolamine was also synthesized by the same procedure.

RESULTS AND DISCUSSION

The procedure for the stereochemical study is outlined in Fig. 1. Reaction of phosphatidylserine (**1**) with PS decarboxylase in D_2O gave [^2H]phosphatidylethanolamine (**2**). In order to determine the configuration of the deuterated carbon, we used phospholipase D to hydrolyze **2**. The desired product, 2-amino-[2-$^2H_1$] ethanol (**3**) was isolated as an HCl salt, as described in the Experimental section. The configuration of **3** could then be analyzed by derivatizing with (−)-camphanoyl chloride[2] to give **4**, followed by ^1H NMR analysis, according to established procedures (*16, 17*).

Figure 2 shows the ^1H NMR analysis of nondeuterated **4** (A) and the product obtained from the above reactions (B). The chemical shift assignment was made according to Gani *et al.* (*17*). It is clear that the deuterium is labeled at the pro-*R* position at carbon 2, as shown in Fig. 2B. The result indicates that the configuration of **3** is 2*R*, and that PS decarboxylase catalyzes the decarboxylation with overall *retention* of configuration. Thus, this integral membrane enzyme appears to follow the same steric course as most of the soluble decarboxylases. The reaction is also fully stereospecific. As shown in the inset of Fig. 2B, the residual ^1H signal at the deutereated position possesses the same coupling pattern as the

[2] The (−)-camphanoyl chloride used was purchased from Aldrich, and was the same optical isomer as the 1S isomer used in Gani *et al.* (*17*). It should be noted that in some of the Aldrich products (e.g., Lot MM 00521EL) (−)-camphanoyl chloride was erroneously labeled as (1*R*)-(−)-camphanoyl chloride. We have checked the optical rotation of the camphanoyl chloride to make sure that it is indeed the (−)-isomer.

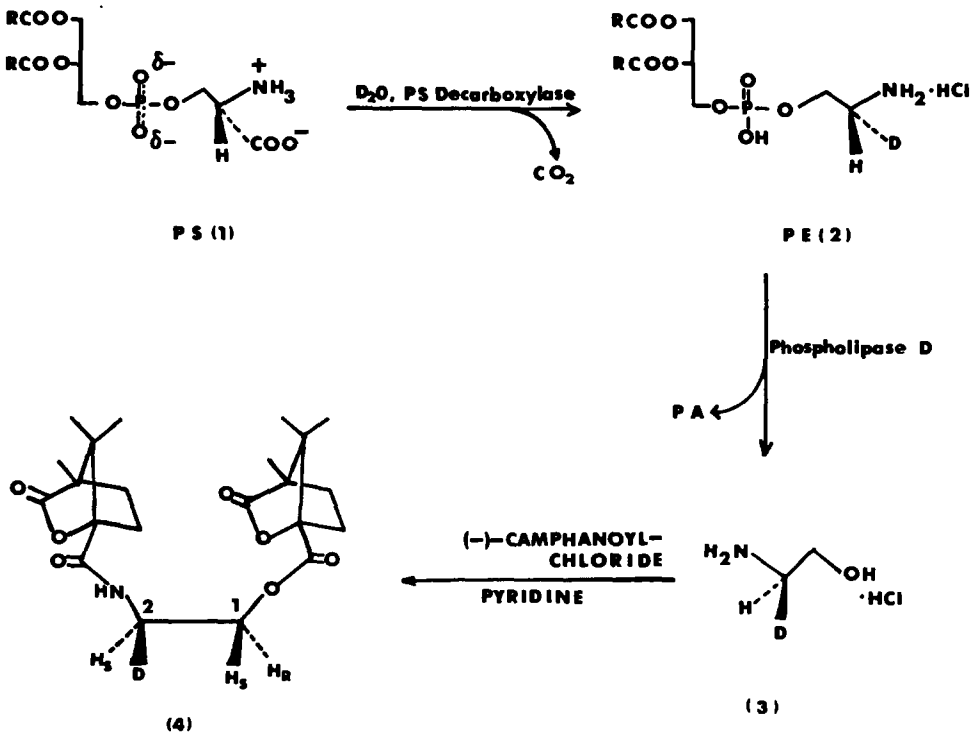

FIG. 1. Outline of the reaction catalyzed by PS decarboxylase and the procedure for stereochemical analysis.

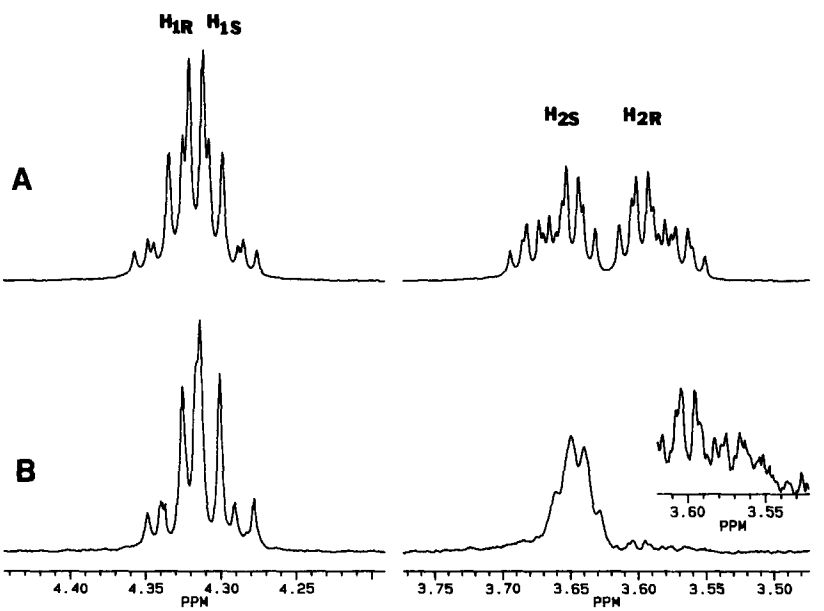

FIG. 2. Partial ^1H NMR spectra (500 MHz, $CDCl_3$) of *N,O*-dicamphanoyl-2-aminoethanols. A is from nonlabeled ethanolamine; B is compound **4** from deuterated ethanolamine as outlined in Fig. 1. The inset in B shows the residual signal with expanded *y* scale.

nondeuterated sample in Fig. 2A. This suggests that the residual signal arises from the totally nondeuterated species (due to residual H_2O and a potential solvent isotope effect in the protonation step), rather than from the 2*S* isomer of **4**.

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