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# Action of the Highly Purified, Membrane-bound Enzyme Phosphatidylserine Decarboxylase *Escherichia coli* toward Phosphatidylserine in Mixed Micelles and Erythrocyte Ghosts in the Presence of Surfactant\*

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Phosphatidylserine decarboxylase, *Escherichia coli*, was purified to near-homogeneity by the procedure of Dowhan, W., Wickner, W. T., and Kennedy, E. P. ((1974) *J. Biol. Chem.* **249**, 3079-3084) and assayed by following the production of CO<sub>2</sub> using gas chromatography. The purified enzyme has an absolute requirement for the surfactant Triton X-100. The function of Triton in the assay is evaluated and a kinetic scheme describing the action of this membrane-bound enzyme in the micellar system provided by the surfactant is presented. According to this scheme, the enzyme first binds to a mixed micelle, composed of phosphatidylserine and Triton, where the dissociation constant is  $K_s^A$ . The enzyme, now part of the mixed micelle, then binds the substrate phosphatidylserine in its active site and this binding is related to the Michaelis constant,  $K_m^B$ .  $K_s^A$ , expressed as the sum of the molar concentrations of Triton and phosphatidylserine, is about 0.04 M.  $K_m^B$ , expressed as the mole fraction of phosphatidylserine in the mixed micelles, is about 0.03. Phosphatidylserine decarboxylase activity toward phosphatidylserine in human erythrocyte ghosts was also determined. The amount of phosphatidylserine converted to phosphatidylethanolamine and CO<sub>2</sub> was found to be related to the amount of phosphatidylserine solubilized from the membrane by Triton X-100. In the absence of Triton, no significant activity of the enzyme toward the ghosts was detected even after subjecting the ghosts to lyophilization, homogenization, or sonication.

*Escherichia coli* phosphatidylserine decarboxylase, which is localized in the inner cytoplasmic membrane (1-3), plays a central role in the biosynthesis of the major membrane lipid of that organism, phosphatidylethanolamine. The enzyme can be solubilized with the nonionic surfactant Triton X-100 (4) and recently Dowhan *et al.* (5) reported the complete purification and partial characterization of the Triton-solubilized enzyme. This enzyme apparently has a requirement for Triton X-100 during assay, as well as purification (5). Since this is the only enzyme involved in the final steps of membrane phospholipid biosynthesis that is available in pure form, its mechanism of action and the nature of its apparent Triton requirement are of great interest. Phosphatidylserine decarboxylase is also of interest because of its potential as a specific probe of membrane structure and function, since it specifically degrades phosphatidylserine<sup>1</sup> and the product of its action, phosphatidylethanolamine, is not disruptive to membrane structure. Furthermore, phosphatidylserine apparently is localized

only on the inside of the erythrocyte membrane (6-9). A preparation of crude *E. coli* phosphatidylserine decarboxylase has been utilized in an attempt to elucidate the lipid requirement of the membrane-bound enzyme (Na<sup>+</sup>, K<sup>+</sup>)-ATPase (sodium and potassium ion-activated ATPase) (10, 11). However, the purified decarboxylase has not yet been exploited as a membrane probe and especially because of the apparent Triton requirement, defined assay conditions which may allow for the utilization of the purified enzyme in further investigations with natural membranes are required.

Before studies on the mechanism of action of this membrane-bound enzyme can be conducted meaningfully, it is essential that the precise function of Triton X-100 in the assay of this enzyme be understood. Recently, we (12) have reviewed our studies on the role of Triton X-100 in the assay of the soluble enzyme phospholipase A<sub>2</sub> where we have shown by a combination of NMR, gel chromatographic, and kinetic studies that Triton serves to convert phosphatidylcholine bilayers into mixed micelles in which the substrate is in the proper form for interaction with the enzyme. Furthermore, we have suggested that kinetic studies of the phospholipases in the presence of surfactants such as Triton X-100 can best be interpreted in terms of a "surface dilution model" (13). Although the action of the decarboxylase toward mixed mi-

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<sup>1</sup> The abbreviations used are: phosphatidylserine, 1,2-diacyl-*sn*-glycero-3-phosphorylserine; Triton, Triton X-100.

celles may be potentially more complex because of its membrane-bound origin, it appears that the general kinetic approaches derived from studies on phospholipase A<sub>2</sub> are directly applicable to the analysis of this enzyme and the considerations are necessary in understanding its action toward erythrocyte membranes; a preliminary report of this work has been presented (14).

#### EXPERIMENTAL PROCEDURE

**Materials**—Phosphatidylserine was extracted from fresh bovine brains by the procedure of Folch (15) and was purified as described previously (16). Triton X-100, a polydisperse preparation of *p,t*-octylphenoxy polyethoxyethanols, (Rohm and Haas Co.) was used without further purification. [<sup>3</sup>H]Triton X-100 (0.286 mCi/g) labeled in the phenyl ring was a gift from Dr. W. R. Lyman, Rohm and Haas Co., and was employed where noted. Bio-Gel A-5m (Bio-Rad) which is a 6% agarose gel, 100 to 200 mesh, was prepared according to the manufacturer's instructions. Blue dextran 2000 (Pharmacia) and KH<sub>2</sub>PO<sub>4</sub> were used to determine the void volume and total volume of the agarose gel column. All other chemicals were of reagent grade and distilled water was used routinely.

**Phosphatidylserine Decarboxylase**—Phosphatidylserine decarboxylase was purified according to the procedure of Dowhan *et al.* (5), except that dialysis was substituted for the final agarose column. Purity of the final protein was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (17) and it was judged to be near-homogeneity. The activity of this material was 0.8 unit ml<sup>-1</sup>, where 1 unit of activity is the amount of enzyme that will decarboxylate 1 μmol min<sup>-1</sup> of substrate at "standard conditions." A different, somewhat less homogeneous preparation of the decarboxylase was employed for the experiments with erythrocyte ghosts. This preparation was carried through the final agarose chromatography step and concentrated to a small volume on a DEAE-column similar to that used in Step 4 of the Dowhan procedure. The activity of this preparation of the decarboxylase was 7 units ml<sup>-1</sup>.

**Erythrocyte Ghosts**—Human blood, collected in blood packs containing citrate-phosphate-dextrose U.S.P. solution, was obtained as packed cells from the San Diego blood bank immediately after drawing. The fresh cells were washed and used immediately to prepare erythrocyte ghosts according to the procedure of Dodge *et al.* (18).

**Assay Procedure**—Phosphatidylserine decarboxylase was assayed by the detection of CO<sub>2</sub> employing a gas chromatographic assay previously described (16). Under "standard conditions," the assay system consisted of 50 mM imidazole-HCl, pH 7.0, 6 mM phosphatidylserine as the free acid, 36 mM Triton X-100, and about 1.65 μg of protein (0.015 unit), in a total volume of 0.5 ml. Incubations were conducted for 20 min at 37°. Standard assay conditions for phosphatidylserine in erythrocyte ghosts were: 50 mM imidazole-HCl, pH 7.0, 0.59 ml of packed ghosts (1 μmol of lipid phosphorus), 5 mM EDTA, and 0.07 unit of phosphatidylserine decarboxylase in a total volume of 1.0 ml. Incubations were conducted at 37° for 1 hour. With phosphatidylserine as substrate, zero time controls were included; with erythrocyte ghosts as substrate, both zero time controls and controls containing ghosts, but incubated without added enzyme were employed. Average of duplicate or triplicate determinations is reported in each case. The average error between triplicate determinations was about ±0.005 μmol of CO<sub>2</sub>. With erythrocyte ghosts, the activity of the enzyme was also determined by extracting and separating the phospholipids on two-dimensional thin layer chromatography employing a modification of the procedure of Turner and Rouser (19). The lipids were detected by development of the thin layer chromatography plates with iodine vapor and the appropriate areas were then scraped from the plates, digested with HClO<sub>4</sub> acid, and analyzed for phosphorus by a modification of the procedure of Chen *et al.* (20). Protein was determined by modification of the procedure of Lowry *et al.* (21) as suggested by Dowhan *et al.* (5).

#### RESULTS

**Kinetic Studies**—Under standard assay conditions, the activity of the decarboxylase is linear with protein and with time in the presence of standard amounts of Triton X-100 as well as at high concentrations. We have found, consistent with the work of Dowhan *et al.* (5), that the enzyme exhibits a broad pH

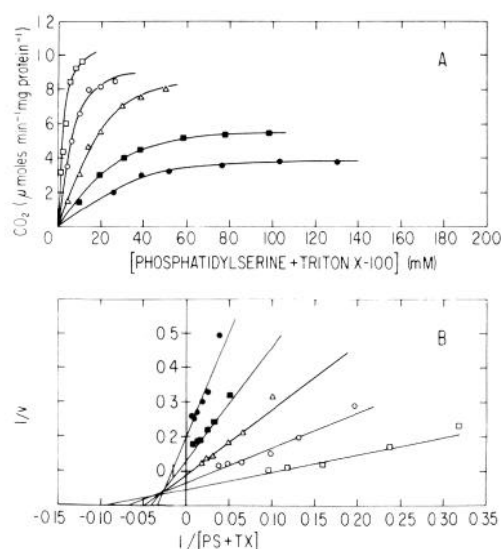


FIG. 1. A, dependence of phosphatidylserine decarboxylase on the sum of the phosphatidylserine and Triton concentrations at varying molar ratios of Triton to phosphatidylserine: (□) 6:1, (○) 16:1, (△) 32:1, (■) 64:1, and (●) 128:1. The phosphatidylserine concentrations employed were similar at all molar ratios, except at 128:1 where the highest concentration was 1.0 mM rather than 1.5 mM. B, Lineweaver-Burk representation of each plot. PS, phosphatidylserine; TX, Triton.

maximum in the region of pH 6.0 to pH 8.0 and that the enzyme has an absolute requirement for the presence of the surfactant Triton X-100. With 6 mM phosphatidylserine, the activity was optimal at a surfactant concentration of 36 mM; this corresponds to a molar ratio of Triton to phosphatidylserine of 6:1. As the concentration of Triton X-100 was increased above 36 mM, the activity of the decarboxylase decreased.

In Fig. 1, the dependence of the activity of the decarboxylase on the phosphatidylserine concentration is shown at various molar ratios of Triton to phospholipid above 6:1. The activity is plotted as a function of the sum of the molar concentrations of Triton and phosphatidylserine (Fig. 1A). Saturation curves are obtained which give linear double reciprocal plots (Fig. 1B). The intercepts on the 1/v axis give a value for the apparent V at each mole ratio. These values correspond to saturation of the enzyme with the sum of Triton X-100 plus phosphatidylserine at that mole ratio. When these apparent V values are plotted as a function of the mole fraction of phosphatidylserine present, a saturation curve is obtained (Fig. 2A) which gives a linear double reciprocal plot (Fig. 2B). The V at an infinite mole fraction of phosphatidylserine, which is the true V for the enzyme, can be obtained from this plot; it is 25 μmol min<sup>-1</sup> mg protein<sup>-1</sup>.

A model for the action of this enzyme toward phosphatidylserine in mixed micelles is presented under "Discussion." This model predicts that replots of the 1/v intercepts and slopes (in Fig. 1B) versus 1/mole fraction of phosphatidylserine would be linear and would give values of V, K<sub>s</sub><sup>A</sup>, and K<sub>m</sub><sup>B</sup> as indicated in Fig. 2, B and C. From these plots, the value of K<sub>s</sub><sup>A</sup> is 0.04 M and the value of K<sub>m</sub><sup>B</sup> is 0.03. The value of V is given above.<sup>2</sup>

**Agarose Chromatography**—Mixtures of phosphatidylserine and Triton X-100 were subjected to agarose gel chromatography in order to monitor the effect of the surfactant on the

<sup>2</sup> It should be noted that in all plots, the lines were drawn through the data points to be consistent with the predictions of the model; least squares treatment of the data gave similar lines and values of V, K<sub>s</sub><sup>A</sup>, and K<sub>m</sub><sup>B</sup>.

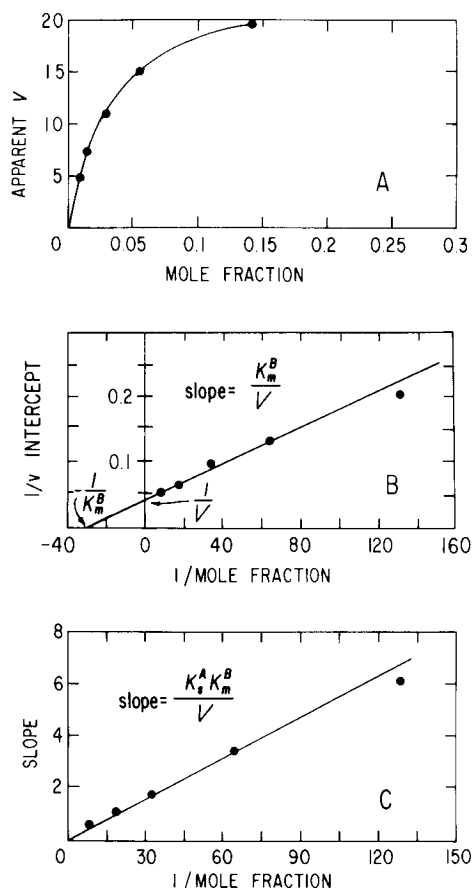


FIG. 2. A, apparent  $V$  obtained from the  $1/v$  intercepts of Fig. 1B plotted as a function of the mole fraction of phosphatidylserine present. B, Lineweaver-Burk representation of the resulting curve which is the same as plotting directly the  $1/v$  intercepts in Fig. 1B versus  $1/\text{mole fraction}$  of phosphatidylserine. C, replot of the slopes in Fig. 1B as a function of  $1/\text{mole fraction}$  of phosphatidylserine.

physical state of the phospholipid. The elution pattern of Triton X-100 micelles is shown in Fig. 3 (A). Mixed micelles at a molar ratio of about 10:1 Triton to phospholipid elute in the same size range as Triton micelles (B), but at lower molar ratios, such as 3.5:1 Triton to phospholipid, the mixed micelles elute somewhat earlier and the peak broadens, indicating an increase in the size and perhaps polydispersity of the mixed micelles (C). At a molar ratio of about 1.4:1 Triton to phospholipid (D) two peaks appear, one near the void volume (Peak I) and one at about the same elution volume as the 3.5:1 structures (Peak II). The very large structures are presumably more nearly similar in structure to phosphatidylserine bilayers which elute in the void volume (E).

**Erythrocyte Ghosts**—Phosphatidylserine decarboxylase activity toward phosphatidylserine in human erythrocyte ghosts was determined as shown in Table I. The total lipid phosphorus in 0.59 ml of packed ghosts used in a standard experiment was about 1  $\mu\text{mol}$  of which about 0.14  $\mu\text{mol}$  was present as phosphatidylserine as determined by thin layer chromatography-phosphorus analysis. As shown in Table I, little or no  $\text{CO}_2$  was released after incubation with the decarboxylase for 1 or 2 hours at 37°. Separation of the phospholipids by thin layer chromatography and analysis of lipid phosphorus confirmed that phosphatidylserine was not degraded by the decarboxylase under these assay conditions, and also indicated that lipid degradation did not occur during the incubation periods. When 18 mM Triton X-100 was included

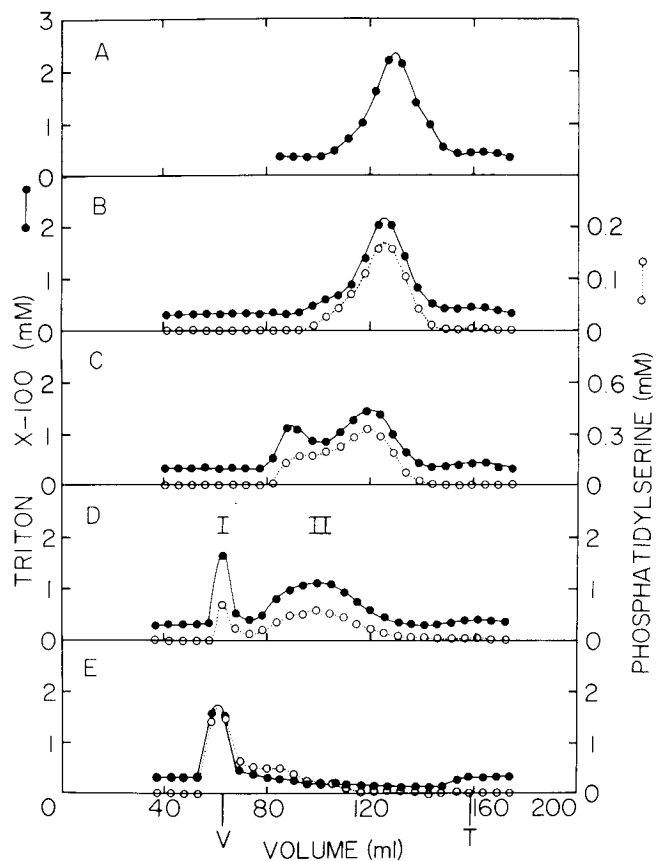


FIG. 3. Agarose gel chromatography of phosphatidylserine and [ $^3\text{H}$ ]Triton X-100 mixtures according to a procedure described elsewhere (22). The column ( $2.5 \times 50$  cm) was run at room temperature with a flow rate of about  $0.5 \text{ ml min}^{-1}$  and was pre-equilibrated with and the elution carried out with buffer consisting of 0.01 M Tris-HCl, pH 8.0, 0.1 M NaCl, 0.02% sodium azide, and 0.35 mM [ $^3\text{H}$ ]Triton X-100. The samples (1 ml) were brought to pH 8.0 with Tris-HCl before application to the column: A, 40 mM Triton, B, 40 mM Triton plus 3.8 mM phosphatidylserine; C, 40 mM Triton plus 11.5 mM phosphatidylserine; D, 40 mM Triton plus 28 mM phosphatidylserine; and E, 35 mM sonicated phosphatidylserine. The void volume (V) and total volume (T) are shown. Recovery was within  $\pm 10\%$  of that applied to the column except for Sample D in which recovery of the phospholipid applied to the column was reduced consistently.

in the assay mixture, 0.14  $\mu\text{mol}$  of  $\text{CO}_2$  was detected after 1 hour of incubation. When the phospholipids were analyzed, little phosphatidylserine could be detected and an increase in the amount of phosphatidylethanolamine consistent with almost total degradation of the phosphatidylserine was found as indicated in Table I. Note that the thin layer chromatography-phosphorus analysis gives slightly different phospholipid ratios in the controls when Triton is included during incubation than when it is not so that different controls were employed with and without Triton.

Homogenization, lyophilization, and sonication (in the presence of the decarboxylase) of ghosts did not lead to significant activity of the decarboxylase toward the phosphatidylserine unless Triton X-100 was present as shown in Table II. Although standard assay conditions included 50 mM imidazole-HCl and 5 mM EDTA, experiments were also conducted without added buffer as well as with 10 mM phosphate and 5 mM EDTA, but these altered conditions also did not lead to  $\text{CO}_2$  production unless Triton X-100 was present. Table III shows that the amount of phosphatidylserine which is decarboxylated is related to the amount of phosphatidylserine solubilized by the

TABLE I

*Phosphatidylserine decarboxylase activity toward phosphatidylserine in erythrocyte ghosts*  
Triton X-100 (18 mM) was included where indicated.

Enzyme	Triton	Time	CO <sub>2</sub> <sup>a</sup>	PE:PC <sup>b</sup>	PS:PC <sup>b</sup>	(PE + PS):PC <sup>b</sup>
		hr	μmol			
-	-	0		0.91	0.56	1.47
-	-	1	0.00	0.91	0.51	1.42
+	-	1	0.00	0.97	0.54	1.51
+	-	2	0.01	0.96	0.48	1.44
-	+	0	0.00	0.83	0.53	1.36
-	+	1	0.00	0.85	0.50	1.35
+	+	1	0.14	1.25	0.07	1.32

<sup>a</sup> Gas chromatographic assay.

<sup>b</sup> Thin layer chromatography assay. Phosphatidylethanolamine (PE) and phosphatidylserine (PS) are reported relative to the phosphatidylcholine (PC) content.

TABLE II

*Phosphatidylserine decarboxylase activity toward treated erythrocyte ghosts*

Erythrocyte ghosts were treated as described and then assayed under standard conditions. Triton X-100 (18 mM) was included during assay when indicated.

Treatment	Triton X-100	CO <sub>2</sub>
		μmol
Homogenization <sup>a</sup>	-	0.00
	+	0.12
Lyophilization <sup>b</sup>	-	0.01
	+	0.16
Sonication <sup>c</sup>	-	0.01
	+	0.15

<sup>a</sup> Subjected to several strokes with the Teflon pestle of a Potter-Elvehjem homogenization apparatus at 4°.

<sup>b</sup> Diluted 5-fold with water, lyophilized, and then resuspended with gentle shaking to its original volume of packed cells.

<sup>c</sup> Complete assay mixture including enzyme was sonicated with an MSE 100-watt Ultrasonic Disintegrator under argon at 4° for five 30-s pulses. Resulting dispersion was opalescent.

Triton X-100. Under these assay conditions, 1 to 3 mM Triton X-100 is required for significant activity and at 18 mM Triton, complete conversion of the phosphatidylserine to phosphatidylethanolamine occurs.

## DISCUSSION

### Surfactant Function

We have found that Triton X-100 stimulates the activity of phosphatidylserine decarboxylase with the maximum activation occurring at a molar ratio of Triton to phosphatidylserine of about 6:1. This stimulatory effect on activity may, in principle, be the result of surfactant-enzyme, or surfactant-substrate interactions, or both. The gel chromatographic studies show that Triton X-100 converts the phosphatidylserine multibilayers into mixed micelles of Triton and phospholipid. Although some surfactant is required to keep the enzyme soluble (5), the simplest and most straightforward explanation for the dependence of the activity on the presence of surfactant, is that the phosphatidylserine in the multibilayers is not

TABLE III

*Relationship between phosphatidylserine solubilized in erythrocyte ghosts and amount decarboxylated by phosphatidylserine decarboxylase*

Triton	CO <sub>2</sub> <sup>a</sup>	Phosphatidylserine solubilized <sup>b</sup>
mM	μmol	μmol
1	0.01	0.01
3	0.10	0.06
18	0.12	0.12

<sup>a</sup> Activity of phosphatidylserine decarboxylase toward unfractionated ghosts. Standard assay conditions were employed, except that Triton was included in the amount indicated.

<sup>b</sup> Assay mixtures without enzyme were centrifuged in a Sorvall model RC2-B centrifuge (35,000 × *g*, 37°, 1 hour). The supernatant was removed carefully and the pellet was resuspended to the original sample volume with the standard assay solution. Phosphatidylserine decarboxylase was added to each fraction and the phosphatidylserine content determined by the gas chromatographic assay under standard conditions. The CO<sub>2</sub> produced in the supernatant fraction is reported.

accessible to the solubilized form of the enzyme, while substrate which is incorporated into mixed micelles is accessible.

### Kinetic Parameters of Phosphatidylserine Decarboxylase

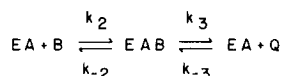
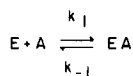
The apparent inhibitory effect of high concentrations of the surfactant on the activity of phosphatidylserine decarboxylase is probably similar to the observed inhibition by the surfactant on the activity of the soluble enzyme phospholipase A<sub>2</sub>. This inhibitory effect has been discussed in terms of a "surface dilution" model (13). According to this model, increasing the surfactant concentration dilutes the phospholipid in the surface of the mixed micelles and this causes the decreased activity of the enzyme. These considerations have led to a model for the action of phosphatidylserine decarboxylase toward mixed micelles which involves two binding steps.

**Enzyme-Micelle Association**—The solubilized phosphatidylserine decarboxylase "binds" to a mixed micelle of Triton X-100 and phosphatidylserine. The term binds is used loosely to describe the association of the solubilized enzyme with the mixed micelle. This association process may involve the integration of a Triton-containing enzyme molecule into the mixed micelle or association of the enzyme with the hydrophilic surface of a mixed micelle. The concentration of micellar binding sites is related to the sum of the molar concentrations of Triton and phosphatidylserine.

**Enzyme-Substrate Binding**—Following the initial binding to the mixed micelle, the bound enzyme binds a phosphatidylserine molecule in its active site and can then convert it to product. The concentration of the phospholipid within the microenvironment of the micelle is the relevant concentration of substrate and this is related to the mole fraction of phosphatidylserine present.

These two binding steps are indicated schematically in Equation 1. *E* is the enzyme, *A* the mixed micelle, *B* the phosphatidylserine in the mixed micelle, and *Q*, the products of decarboxylase action (phosphatidylethanolamine + CO<sub>2</sub>). An analogous scheme has been developed for the action of phospholipase A<sub>2</sub> toward mixed micelles of Triton and phosphatidylcholine and the kinetic equations for that system have been derived and analyzed in detail elsewhere.<sup>3</sup> A simplified

<sup>3</sup>R. A. Deems, B. R. Eaton, and E. A. Dennis, manuscript in preparation.



EQUATION 1

$$v = \frac{V(A)(B)}{K_s^A K_m^B + K_m^B(A) + (A)(B)}$$

$$K_s^A = \frac{k_{-1}}{k_1}$$

$$K_m^B = \frac{k_{-2} + k_3}{k_2}$$

EQUATION 2

form of this solution is shown in Equation 2.  $(A)$  is the sum of the molar concentrations of Triton and phosphatidylserine;  $(B)$  is the mole fraction of phosphatidylserine present.

Equation 2 predicts that plots of  $1/v$  versus  $1/(A)$  at constant  $(B)$  will be linear and that replots of the  $1/v$  intercepts and slopes versus  $1/(B)$  will give linear replots and values of  $V$ ,  $K_s^A$ , and  $K_m^B$  directly.  $V$ , obtained from this approach, is that which occurs at an infinite micelle concentration and an infinite concentration of phosphatidylserine in the micelles; it was found to be about  $25 \mu\text{mol min}^{-1} \text{mg protein}^{-1}$ .  $K_s^A$  which expresses the association with mixed micelles was found to be about  $0.04 \text{ M}$  and  $K_m^B$  which reflects the binding to phosphatidylserine in the mixed micelles (but is not necessarily a binding constant) was found to be about  $0.03$ . The model and kinetic scheme presented allows for a clear conceptualization and interpretation of the kinetic parameters obtained from the decarboxylase system and illustrate the type of kinetic studies required in order to obtain meaningful kinetic constants for the action of membrane enzymes in the presence of surfactant.

#### Phosphatidylserine Decarboxylase Action toward Erythrocyte Ghosts

Incubation of erythrocyte ghosts in the presence of the decarboxylase and in the absence of added surfactant does not result in the decarboxylation of the phosphatidylserine in the ghost membrane. Phosphatidylserine has been suggested to reside in the interior portion of the erythrocyte membrane bilayer (6-9). The lack of activity of the decarboxylase toward the phosphatidylserine in the ghost membranes may result from the inaccessibility of this protein to the interior portion of the erythrocyte ghost. Presumably, however, erythrocyte ghosts prepared according to the procedure of Dodge *et al.* (18) and maintained at  $4^\circ$  are permeable to large macromolecules and therefore the interior portion of the ghost membrane should be available to the decarboxylase (23-25). In order to increase the probability that the phosphatidylserine of the ghost membrane was exposed to the decarboxylase, suspensions of the erythrocyte ghosts were also homogenized, sonicated (in the presence of the enzyme), or lyophilized. However, none of these treatments resulted in significant decarboxylation of the phosphatidylserine in the ghost preparations. Thus,

the lack of activity of the enzyme toward the phosphatidylserine in the ghosts structures is probably not due to the inaccessibility of the decarboxylase to the phosphatidylserine in the bilayer and this suggests that the solubilized, purified enzyme does not interact significantly with phosphatidylserine in the intact membrane, at least under the experimental conditions employed.

A recent report showed that treatment of a lyophilized preparation of ghost membranes with a crude preparation of the decarboxylase, in the apparent absence of added surfactant, converts about 88% of the phosphatidylserine to phosphatidylethanolamine (11). Although it is apparent that the crude preparation of this enzyme will act on phosphatidylserine in ghosts while the purified enzyme will not, the degree of disruption of the ghost membrane under the assay conditions employed in that report was not elucidated. It is possible that the crude preparation of the enzyme that was employed may have contained enough residual Triton X-100 from the solubilization procedure to solubilize the phosphatidylserine in the ghosts. We have found that the purified enzyme can interact effectively with the phosphatidylserine of the ghost membrane only when Triton X-100 is present. Triton removes phosphatidylserine from the membrane and converts it into mixed micelles. Use of the phosphatidylserine decarboxylase as a probe in membrane studies would be invaluable (26), but these results suggest that any use of the purified enzyme in membrane structure studies will require a close correlation with the degree of membrane disruption.

*Acknowledgments*—We wish to thank Dr. William Dowhan and co-workers for making their manuscript (5) available to us prior to publication and for many helpful discussions with Dr. Dowhan concerning the details of the purification procedure. Also, we wish to thank Dr. Irving Crawford, Scripps Clinic and Research Foundation, for the *Escherichia coli* culture and use of their fermentor.

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