Kinetics and Protein Subunit Interactions of *Escherichia coli* Phosphatidylserine Decarboxylase in Detergent Solution†

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ABSTRACT: Phosphatidylserine decarboxylase from *Escherichia coli*, an intrinsic membrane protein, catalyzes the conversion of phosphatidylserine to phosphatidylethanolamine. The physical and kinetic properties of the purified enzyme were studied in several detergents under assay conditions. The active form of the enzyme is an oligomer, probably a trimer, and the enzyme activity was unaffected by the concentration of the nonionic poly(oxyethylene) ether detergent present in the assay medium, so long as the detergent micelle/substrate mole ratio was less than one. When this ratio was greater than one, the detergent acted as an inhibitor by competing with enzyme-containing micelles for substrate. The zwitterionic and bile salt detergents that were tested inactivated the enzyme by dissociating the oligomer. The native, Triton X-100 solubilized, enzyme was modified with a cross-linking reagent. Activity of the cross-linked enzyme was retained after the Triton X-100 was replaced by a zwitterionic sulfobetaine detergent and conformed to the same kinetic model as with the poly(oxyethylene) ether detergents. The cross-linked enzyme was also active when solubilized by the bile salt detergents although the activity did not conform to any simple kinetic model. These data indicate that the oligomer is the active form of the enzyme under assay conditions and that certain nondenaturing detergents can inactivate this enzyme by dissociating the enzyme complex.

Detergents have many diverse applications in membrane protein chemistry, but the literature describing protein–detergent interactions remains incomplete [see Helenius & Simons (1975), Tanford & Reynolds (1976), and Helenius et al. (1979)]. It is important to understand the influence of detergent on protein structure, even if detergent is used only transiently to maintain proteins in solution during experimental manipulations. This paper examines the quaternary structure and enzymatic activity of the *Escherichia coli* integral membrane protein phosphatidylserine decarboxylase solubilized by seven detergents: one alkyl zwitterion, two bile salts, and four nonionic poly(oxyethylene) ethers.

Phosphatidylserine decarboxylase catalyzes the conversion of phosphatidylserine to phosphatidylethanolamine. The enzyme, which migrates as one band with an apparent molecular weight of 30 000 upon $NaDodSO_4$[1]–polyacrylamide gel electrophoresis, has been purified to homogeneity in the presence of Triton X-100 by Dowhan et al. (1974). Tyhach et al. (1979) have characterized an *E. coli* plasmid bearing strain that overproduces the enzyme 10–20-fold. The enzyme activity, a sensitive criterion of native structure, was characterized only in the presence of Triton X-100, which was required to solubilize both the enzyme and the substrate.

Dowhan et al. (1974) found that high concentrations of Triton X-100 inhibit the enzyme. Warner & Dennis (1975) have examined the role of Triton X-100 in the phosphatidylserine decarboxylase assay system and explained the enzyme kinetics in terms of a "surface dilution" model. In a different system, Weiss & Wingfield (1979) studied the interactions between the ubiquinone-utilizing electron-transfer enzymes from mitochondria and their hydrophobic substrates, and have proposed an alternative model to explain this kind of kinetic data. Both groups assumed the detergent did not affect protein structure but did affect the availability of substrate to the enzyme.

The present study reexamines the role of the detergent in the phosphatidylserine decarboxylase assay system and considers its effect on protein structure. By variation of the head group and the hydrophobic tail of the detergent, the sensitivity of the enzyme to its microenvironment was determined. It appears that an oligomer is the active form of the enzyme. All of the nonionic detergents tested can serve as inert solvents while the other detergents can inactivate the decarboxylase by dissociating the oligomer.

Materials and Methods

Materials. Triton X-100 and Triton X-102 were obtained from Rohm and Haas, and Brij 97 was obtained from ICI United States, Inc. Dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$) was a nominally pure compound prepared by Nikko Chemicals Co., Tokyo, and $C_{12}SB$ was obtained under the trade name Zwittergent from Calbiochem–Behring Co. Sodium deoxycholate (DOC) (Fisher Scientific Co.) and sodium cholate (Sigma Corp.) were purified by charcoal and Celite treatment and recrystallized from 80% acetone. Phosphatidylserine (more than 95% pure as judged by thin-layer chromatography) from bovine brain was obtained from Sigma Corp., and phosphatidyl[1-^{14}C]serine, prepared as described by Tyhach et al. (1979), was the gift of Richard Tyhach. Dithiobis(succinimidyl propionate) (DTSP) was a product of Pierce Chemical Co. Sepharose 4B and 6B and Sephacryl S200 were products of Pharmacia Fine Chemicals.

For preparation of tritium-labeled phosphatidylserine decarboxylase, L-[2,3-^{3}H]arginine, L-[4,5-^{3}H(N)]leucine, L-[*alanine*-2,3-^{3}H]phenylalanine, and L-[^{3}H(G)]proline, obtained from New England Nuclear, were used.

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[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; DOC, deoxycholate; $C_{12}SB$, *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate; $C_{12}E_8$, dodecyl octaoxyethylene glycol monoether; DTSP, dithiobis(succinimidyl propionate); PS, phosphatidylserine; PE, phosphatidylethanolamine; K_d, distribution coefficient; PSD, phosphatidylserine decarboxylase.

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E. coli Growth Conditions. Strain EH503, a derivative of *E. coli* K12 carrying the plasmid pLC8-47 *psd*$^+$, was used in these studies. The growth conditions and characteristics of this strain were described by Tyhach et al. (1979). Cell growth was monitored by the absorbance at 650 nm (A_{650} = 0.1 corresponds to 3.8 × 10^8 cells/mL). For preparation of unlabeled protein, cells were grown at 37 °C in L-broth medium (Miller, 1972) containing colicin E1. For preparation of tritium-labeled protein, cells were grown at 37 °C in 1.0 L of medium 63 (Cohen & Rickenberg, 1956) supplemented with 0.5% (w/v) glucose, 1 μg/mL thiamin, 0.1% (w/v) casamino acids, and colicin E1; 1 mCi each of tritiated arginine, leucine, phenylalanine, and proline was added when the absorbance at 650 nm reached 0.2, and cells were harvested when the absorbance reached 0.5.

Preparation of Phosphatidylserine Decarboxylase. Phosphatidylserine decarboxylase was purified to homogeneity by a modification of the procedure of Dowhan et al. (1974). Since EH503 overproduces the decarboxylase, the QAE-Sephadex and sucrose gradient centrifugation steps could be omitted. The final preparation was free of phospholipids as judged by the method of Bartlett (1959).

Enzyme Assay. The standard enzyme assay, which includes Triton X-100, was performed as described by Hawrot & Kennedy (1975), except that the prior incubation of enzyme without substrate was omitted. Conditions for assays performed in the presence of other detergents are described in the figure legends. One unit of decarboxylase activity is the amount of enzyme which catalyzes the decarboxylation of 1 nmol of phosphatidylserine per min at 37 °C.

Other Analytical Methods. Protein concentrations were determined by the method of Lowry et al. (1951) by using bovine serum albumin as the standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). Phosphatidylserine decarboxylase has an M_r of 30 000 when analyzed by this gel system, compared to 36 000 M_r when analyzed by the gel system employed by Dowhan et al. (1974). Fluorography of $NaDodSO_4$ gels was performed by the method of Bonner & Laskey (1974), and densitometry tracings of the fluorograms were made by using a Schoeffel SD 3000 spectrodensitometer set at 546 nm.

Results

In vivo phosphatidylserine decarboxylase and its substrate, phosphatidylserine, are located within a continuous lipid bilayer, and diffusion in the plane of the membrane will bring enzyme and substrate together. In the assay medium, enzyme and substrate are inserted in discrete micelles, and catalysis occurs only when substrate is transferred from substrate-containing micelles to enzyme-containing micelles according to the following reaction scheme, as proposed by Weiss & Wingfield (1979):

$$M_{PSD} + M_{PS} \rightleftarrows M_{PSD+PS} + M \quad (1)$$

$$M_{PSD+PS} \rightleftarrows M_{PSD+PE} + CO_2 \quad (2)$$

$$M_{PS}^1 + M^2 \rightleftarrows M^1 + M_{PS}^2 \quad (3)$$

where M is a detergent micelle. Reaction 1 is the substrate-transfer reaction, and reaction 2 is the enzyme reaction. If there is a significant population of pure detergent micelles, reaction 3 can also occur. When reaction 3 occurs, the detergent acts as an inhibitor since, at constant substrate concentration, an increase in the free-micelle concentration will effectively dilute the substrate. The following studies test the applicability of this model to phosphatidylserine decarboxylase solubilized by several different detergents.

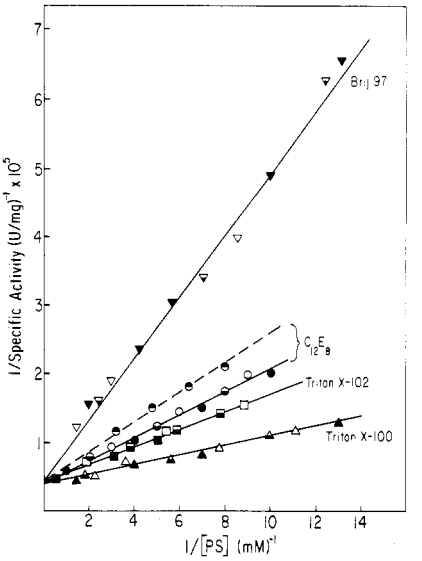

FIGURE 1: Enzyme activity of nonionic detergent solubilized phosphatidylserine decarboxylase. Decarboxylase (50 ng) was solubilized by the detergent indicated in 0.5 mL of 0.1 M potassium phosphate buffer, pH 7.0, with varying amounts of phosphatidylserine and detergent, and assayed at 37 °C. The symbols indicate the detergent/phosphatidylserine mole ratio: Brij 97 ($C_{18:1}E_{10}$) 20:1 (▼), 25:1 (▽), 30:1 (▽); ($C_{12}E_8$) 10:1 (●), 20:1 (◒), 30:1 (○), 150:1 (◓); Triton X-102 (t-$C_8PhE_{12.5}$) 20:1 (■), 50:1 (◨), 75:1 (□); Triton X-100 (t-$C_8PhE_{9.6}$) 10:1 (▲), 25:1 (▲), 50:1 (△). Alkyl poly(oxyethylene) ethers, C_xE_n, contain an alkyl chain with "x" carbon atoms and a poly(oxyethylene) chain with "n" monomers, $(OCH_2CH_2)_nOH$; the Tritons contain a phenyl ring between the alkyl groups and the poly(oxyethylene) chain.

Nonionic Detergents. Phosphatidylserine decarboxylase was active in those nonionic detergents capable of readily solubilizing phosphatidylserine. Enzyme activity, measured as a function of substrate concentration, is plotted in Figure 1 in the form of a double-reciprocal plot. Several assay conditions with negligible concentrations of pure detergent micelles were selected. In each experiment, the detergent/substrate ratio was held constant, and three different ratios were tested for each detergent. For each detergent, the enzyme activity was independent of the detergent concentration, and all of the data could be described by one linear equation (Figure 1, solid lines). Although the apparent K_m was different for each detergent, the V_{max}, given by the reciprocal of the ordinate intercept, was the same for all of the detergents, 250 000 ± 7500 units/mg of protein. The data indicate that the substrate-transfer reaction (eq 1) is the rate-limiting step, since the enzyme activity was insensitive to the number of phosphatidylserine molecules present in the average detergent micelle. The apparent K_m reflects a property of the detergent as well as of the enzyme. Detergent had an inhibitory effect only at high detergent/substrate mole ratios where the free-micelle concentration is likely to be appreciable. For example, when the ratio for $C_{12}E_8$ was 150:1, the detergent acted as a competitive inhibitor (Figure 1, dashed line). Unfortunately, the data for micelle aggregation numbers at 37 °C are sparse, making it difficult to estimate the free-micelle concentration (Helenius et al., 1979). The ordinate intercept thus reflects the true V_{max} of the enzyme, which is insensitive to the microenvironment of the micelle. The branching, degree of saturation, and length of the alkyl chain, or the size of the head group, affect only reaction 1.

The physical properties of phosphatidylserine decarboxylase in Triton X-100 were examined in further detail. The enzyme formed large soluble aggregates in dilute protein solution when

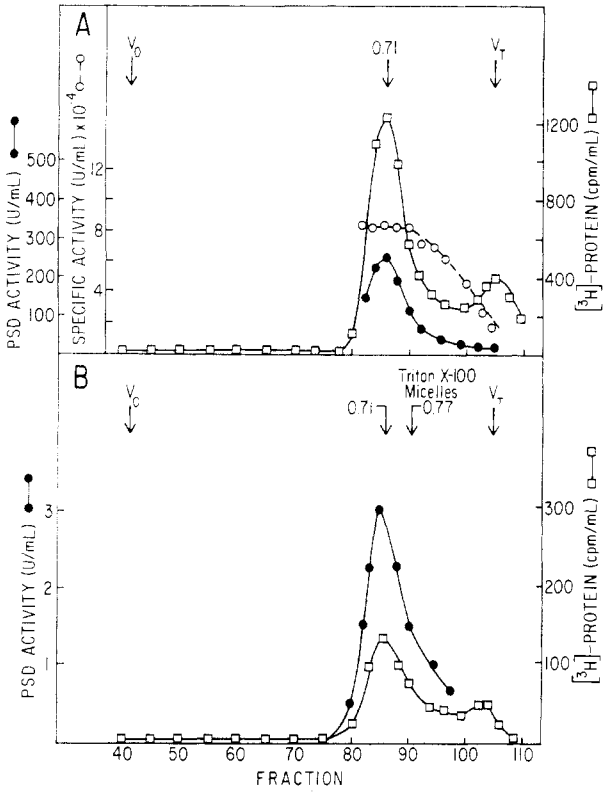

FIGURE 2: Gel filtration of decarboxylase in Triton X-100. ^{3}H-Labeled decarboxylase was incubated in eluent buffer for 1 h at room temperature. The sample was loaded on a Sepharose 4B column (1.1 × 90 cm) which was equilibrated and eluted with 0.1 M potassium phosphate buffer, pH 7.0, 1.0% (w/v) Triton X-100, and 10% (w/v) glycerol at 4 °C. V_0 marks the void volume, V_T marks the total volume, and the numbers refer to the K_d. Enzyme activity was assayed at 37 °C in the standard assay medium. (A) Decarboxylase (0.14 mg), micelle/polypeptide chain mole ratio equals 1000:1; (B) decarboxylase (0.023 mg), micelle/polypeptide chain mole ratio equals 15 000:1.

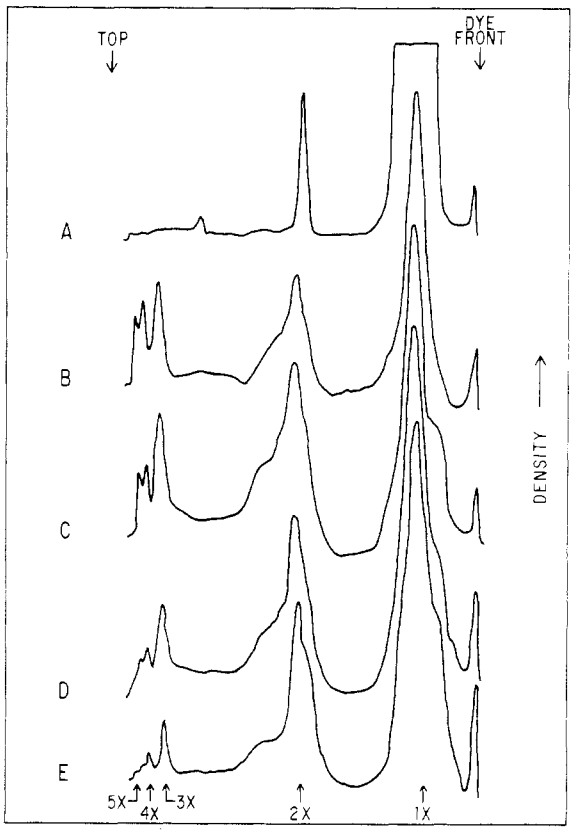

FIGURE 3: NaDodSO$_4$–polyacrylamide gel electrophoresis of cross-linked phosphatidylserine decarboxylase. Decarboxylase (1.9 μg in 100 μL of 50 mM potassium phosphate buffer, pH 7.0) was modified with two additions of 5 × 10^{-8} mol of DTSP over the course of 1 h at room temperature. The mole ratio of Triton X-100 micelle/polypeptide chain was (A) 10:1, (B) 10:1, (C) 360:1, (D) 700:1, or (E) 1300:1. The reaction was quenched by the addition of 10 μL of 0.05 M lysine. An aliquot of 100 μL of 10% (w/v) NaDodSO$_4$ was added, and the samples were incubated at 37 °C for 0.5 h before loading on 8.75% polyacrylamide gel. Sample (A) was treated with 2-mercaptoethanol during the heat step. Fluorography and densitometry were performed as described under Materials and Methods. The migration distance of monomer through pentamer is indicated in the figure.

there was a 10-fold molar excess of micelles over protein polypeptide chains. Gel filtration experiments performed on Sepharose 4B showed this aggregate could be gradually dissociated by increasing the mole ratio of Triton X-100 micelles to decarboxylase peptide chains up to 1000:1. Figure 2 shows that most of the enzyme remains oligomeric after increasing the mole ratio to that of assay conditions, 15 000:1. Most of the protein in Figure 2A,B was eluted with a K_d of 0.71, indicating the Triton X-100–protein complex is larger than pure Triton X-100 micelles, which were eluted with a K_d of 0.77. A small amount of contaminating low molecular weight protein was eluted with the total volume marker. When the higher detergent/protein mole ratio was used, a small amount of protein was eluted with Triton X-100 micelles. The specific activity of the decarboxylase determined in the experiment described in Figure 2A was constant in fractions taken from the major peak, but decreased in fractions taken from the trailing edge. Furthermore, when stored for several days at room temperature, enzyme activity from peak fractions was more stable than that taken from fractions of the trailing edge.

Since the contribution of Triton X-100 to the Stokes radius of the enzyme–detergent complex is unknown, it is not possible to infer molecular weights from the gel filtration data. Instead, the enzyme was modified with the cross-linking reagent DTSP in assay media containing varying concentrations of Triton X-100, and the data were analyzed as described by Lomant & Fairbanks (1976). Under the reaction conditions, the enzyme is not completely cross-linked, and all possible intermediate products are formed. Increasing the detergent micelle concentration effectively dilutes the protein dissolved in the detergent micelles, and only cross-links between protomer units of the same oligomer should be formed. The number of bands seen after polyacrylamide gel electrophoresis in NaDodSO$_4$ will then correspond to the number of subunits in the native enzyme. Under the conditions described in the legend to Figure 3, approximately 30–50% of the decarboxylase was cross-linked. As the concentration of Triton X-100 was increased, the area under the 5X and 4X peaks was diminished relative to the area under the 3X peaks, indicating the decarboxylase is a trimer.

The cross-linked oligomer may be reduced with 2-mercaptoethanol, yielding decarboxylase monomers plus protein migrating on NaDodSO$_4$ gel electrophoresis with an apparent molecular weight of 60 000. The latter reflects the propensity of the enzyme to dimerize in NaDodSO$_4$ rather than the presence of an impurity. When freshly purified in NaDodSO$_4$, by gel filtration, the enzyme yielded one band at M_r 30 000 when analyzed by NaDodSO$_4$–polyacrylamide gel electrophoresis. When the same sample was analyzed 1 day later, a second band, M_r 60 000, was observed, even if the sample was heated in the presence of 2-mercaptoethanol for 3 min at 100 °C before electrophoresis. Dimer formation could not be promoted by using the *o*-phenanthroline–cupric ion complex to catalyze the oxidation of intrinsic sulfhydryl groups, according to the method of Wang & Richards (1974). Stable

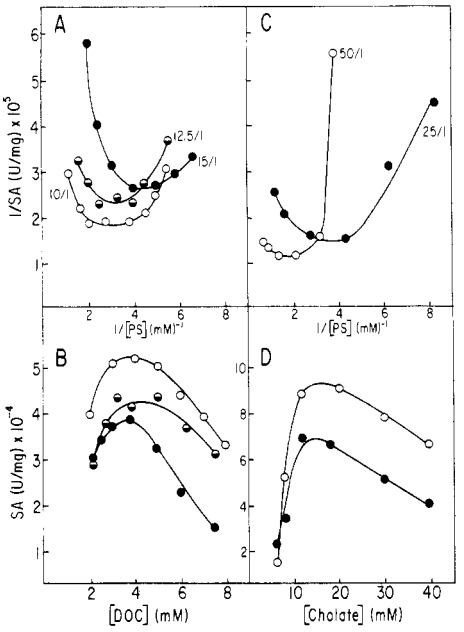

FIGURE 4: Enzyme activity in bile salt detergent. Decarboxylase (50 ng) was assayed at 37 °C in 0.5 mL of 0.1 M potassium phosphate buffer, pH 8.0, and DOC or in 0.5 mL of 0.1 M potassium phosphate buffer, pH 7.0, and cholate. The detergent/substrate mole ratio in the assay medium is indicated in the figure: (A and B) DOC; (C and D) cholate.

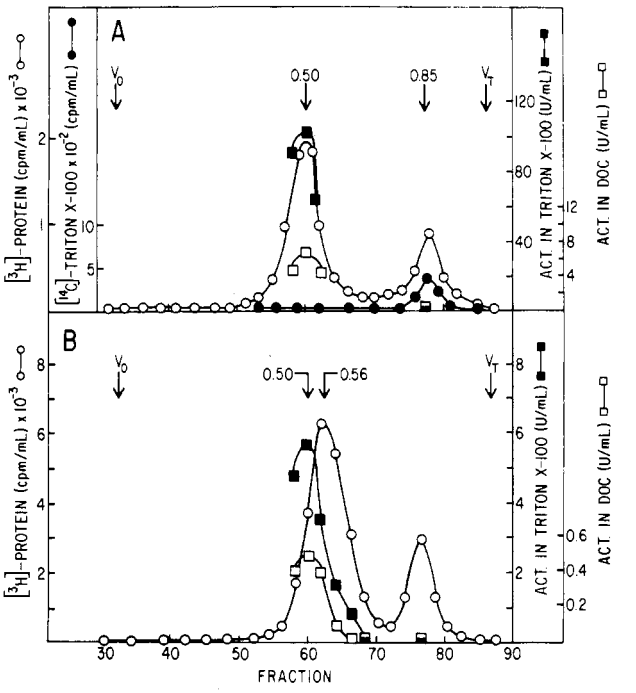

FIGURE 5: Gel filtration of decarboxylase in DOC. ^{3}H-Labeled decarboxylase solubilized in Triton X-100 was bound to DEAE-cellulose, washed with 10 mM potassium phosphate buffer, pH 7.0, and eluted with 10 mM potassium phosphate buffer, pH 8.0, 0.6 M NaCl, and either (A) 3 mM DOC or (B) 15 mM DOC. The sample was then loaded on a Sepharose 6B (0.9 × 90 cm) column equilibrated and eluted with 0.1 M potassium phosphate, pH 8.0, and either (A) 3 mM DOC or (B) 15 mM DOC. Enzymatic activity was assayed at 37 °C in either Triton X-100 under standard conditions or 0.5 mL of 0.1 M potassium phosphate buffer, pH 8.0, 0.3 mM PS, and 3 mM DOC. Trace amounts of ^{14}C-labeled Triton X-100 were added to the experiment described in (A).

oligomers in $NaDodSO_4$ have also been observed with rhodopsin (Albert & Litman, 1978) and glycophorin (Marton & Garvin, 1973). Un-cross-linked dimer comprised approximately 30% of the protein included in the 2X peaks of Figure 3. The specific activity of cross-linked phosphatidylserine decarboxylase varied from 20 to 50% of that of native enzyme, while studies performed in DOC and $C_{12}SB$ indicated that at least a portion of this activity must be ascribed to cross-linked enzyme (see below).

Bile Salt Detergents. The enzyme kinetics in DOC and cholate were studied by diluting phosphatidylserine decarboxylase, purified in Triton X-100, into assay medium containing a molar excess of bile salt detergent micelles over Triton X-100 monomers. Under these conditions, a double-reciprocal plot of the data was nonlinear, and activity was dependent on the detergent/substrate ratio (Figure 4). A plot of specific activity as a function of detergent concentration shows that inhibition began at a specific detergent concentration irrespective of the detergent/substrate ratio. This inhibition may reflect a concentration-dependent change in the physical properties of the bile salt–phosphatidylserine mixed micelles (which is likely to occur) or an effect of the detergent on protein structure. The dependence of the physical properties of pure bile salt micelles on detergent concentration has been examined (Small, 1971), but little is known of the properties of mixed micelles. The effect of bile salt detergent on protein structure was easily tested.

In order to test the hypothesis that high concentrations of bile salt detergent dissociate the oligomer to protomer units which are inactive or have a low specific activity, decarboxylase solubilized by DOC was analyzed by gel filtration on Sepharose 6B equilibrated and eluted with either 3 or 15 mM DOC in assay buffer. Triton X-100 was removed by binding the enzyme to DEAE-cellulose, washing the resin with detergent-free buffer, and eluting the protein with DOC-containing buffer. Figure 5 shows that in 3 mM DOC most of the proteins were eluted as a symmetrical peak that coincided with the enzyme activity peak, assayed in either Triton X-100 or 3 mM DOC. The specific activity, assayed in each detergent, was constant across the protein peak. Trace amounts of ^{14}C-labeled Triton X-100, added before DOC was exchanged for Triton X-100, were completely resolved from the major protein peak.

When the DOC concentration was increased to 15 mM, the protein peak shifted to a higher K_d, indicating dissociation of the oligomer, but active enzyme was still eluted with a K_d of 0.50 (Figure 5B). Enzyme activity of the smaller protein–DOC complexes could not be recovered by simply diluting the samples in assay buffer containing Triton X-100, indicating the dissociation of oligomers was not readily reversible. Prolonged incubation of the enzyme in 15 mM DOC resulted in further dissociation of the oligomer with a concomitant loss of activity.

Modifying phosphatidylserine decarboxylase with DTSP in Triton X-100 before assaying in DOC protected the enzyme from inactivation at moderate concentrations of detergent as shown in Figure 6. The enzyme activity was still dependent on the detergent/substrate ratio, resulting in a double-reciprocal plot of the data which did not conform to any simple kinetic model, and that probably reflects changes in physical properties of the mixed micelle as a function of both detergent concentration and detergent/substrate ratio.

The enzyme activity of cross-linked decarboxylase in bile salt detergent and in Triton X-100 might be attributed to phosphatidylserine decarboxylase monomers whose structure had been stabilized by intramolecular cross-links. This possibility was eliminated by repeating the experiment described in Figure 5B, using enzyme modified with DTSP in Triton X-100 as the starting material. Figure 7 shows that most of the enzyme activity was eluted with the protein of large mo-

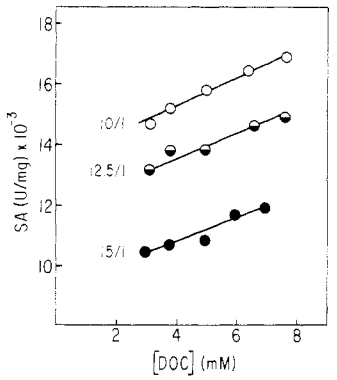

FIGURE 6: Enzyme activity of cross-linked decarboxylase in DOC. Decarboxylase was modified with DTSP as described in the legend to Figure 3C. Assays were performed as described in the legend to Figure 4. The detergent/substrate mole ratio in the assay medium is indicated in the figure.

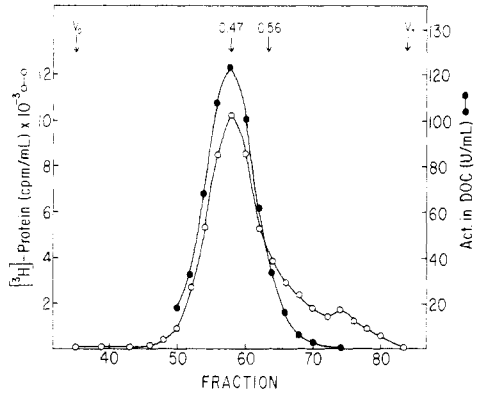

FIGURE 7: Gel filtration of cross-linked decarboxylase in DOC. Decarboxylase was modified with DTSP as described in the legend to Figure 3. DOC was exchanged for Triton X-100, and the chromatography was performed as described in the legend to Figure 5B.

lecular size and that the specific activity of dissociated enzyme was lower than that of the oligomer.

Zwitterionic Detergent. $C_{12}SB$ completely inactivated phosphatidylserine decarboxylase, but this inactivation could be prevented by modifying the enzyme with DTSP in Triton X-100 before exchanging detergents. Figure 8 shows that a double-reciprocal plot of the enzyme kinetic data is linear, and the apparent K_m is comparable to that with Triton X-102. The significance of the low apparent V_{max} cannot be evaluated since the percentage of active enzyme and the effect of cross-linking on V_{max} cannot be determined from these data.

Discussion

This study considers the effect of detergent on protein structure and enzyme activity in its examination of the physical properties of phosphatidylserine decarboxylase. Detergents are widely used in the study of membrane proteins, yet detergent–protein interactions are poorly understood, largely because of the variety of detergents with diverse physical properties, and because of the inability to classify detergents as denaturing or nondenaturing. For example, rhodopsin can be reversibly bleached in cholate (Henselman & Cusanovich, 1974) while the calcium pump protein from sarcoplasmic reticulum is irreversibly denatured in this detergent (Rizzolo & Tanford, 1978). In addition, some detergents may inhibit enzyme activity without denaturing the enzyme; the bulky head groups of Tween 80 inhibit the calcium pump protein by steric interference of the active site (Dean & Tanford, 1978). In order to distinguish properties resulting from the presence of detergent from properties intrinsic to the enzyme, phosphatidylserine decarboxylase was characterized in a variety of detergent solutions.

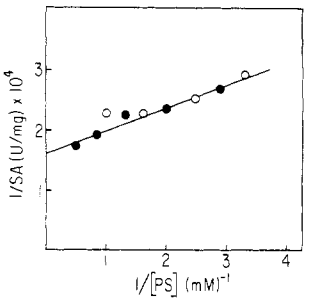

FIGURE 8: Enzyme activity of cross-linked decarboxylase in $C_{12}SB$. Decarboxylase was modified with DTSP as described in the legend to Figure 3C, and 500 ng was assayed at 37 °C in 0.5 mL of 0.1 M potassium phosphate buffer, pH 7.0, with varying amounts of phosphatidylserine and $C_{12}SB$. The symbols indicate the $C_{12}SB$/phosphatidylserine mole ratio: 5:1 (●); 10:1 (○).

In the assay medium, the decarboxylase and phosphatidylserine are solubilized in discrete micelles, and most of the substrate is not readily accessible to the enzyme. For catalysis to occur, phosphatidylserine must be transferred into a micelle containing the enzyme. The decarboxylase has a hydrophobic anchor which should insert into a detergent micelle without greatly perturbing the micelle size [see Tanford (1980)]. The average volume of a Triton X-100 micelle, which has a partial specific volume of 0.908 mL/g (Steele et al., 1978), is 1.4×10^{-22} L, and the concentration of one enzyme molecule in such a micelle would be about 10 mM. If one phosphatidylserine molecule were also present, its concentration would likewise be about 10 mM. From these considerations, it is not surprising that a detergent–substrate–enzyme complex is very short-lived and that the rate-limiting step for catalysis is the exchange of substrate between micelles. Weiss & Wingfield (1979) observed that the exchange of ubiquinone between nonionic detergent micelles was rate limiting for ubiquinone-utilizing electron-transfer complexes isolated from mitochondria. These authors also found that the substrate-exchange rate depended on the detergent, although in their study the rate of exchange was faster with detergents containing longer poly(oxyethylene) head groups. While it is not possible to discuss the effect of the microenvironment on the K_m of the decarboxylase, it is clear that the V_{max} was unaffected by the length, degree of saturation, or branching of the alkyl chains in the hydrophobic core of the detergent micelle. Thus, the V_{max} reported here is most likely the same as that of the enzyme in vivo. With the molecular weight determined by $NaDodSO_4$–polyacrylamide gel electrophoresis, the turnover number of the enzyme is 2500, in agreement with the estimate of Larson & Dowhan (1976).

Only oligomeric enzyme was active in the various assay systems employed. The purified enzyme has a strong tendency to form large protein complexes as was demonstrated by gel filtration and cross-linking experiments performed as a function of Triton X-100 micelle/polypeptide chain ratio. Two modes of self-association are evident. The weaker mode of association results in large aggregates comprised of multimeric units, which could be progressively disrupted by gradually increasing the relative amount of Triton X-100 up to a Triton X-100 micelle/polypeptide chain ratio of 1000:1. This is not simply a hydrophobic association between polypeptide chains, since Triton X-100 concentrations in excess of that required to disrupt decarboxylase–lipid associations were required to dissociate the protein complex. The cross-linking experiments, which might underestimate the size of the oligomer, indicated the multimeric protein unit is a trimer. The second mode of

association, which holds this trimer together, is very strong. Increasing the Triton X-100 micelle/polypeptide chain ratio by an order of magnitude to 15 000:1 resulted in the dissociation of only a small fraction of trimers. By contrast, active monomers of the calcium pump protein from sarcoplasmic reticulum can be completely dissociated with a $C_{12}E_8$ micelle/polypeptide chain ratio of only 10:1 (Dean & Tanford, 1978). Larson & Dowhan (1976) estimated there are 1800 copies of the decarboxylase monomer in the inner membrane of *E. coli*. Together with the data presented here, this fact indicates that the enzyme must be oligomeric in vivo, since the concentration of the enzyme in the inner membrane of *E. coli* is greater than the concentration of the enzyme in the solvent space provided by the Triton X-100 micelles in these experiments.

The kinetics of DOC- and cholate-solubilized phosphatidylserine decarboxylase differ in several ways from that of the nonionic detergent-solubilized enzyme. First, increasing concentrations of the bile salt detergents progressively inactivated the enzyme by dissociating active trimers to inactive protomers. The dissociation depended only upon the detergent concentration and was unaffected by varying the detergent to substrate ratio. The inactivation could be prevented by first joining the enzyme subunits together with the chemical cross-linker DTSP. Second, enzyme activity was dependent on the detergent/substrate mole ratio even after modification by DTSP. Unlike the nonionic detergents, the size and structure of pure bile salt micelles are dependent on detergent concentration, and these properties are significantly altered when phospholipid is added [for review, see Small (1971)]. These considerations make it difficult to evaluate the kinetic data.

Although the bile salt detergents are inappropriate for kinetic studies, they were useful for elucidating protein structure. In DOC-containing buffer, active oligomers could be separated from inactive monomers by gel filtration chromatography. This technique allowed verification of the fact that protein modification by DTSP prevented enzyme inactivation by joining together enzyme subunits rather than by stabilizing the tertiary structure of the monomer via intramolecular cross-links. The ability of DOC to dissociate membrane protein subunits has also been observed in several other systems (Helenius et al., 1979), and both DOC and cholate can alter the secondary and tertiary structure of the calcium pump from sarcoplasmic reticulum (Rizzolo & Tanford, 1978).

$C_{12}SB$ also inactivated phosphatidylserine decarboxylase by dissociating the enzyme into subunits, but in contrast to the bile salt detergents, the activity of the cross-linked enzyme was independent of the detergent/substrate ratio. Of all the detergents included in this study, $C_{12}SB$, containing a relatively small zwitterionic head group, most resembles a phospholipid and, therefore, might be an ideal detergent to use when characterizing a membrane protein in solution. Gonenne & Ernst (1978) have shown that this detergent is an effective membrane protein solubilizing agent which does not inhibit the activity of several membrane and water-soluble enzymes. The studies performed here, however, indicate that $C_{12}SB$ was even more effective at disrupting the quaternary structure of phosphatidylserine decarboxylase than the bile salt detergents. The low V_{max} of the cross-linked enzyme determined in this detergent may indicate that tertiary structure is altered as well.

This study supports the notion that nonionic detergents can serve as inert solvents for membrane proteins. Cholate, DOC, and $C_{12}SB$, however, are capable of disrupting native protein structure.

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