Phosphatidylserine decarboxylase from *Clostridium butyricum*

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Abstract Phosphatidylserine decarboxylase activity has been characterized in membrane preparations from *Clostridium butyricum* ATCC 19398. A particulate fraction was shown to catalyze the formation of phosphatidylethanolamine and plasmenylethanolamine when vesicles containing phosphatidylserine and plasmenylserine were used as substrate. No plasmenylethanolamine was formed when phosphatidylserine alone was used as substrate. The activity with phosphatidylserine was activated by divalent cations and was optimal under anaerobic conditions. Ionic detergents inhibited phosphatidylethanolamine formation strongly and nonionic detergents inhibited partially. In the presence of Triton X-100, phosphate from [^{32}P]phosphatidylserine appeared in three unidentified lipid products, in addition to phosphatidylethanolamine. The formation of these products was time- and Triton X-100 concentration-dependent. Hydroxylamine inhibited phosphatidylserine decarboxylase, but did not prevent the reactions stimulated by Triton X-100. —**Verma, J. N., and H. Goldfine.** Phosphatidylserine decarboxylase from *Clostridium butyricum*. *J. Lipid Res.* 1985. **26:** 610–616.

Supplementary key words phosphatidylserine • plasmenylserine • phosphatidylethanolamine • plasmenylethanolamine • plasmalogens • phospholipid metabolism

Studies on the pathways for the biosynthesis of phosphatidylethanolamine and plasmenylethanolamine in anaerobic bacteria have been carried out in this laboratory (1, 2). Kennedy and his coworkers demonstrated that the de novo synthesis of phosphatidylethanolamine in *Escherichia coli* involved the following steps (for references see 3).

1. Phosphatidic acid + CTP $\xrightarrow{Mg^{2+}}$ CDP-diglyceride + PPi.
2. CDP-diglyceride + L-serine → phosphatidylserine + CMP.
3. Phosphatidylserine → phosphatidylethanolamine + CO_2.

Reactions 1 and 2, which result in the formation of phosphatidylserine, have been demonstrated in this laboratory in a number of anaerobic bacteria containing plasmalogens, including: *Clostridium butyricum* (1), *Megasphaera elsdenii*, and *Veillonella parvula* and one devoid of plasmalogens, *Desulfovibrio vulgaris* (2). In these studies, phosphatidylserine decarboxylase, which catalyzes reaction *3*, was readily detected only in *D. vulgaris*.

There is evidence suggesting the presence of serine phospholipid decarboxylase activity in *Clostridium butyricum*. Experiments in our laboratory (4) showed that both phosphatidylserine and plasmenylserine, which are not present under normal growth conditions, accumulate in the presence of hydroxylamine, an inhibitor of phosphatidylserine decarboxylase. Further, it was demonstrated that the accumulated [^{32}P]phosphatidylserine and [^{32}P]plasmenylserine undergo rapid turnover and the label appears sequentially in phosphatidylethanolamine, plasmenylethanolamine, and the glycerol acetal of plasmenylethanolamine.

In this investigation we have identified and characterized enzyme activities from *C. butyricum* which decarboxylate both diacyl and alk-1-enyl acyl serine phospholipids to diacyl and alk-1-enyl acyl ethanolamine phospholipids, respectively.

MATERIALS AND METHODS

Materials

Sources of supplies were: Amersham, L-α-phosphatidyl-L-[U-^{14}C]serine, 60 mCi/mmol, [^{32}P]orthophosphoric acid, carrier-free, OCS scintillation mixture; Sigma, bovine brain phosphatidylserine; Whatman, CM-cellulose (CM-52); E. Merck, silica gel 60-pre-coated TLC plastic sheets. All other solvents and chemicals were of analytical grade. Cutscum® was obtained from Fischer Scientific.

Clostridium butyricum ATCC 19398, growth conditions and preparation of extracts

An actively growing inoculum (10%) was added to standard Broquist and Snell casamino acid medium (5) and the cells were grown anaerobically to the logarithmic

Abbreviations: TLC, thin-layer chromatography; MES, 2-(N-morphilino)ethanesulfonic acid; Tris, Tris(hydroxymethyl)aminomethane; pHMB, *p*-hydroxymercuribenzoate; NEM, N-ethylmaleimide.

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phase (60–80 klett units, red filter). The cells were harvested by centrifugation at 9000 *g* for 15 min at 20°C and washed once with 10 mM potassium phosphate buffer (pH 7.5). The cells were broken by two passages through a French pressure cell at 12,000 psi. The lysate was centrifuged at 105,000 *g* for 1 hr at 4°C, the pellet was washed once with and suspended in 10 mM potassium phosphate buffer (pH 7.5), and stored in liquid nitrogen.

Preparation and lysis of protoplasts

Cells grown as described above were washed twice with protoplasting buffer, 10 mM potassium phosphate buffer containing 0.4 M sucrose and 15 mM $MgCl_2$ (6), and concentrated 100-fold in the same buffer. One hundred μl of lysozyme (60 mg/ml) in 50 mM potassium phosphate buffer (pH 7.5) with 15 mM $MgCl_2$ was added per ml of cell suspension and the suspension was incubated under anaerobic conditions (N_2 atmosphere) at 37°C for 30–45 min. The protoplasts were lysed by addition of 4 ml of 10 mM potassium phosphate buffer (pH 7.5) containing 15 mM $MgCl_2$ per ml of protoplast suspension. The lysate was centrifuged at 105,000 *g* for 1 hr, the pellet was washed once with and suspended in 10 mM potassium phosphate buffer (pH 7.5), and aliquots were stored in liquid N_2. Both types of frozen membrane preparations were thawed only once and any remaining material was discarded.

Preparation of [^{32}P]phosphatidylserine and [^{32}P]plasmenylserine

For the preparation of ^{32}P-labeled serine phospholipids containing a mixture of diacyl and plasmalogen forms, *M. elsdenii* ATCC 17752 was grown in the synthetic medium described by Forsberg (7). As detailed previously (2), the medium contained: minerals 1, minerals 2, resazurin, glucose, amino acids (groups A, B, and C), volatile fatty acids, vitamins, and sodium carbonate. The phosphate of minerals 1 was reduced to 10% of the original and it was replaced by an equivalent amount of HEPES (N-2-hydroxyethylpiperazine-N'2-ethane sulfonate). Cells were grown in 2 l of low phosphate medium containing 400–800 μCi of $^{32}P_i$. The cells were washed in 50 mM phosphate buffer, pH 7.2, and the washed cells were extracted by the procedure of Folch, Lees, and Sloane Stanley (8). The chloroform phase was concentrated, and the lipids were purified by CM-52 column chromatography using stepwise elution with solvents containing increasing percentages of methanol in chloroform according to the method described by Comfurius and Zwall (9) with minor variations. Elution was as follows: fraction 1, chloroform, 100 ml; fraction 2, 25% methanol in chloroform, 200 ml; fraction 3, 40% methanol in chloroform, 200 ml; fraction 4, methanol, 100 ml. Fraction 2 contained the ethanolamine phospholipids and fraction 3 contained most of the serine phospholipids. The latter was enriched for plasmenylserine by selectively hydrolyzing phosphatidylserine with phospholipase A according to Waku and Nakazawa (10). [^{32}P]Phosphatidylserine and [^{32}P]phosphatidylethanolamine (diacyl forms) were prepared in a similar manner from *M. elsdenii* ATCC 17752 after it had been subcultured several times in another medium (11), which eventually yielded a strain without plasmalogens. The isolation and characterization of this strain will be described in a subsequent publication.

Preparation of vesicles

Phospholipid vesicles were prepared in 10 mM potassium phosphate buffer (pH 7.5) by sonication for 22 min (Heat Systems Ultrasonics Model W220-F, setting between 4 and 5), under nitrogen, in a test tube surrounded by a chilled water bath.

Enzyme assays

Unless otherwise specified for individual experiments, the assay contained in 1.1 ml: potassium phosphate buffer (pH 7.5), 10 mM; $MgCl_2$, 15 mM; 2-mercaptoethanol, 4.5 mM; serine phosphatide, 200 nmol; and membrane particles or cell extract of *C. butyricum* ATCC 19398 (usually 1–5 mg protein). They were incubated under nitrogen at 37°C in a reciprocal shaking water bath for 30 min. The reaction was terminated by the addition of 4 ml of methanol and 2 ml of chloroform and the lipids were extracted by the procedure of Bligh and Dyer (12).

Thin-layer chromatography

The radioactive phospholipids were separated on plastic plates pre-coated with silica gel 60 (E. Merck). The solvent systems used were: (A) chloroform–methanol–7 N ammonia 60:35:5 (v/v) for one-dimensional chromatography and for the first dimension of two-dimensional chromatography; (B) chloroform–methanol–acetic acid 65:25:8 (v/v) for the second dimension of two-dimensional chromatograms. Alk-l-enyl ether lipids were hydrolyzed between the first and second dimension runs by exposing the plates to HCl fumes for 5 min in a glass chromatography tank, prewarmed in an 80°C oven for 5 min (4). The plates were exposed to Kodak AR film, and the radioactive spots were cut out and counted in OCS liquid scintillation fluid (Amersham).

RESULTS

As will be shown below, phosphatidylserine decarboxylase of *C. butyricum* catalyzed the decarboxylation of both phosphatidylserine and plasmenylserine. Unless otherwise noted, the studies reported here were conducted

using phosphatidylserine as substrate. For optimum decarboxylase activity, a divalent cation (see below), 2-mercaptoethanol, which stimulated the activity of lysed protoplasts approximately 20%, and anaerobic conditions were routinely used. Decarboxylation catalyzed by lysed protoplasts was 1.8 and 2.5 times greater under N_2 than in air in 1- and 2-hr incubations, respectively. The activity was characterized in cell extracts obtained by osmotic disruption of protoplasts or by use of a French pressure cell. No significant differences were noted in these activities except that the latter gave more consistent specific activities, presumably because the extent of protoplast formation was not reproducible from one preparation to the next. Intact protoplasts did not decarboxylate phosphatidylserine. Upon treatment at 60°C for 15 min, membrane particles lost decarboxylase activity completely. Under standard conditions, the decarboxylation of phosphatidylserine was linear for 30 min of incubation and enzyme activity was usually linear up to 4 mg of protein per 1.1 ml assay. The initial velocity of the reaction was proportional to the substrate concentration up to 180 μM phosphatidylserine.

The enzyme activity was localized in the membrane fraction (105,000-*g* pellet); no activity was detected in the soluble 105,000-*g* supernatant fraction. Reconstitution of cell extracts after centrifugation at 105,000 *g* by adding the supernatant fraction, with or without heat treatment, to the particulate fraction did not change the activity (data not shown), suggesting that the soluble fraction did not contain any co-factor required for the activity.

Several bivalent metal ions increased the enzyme activity. Among the metal ions tested, Ba^{2+} was more effective than Mg^{2+}, and Cu^{2+} completely inhibited the enzyme activity at 15 mM. Mg^{2+} produced optimum activity at 15 mM (**Fig. 1**). In a separate experiment with lysed protoplasts, Mn^{2+} at 15 mM stimulated the enzyme 24% compared with 102% for Mg^{2+}, 170% for Ca^{2+}, and 247% for Ba^{2+} at the same concentration.

The effect of varying the potassium phosphate concentration in the assay from 5 mM to 50 mM using a buffer of pH 6.5 was studied. The activity remained unchanged between 5 mM and 30 mM potassium phosphate; however, further increases to 50 mM resulted in a 30% increase in activity. Using 50 mM potassium phosphate buffer, the activity had a broad pH optimum range around pH 6.8. At pH 6.3 and pH 7.35 the activity was 65% and 69%, respectively, of the optimum activity at pH 6.8. MES-KOH and Tris-HCl, the two other buffers tested at 50 mM in the pH range overlapping that of KPO_4, showed inhibitory effects resulting in approximately 50% lower activity compared to that of phosphate buffer.

In addition to measuring the decarboxylation product, [^{32}P]phosphatidylethanolamine, the stoichiometry of the

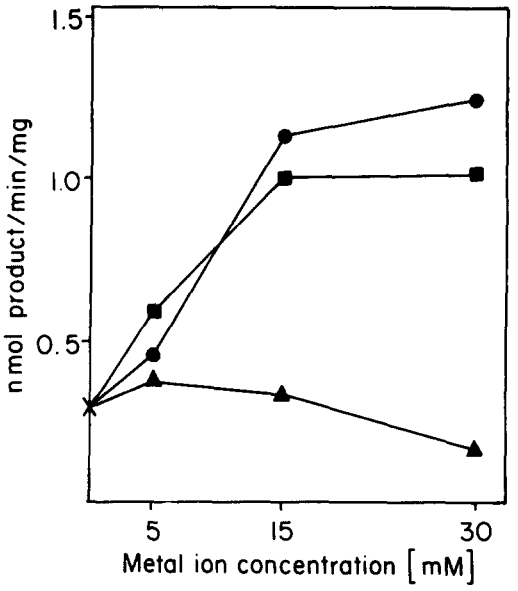

Fig. 1. Bivalent metal ion dependence of phosphatidylserine decarboxylase. Bivalent metal ions (● — ●, Ba^{2+}; ■ — ■, Mg^{2+}; ▲ — ▲, Ca^{2+}) were added at the concentrations shown. Phosphatidylserine isolated from ^{32}P-labeled *Megasphaera elsdenii* was used for the preparation of substrate vesicles.

assay was examined by measuring $^{14}CO_2$ release and [^{14}C]phosphatidylethanolamine formed by decarboxylation of dioleoyl phosphatidyl-[U-^{14}C]serine diluted with bovine brain phosphatidylserine. All three products were formed in parallel (**Fig. 2**), however at any time of incubation, the activity using [^{32}P]phosphatidylserine isolated from *M. elsdenii* as substrate, was twofold higher compared to dioleoyl phosphatidylserine diluted with bovine brain phosphatidylserine. A similar inhibition was also observed when phosphatidylserine isolated from *M. elsdenii* was diluted with bovine brain phosphatidylserine (**Fig. 3**).

Inhibition of the decarboxylase was observed at 1 mM and 5 mM concentrations of hydroxylamine, pHMB, and NEM. As shown in **Table 1**, hydroxylamine completely inhibited the enzyme activity at 1 mM, pHMB did not inhibit at 1 mM but completely inhibited at 5 mM, whereas NEM partially inhibited to the extent of 20% and 47% at 1 mM and 5 mM, respectively.

Under the assay conditions for optimum activity, no detectable amounts of plasmenylethanolamine were formed when phosphatidylserine was used as substrate. However, when plasmenylserine was present in the substrate, it was decarboxylated to plasmenylethanolamine (**Table 2**). The proportion of plasmenylethanolamine formed was higher than the proportion of plasmenylserine present, when the two substrates were present in approximately equal amounts (Table 2A, lines 1 and 2; Table 2B, lines 1 and 2), but not at higher ratios of diacyl to plasmalogen substrate (Table 2A, lines 3 and 4, Table 2B, line 3).

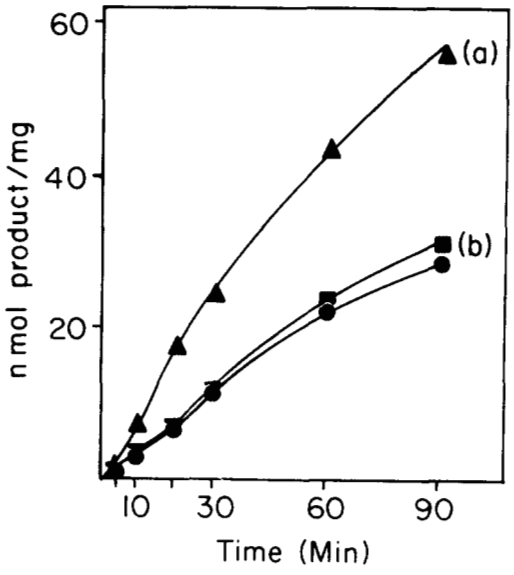

Fig. 2. Stoichiometry of phosphatidylserine decarboxylase. The assays contained in 1.1 ml: potassium phosphate buffer (pH 7.5), 10 mM; $MgCl_2$, 15 mM; 2-mercaptoethanol, 4.5 mM; phosphatidylserine (a) isolated from ^{32}P-labeled *Megasphaera elsdenii* and (b) dioleoyl phosphatidyl-[U-^{14}C]serine diluted with bovine brain phosphatidylserine (1:545 molar ratio), 200 nmol; membrane particles obtained from a French press lysate, 1.45 mg of protein. Incubations were in a nitrogen atmosphere at 37°C. (a) With ^{32}P-labeled phosphatidylserine vesicles, the reaction was terminated by addition of 4 ml of methanol and 2 ml of methanol-chloroform and the lipids were extracted by the Bligh and Dyer procedure. (b) With dioleoyl phosphatidyl[U-^{14}C]serine; incubations were done in Warburg flasks. Released [^{14}C]CO_2 was trapped in 0.2 ml of hydroxide of hyamine placed in the central well. The reaction was stopped by addition of 1 ml of 2 N H_2SO_4 contained in the side arm. The flasks were kept at room temperature after terminating the reaction, to allow all the CO_2 to be trapped: ▲—▲, [^{32}P]phosphatidylethanolamine; ■—■ phosphatidyl[U-^{14}C]ethanolamine; ●—●, [^{14}C]CO_2.

Effects of detergents

The effects of several detergents on phosphatidylserine decarboxylase activity were tested. As shown in **Table 3**, all detergents at all concentrations tested resulted in decreased yields of phosphatidylethanolamine. The ionic detergents, sodium dodecyl sulfate and cetyl trimethylammonium bromide, were strongly inhibitory. The nonionic detergents, Brij 35 and Tween 80, inhibited partially. The effects of the nonionic detergents, Triton X-100 and Cutscum, were complex. At higher concentrations of these detergents, large amounts of ^{32}P were found in lipids other than phosphatidylethanolamine. The amounts of radioactivity in these products increased with increasing detergent concentration above 0.2 mg/ml. The percentage input radioactivity found in these compounds at 1.2 mg/ml Triton X-100 and their chromatographic mobilities are shown in **Table 4**. Similar results were obtained with Cutscum (not shown). Although decreased formation of phosphatidylethanolamine was observed at 0.2 mg/ml Triton X-100, at which there was no significant diminution of substrate concentration as a result of other reactions (data not shown), part or all of the apparent inhibition of phospatidylserine decarboxylation could have been due to the conversion of phosphatidylethanolamine to other products at this and at higher concentrations of Triton X-100. Additional experiments will be needed to elucidate the kinetics of Triton X-100 inhibition. The major unknown (I) ran near the solvent front in solvent A and its mobility resembled that of Triton X-100. In order to determine whether the formation of these products depended on phosphatidylserine as precursor, similar experiments were carried out with [^{32}P]phosphatidylethanolamine. Products of approximately the same mobility in solvent A were formed. A similar set of products was obtained when the assay was carried out with [^{32}P]phosphatidylserine, Triton X-100 (1.2 mg/ml), and hydroxylamine (5 mM). These observations strongly suggest that these reactions were not dependent on the decarboxylation of phosphatidylserine and that the phosphate group of phosphatidylserine and phosphatidylethanolamine can be transferred to similar acceptors or the parent lipids can undergo similar transformations in the presence of these nonionic detergents.

DISCUSSION

The membranes of *C. butyricum* decarboxylated plasmenylserine and phosphatidylserine. From the results shown in Table 2A, it appears that total decarboxylation was little affected by variation in the plasmenylserine:phosphatidylserine ratio in the substrate vesicles. This and the

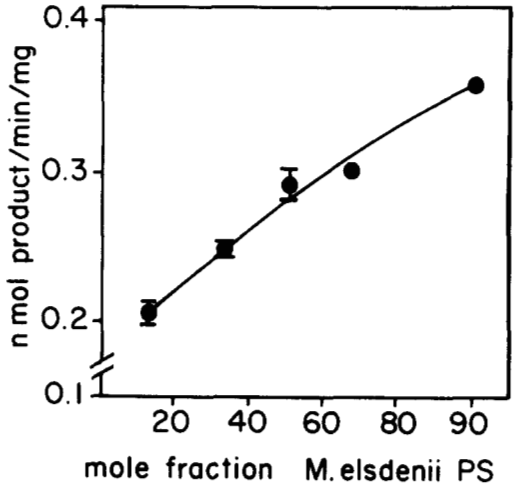

Fig. 3. Phosphatidylserine decarboxylase activity as a function of the molar ratios of phosphatidylserine from bovine brain and that from *Megasphaera elsdenii*. A total of 200 nmol of phosphatidylserine was added in varying molar ratios as shown. Particles, obtained from a French press lysate, 1.5 mg protein. Incubations, in duplicate, were for 1 hr.

TABLE 1. Effect of enzyme inhibitors on phosphatidylserine decarboxylase

Inhibitor	Inhibitor Concentration	Activity
	mM	*% of control*
Hydroxylamine	1	0.31 ± 0.10
	5	0.38 ± 0.31
pHMB	1	99.2 ± 3.6
	5	0.18 ± 0.17
NEM	1	80.9 ± 2.0
	5	52.8 ± 0.9

Assay conditions were the same as the standard assay except that the potassium phosphate buffer added was 50 mM. Membrane particles, 1.4 mg of protein, were obtained from a French press lysate. Incubations, in duplicate, were for 30 min. The specific activity of the control was 0.69 nmol phosphatidylethanolamine formed/mg protein per min.

similar turnover rates of phosphatidylserine and plasmenylserine in vivo (4) suggest that either the same protein catalyzes the decarboxylation of both serine phospholipids in *C. butyricum* or that there are two proteins with very similar specific activities. An argument for the former is that the *E. coli* enzyme actively decarboxylates plasmenylserine (11), a lipid which is not a natural substrate for that organism.

The enzyme from *C. butyricum* was different from that of *S. ruminantium* (13) and *E. coli* (14, 15) in its response to bivalent metal ions and Triton X-100. Bivalent metal ions inhibited the activity from *S. ruminantium* and were not required for the *E. coli* enzyme. The enzyme from both of these gram-negative species was activated by Triton X-100. Similar requirements were found in other gram-negative species (16). Among gram-positive organisms, the enzyme has been characterized in two species of *Bacillus*. In an unidentified *Bacillus* sp. the activity was stimulated by Mg^{2+} and detergent was required when the substrate was presented in the form of lipid dispersions, but not when the substrate was generated endogenously (17). The enzyme from *Bacillus megaterium* did not require a divalent cation and was inhibited by Triton X-100 levels above 0.2 mg/ml (18). The enzyme in *Clostridium perfringens* was found to be active in the presence of Triton X-100 when phosphatidylserine was generated in vitro by envelope particles (19).

The involvement of a carbonyl group in the decarboxylation reaction is indicated by its hydroxylamine sensitivity in vitro and by accumulation of phosphatidylserine in the presence of hydroxylamine in vivo (4). This characteristic is shared by all of the bacteria that have been studied (12, 16, 19). NEM and pHMB also inhibited the enzyme activity (Table 1) indicating the presence of a sulfhydryl group that is required for activity in the *C. butyricum* decarboxylase. Stimulation of decarboxylation by mercaptoethanol may be related to the sulfhydryl group requirement.

TABLE 2. Decarboxylation of phosphatidylserine and plasmenylserine mixtures of varying composition

A. Decarboxylation by membrane particles obtained from a French press lysate

Serine Phosphatide Diacyl:Plasmalogen Molar Ratio in Substrate Vesicles	15 Minutes		30 Minutes	
	Phosphatidyl-ethanolamine	Plasmenyl-ethanolamine	Phosphatidyl-ethanolamine	Plasmenyl-ethanolamine
	nmol formed/mg protein			
42:58	6.9	16.6	17.8	25.6
50:50	8.4	12.5	17.3	21.7
75:25	17.3	5.9	31.3	10.1
90:10	19.3	1.4	38.9	3.8

B. Decarboxylation by membrane particles obtained from a protoplast lysate

Serine Phosphatide Diacyl:Plasmalogen Molar Ratio in Substrate Vesicles	1 Hour	
	Phosphatidyl-ethanolamine	Plasmenyl-ethanolamine
	nmol formed/mg protein	
41:59	3.0	6.1
54:46	3.0	3.6
96:4	6.0	0.3

Assay conditions were the same as the standard assay. A: The assay volume was 0.55 ml and varying ratios of phosphatidylserine and plasmenylserine were made by adding *M. elsdenii* phosphatidylserine to the mixture of diacyl and plasmalogen obtained from wild type *M. elsdenii*. Protein used was 0.375 mg/assay. B: The substrate from wild type *M. elsdenii* was enriched with plasmenylserine by selective hydrolysis of phosphatidylserine by phospholipase A_1. The assay volume was 1.1 ml and the protein added was 3.85 mg/assay.

The decarboxylation of phosphatidylserine in *C. butyricum* is sufficiently rapid to result in no measurable pool of phosphatidylserine in cells (4). This is also true for *E. coli*, in which there appears to be a large excess of the enzyme (20). The specific activity of the enzyme in crude membranes of *E. coli* is 40- to 50-fold greater than that measured in the most active preparations from *C. butyricum* (40 to 50 vs 1 nmol/min per mg of protein) (20). The activities we have measured in *C. butyricum* ATCC 19398 and in the closely related *C. beijerinckii* (*butyricum*) ATCC

TABLE 3. Effects of detergents on phosphatidylserine decarboxylation

Detergent	Concentration		
	0.2 mg/ml	0.6 mg/ml	1.2 mg/ml
	nmole phosphatidylethanolamine formed		
SDS	0	0	0
CTMAB	0	0	0
Triton X-100	0.86 (13)	1.8 (26)	1.7 (25)
Brij 35	0.94 (14)	0.26 (4)	1.8 (26)
Tween 80	2.8 (41)	1.1 (16)	1.0 (15)
Cutscum	2.8 (41)	3.7 (54)	2.4 (36)

The standard assays contained membranes from lysed protoplasts, 1.96 mg of protein. Incubations were done in duplicate for 1 hr. The control without detergent gave 6.85 nmol phosphatidylethanolamine. The numbers in parentheses represent percent of control value. Abbreviations: SDS, sodium dodecyl sulfate; CTMAB, cetyl trimethylammonium bromide.

TABLE 4. Formation of unidentified reaction products in the presence of [^{32}P]phosphatidylserine and Triton X-100

Reaction Products	R_f	% Composition of ^{32}P-Labeled Unidentified Reaction Products	
		Without Triton X-100	With 1.2 mg/ml Triton X-100
Unknown I	0.85	n.d.	27.1
Unknown II	0.52	0.03	9.0
Unknown III	0.15	0.22	2.8

The assay conditions were the same as the standard assay except that Triton X-100 was added to the assays at the concentration shown. Results were calculated as percentage of radioactivity added to the assay in the form of [^{32}P]phosphatidylserine. Lysed protoplast membranes, 2.8 mg of protein, were added to each incubation. Duplicate incubations were done for 30 min. The R_f of phosphatidylethanolamine was 0.43; n.d., not determined.

6015 (data not shown) in this study are about 100-fold greater than those found in our earlier studies of the latter strain (1, 2). Some of the differences in the results of the two studies can be ascribed to the presence of inhibitory concentrations of Triton X-100 in the coupled assays used in most of our earlier studies, the use of lower concentrations of phosphatidylserine previously, and the use of extracts and particles stored at −20°C. We have observed that storage of membrane preparations from *C. butyricum* ATCC 19398 at −20°C results in a loss of 80% of the activity in 7 days compared to no loss in liquid nitrogen.

In contrast to most bacteria that have phosphatidylethanolamine and no or very little phosphatidylserine, several anaerobic bacteria including *S. ruminantium*, *Anaerovibrio lipolytica*, *M. elsdenii*, and *V. parvula* have 23 to 36% phosphatidylserine (14, 21). These organisms, understandably, have low phosphatidylserine decarboxylase activities (2, 13). Langley, Yaffe, and Kennedy (18) have demonstrated an inverse relationship between the phosphatidylserine content of various strains of *Bacillus megaterium* and their decarboxylase activities. The regulation of the activity of this enzyme and the expression of the gene in bacteria clearly need to be explored further.

The formation of a series of radioactive lipid products other than phosphatidylethanolamine from [^{32}P]phosphatidylserine in the presence of Triton X-100 and Cutscum is a novel finding of our study. The nature of these products and the mechanism of their formation are of interest. Although they may represent artifacts induced by the presence of these surfactants, it is possible that the reactions observed represent unmasking of activities normally present in these cells, which are cryptic in membrane preparations incubated without detergent. These reactions will be the subject of future studies.

This research was supported by U.S. Public Health Service Grant AI-0893, from the National Institute of Allergy and Infectious Diseases. The authors would like to express appreciation to Allan Kaufman for his help with various aspects of this work.

Manuscript received 17 October 1984.

REFERENCES

1. Silber, P., R. P. Borie, and H. Goldfine. 1980. The enzymes of phospholipid synthesis in *Clostridium butyricum*. *J. Lipid Res.* **21:** 1022–1031.
2. Silber, P., R. P. Borie, E. J. Mikowski, and H. Goldfine. 1981. Phospholipid biosynthesis in some anaerobic bacteria. *J. Bacteriol.* **147:** 57–61.
3. Raetz, C. R. H. 1978. Enzymology, genetics and regulation of membrane phospholipid synthesis in *Escherichia coli*. *Microbiol. Rev.* **42:** 614–659.
4. Koga, Y., and H. Goldfine. 1984. Biosynthesis of phospholipids in *Clostridium butyricum*: the kinetics of synthesis of plasmalogens and the glycerol acetal of ethanolamine plasmalogen. *J. Bacteriol.* **159:** 597–604.
5. Broquist, H. P., and E. E. Snell. 1951. Biotin and bacterial growth. I. Relation to aspartate, oleate and carbon dioxide. *J. Biol. Chem.* **188:** 431–444.
6. Bishop, D. G., J. A. F. Op den Kamp, and L. L. M. van Deenen. 1977. The distribution of lipids in the protoplast membranes of *Bacillus subtilis*. A study with phospholipase C and trinitrobenzenesulphonic acid. *Eur. J. Biochem.* **80:** 381–391.
7. Forsberg, C. W. 1978. Nutritional characteristics of *Megasphaera elsdenii*. *Can. J. Microbiol.* **24:** 981–985.
8. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
9. Comfurius, P., and R. F. A. Zwall. 1977. The enzymatic synthesis of phosphatidylserine and purification by CM-cellulose column chromatography. *Biochim. Biophys. Acta.* **488:** 36–42.
10. Waku, K., and Y. Nakazawa. 1972. Hydrolyses of 1-O-alkyl-, 1-O-alkenyl-, and 1-acyl-2-1-[^{14}C]-linoleoyl-glycero-3-phosphorylcholine by various phospholipases. *J. Biochem.* **72:** 149–155.
11. van Golde, L. M. G., R. A. Prins, W. Franklin-Klein, and J. Akkermans-Kruyswijk. 1973. Phosphatidylserine and its plasmalogen analogue as major lipid constituents in *Megasphaera elsdenii*. *Biochim. Biophys. Acta.* **326:** 314–323.
12. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
13. Watanabe, T., S. Okuda and H. Takahashi. 1984. Turn-over of phospholipids in *Selenomonas ruminantium* *J. Biochem.* **95:** 521–527.
14. Hawrot, E. 1981. Phosphatidylserine decarboxylate from *Escherichia coli*. *Methods Enzymol.* **71:** 571–576.
15. Dowhan, W., W. T. Wickner, and E. P. Kennedy. 1974. Purification and properties of phosphatidylserine decarboxylase from *Escherichia coli*. *J. Biol. Chem.* **249:** 3079–3084.
16. Dutt, A., and W. Dowhan. 1977. Intracellular distribution of enzymes of phospholipid metabolism in several gram-negative bacteria. *J. Bacteriol.* **132:** 159–165.
17. Patterson, P. H., and W. J. Lennarz. 1971. Studies on the membranes of bacilli. I. Phospholipid biosynthesis. *J. Biol. Chem.* **246:** 1062–1072.
18. Langley, K. E., M. P. Yaffe, and E. P. Kennedy. 1979. Biosynthesis of phospholipids in *Bacillus megaterium*. *J. Bacteriol.* **140:** 996–1007.
19. Carman, G. M., and D. S. Wieczorek. 1980. Phosphatidylglycerophosphate synthase and phosphatidylserine synthase activities in *Clostridium perfringens*. *J. Bacteriol.* **142:** 262–267.
20. Hawrot, E., and E. P. Kennedy. 1978. Phospholipid composition and membrane function in phosphatidylserine decar-

boxylase mutants of *Escherichia coli*. *J. Biol. Chem.* **253:** 8213–8220.
21. van Golde, L. M. G., J. Akkermans-Kruyswijk, W. Franklin-Klein, A. Lankhorst, and R. A. Prins. 1975. Accumulation of phosphatidylserine in strictly anaerobic lactate fermenting bacteria. *FEBS Lett.* **53:** 57–60.