Partial Purification and Properties of Phosphatidylserine Synthase from *Clostridium perfringens*†

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The membrane-associated phospholipid biosynthetic enzyme cytidine 5'-diphospho-1,2-diacyl-*sn*-glycerol:L-serine *O*-phosphatidyltransferase (phosphatidylserine synthase; EC 2.7.8.8) was partially purified 337-fold from a cell-free extract of the gram-positive pathogenic anaerobe *Clostridium perfringens* (ATCC 3624). The purification procedure included extraction from the cell envelope with the nonionic detergent Triton X-100, followed by affinity chromatography on cytidine 5'-diphosphate-diacylglycerol-Sepharese. When the partially purified enzyme was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, two major bands were evident with apparent minimum molecular weights of 39,000 and 31,000. Activity of phosphatidylserine synthase was dependent on the addition of manganese ions (3 mM) and Triton X-100 (2.7 mM) for maximum activity. The rate of catalysis was maximal at 40°C (with rapid thermal inactivation above this temperature), and the pH optimum was 8.5. The apparent K_m values for cytidine 5'-diphosphate-diacylglycerol and L-serine were 0.24 and 0.26 mM, respectively. The synthetic (forward) reaction was favored, as indicated by an equilibrium constant of 82, and the energy of activation was found to be 18 kcal/mol (75,362 J/mol).

Clostridium perfringens is a gram-positive, endosporeforming, nonmotile, rod-shaped anaerobic bacterium (38). Though its natural habitats are soil and the intestinal tracts of humans and animals, it has been isolated from virtually every environment examined for its presence (39). The significance of this is, of course, that *C. perfringens* is a human pathogen, capable of causing illness either through wound infection (3, 27) or food-borne intoxication (9, 16, 29). In the United States, this organism is a major cause of bacterial food poisoning, third only to salmonellae and staphylococci in terms of total number of cases (40). As such, elucidation of the processes which govern its growth and proliferation is of some importance. One of these governing factors is the synthesis of the bacterial membrane, important both as a selective permeability barrier and as the site of numerous essential metabolic processes (1, 15, 25). Since phospholipids are the chief structural components of the membrane bilayer (37, 41), the purification and characterization of those enzymes which catalyze their formation would be a logical area for investigation. Since these enzymes are themselves membrane associated, their activity

should provide an index of both membrane structure and function.

One such enzyme, cytidine 5'-diphospho-1,2-diacyl-*sn*-glycerol:L-serine *O*-phosphatidyltransferase (phosphatidylserine [PS] synthase; EC 2.7.8.8) catalyzes the formation of PS and CMP from cytidine 5'-diphosphate (CDP)-diacylglycerol and L-serine (20). The enzyme has been studied extensively in bacteria and has been purified to apparent homogeneity from two strains of *Escherichia coli* by means of substrate affinity chromatographic techniques (23, 35). The enzyme has never been purified from a gram-positive organism, though it has been identified and studied in cell-free extracts of several *Bacillus* species (14, 22, 30) and two *Clostridium* species (6, 8, 36). The significance for investigating this particular phospholipid biosynthetic enzyme is twofold. Because it lies at the branchpoint in the bacterial phospholipid biosynthetic pathway, it may be involved in regulating the ratio of dipolar ionic phospholipids to acidic phospholipids in the bacterial membrane (23), thus affecting cell growth (17, 33) and sensitivity to antibiotics (32). Secondly, PS synthase derived from *E. coli* and other gram-negative bacteria is unique among the phospholipid biosynthetic enzymes in having an apparent peripheral membrane association (10, 13, 26, 31). The en-

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zyme in gram-positive organisms, on the other hand, is an integral membrane protein (6, 8, 14, 22, 30, 36).

This paper reports on the partial purification and properties of PS synthase from *C. perfringens*. The results of this work should provide a better understanding of PS synthesis in *C. perfringens* and expand the body of knowledge which examines differences in phospholipid synthesis between gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Materials. All chemicals were reagent grade or better. Bovine serum albumin, DL- α -glycerophosphate, L-serine, sodium *m*-periodate, and Trizma Base (Tris) were purchased from Sigma Chemical Co. 2-(*N*-morpholino)ethanesulfonic acid was a product of Calbiochem-Behring Corp. Triton X-100 (*p*-tert-octylphenoxypolyethoxy ethanol), assumed to have a molecular weight of 624, was obtained from Rohm and Haas Co. Adipic acid dihydrazide was purchased from Eastman Kodak Co. Acetonitrile was obtained from J. T. Baker Chemical Co. Cyanogen bromide was a product of Fisher Scientific Co. Standard PS was purchased from Supelco, Inc. Radiochemicals were products of New England Nuclear Corp. Precoated silica gel analytical thin-layer plates were obtained from E. Merck AG. All reagents and molecular-weight standards for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as well as Bradford protein assay dye concentrate were purchased from Bio-Rad Laboratories. Sepharose 4B and Sephacryl S-300 were products of Pharmacia Fine Chemicals, Inc. Insta-Fluor organic scintillation fluid was obtained from Packard Instrument Co., Inc. CDP-diacylglycerol derived from soybean lecithin was prepared by the method of Carman and Fischl (5).

Preparation of CDP-diacylglycerol-Sepharose affinity resin. The sodium periodate-oxidized derivative of CDP-diacylglycerol was covalently attached to Sepharose 4B via an adipic acid dihydrazide spacer arm, as described by Larson et al. (24). The procedure was modified by activating the Sepharose 4B with cyanogen bromide in acetonitrile as described by March et al. (28).

Growth conditions. A freeze-dried culture of *C. perfringens* (ATCC 3624) was obtained from the American Type Culture Collection. Stock cultures of the organism were maintained at 4°C in 10 ml of cooked-meat medium (Difco Laboratories). A 1-ml sample of the stock culture was inoculated into 100 ml of TGY medium, pH 7.4 (3% Trypticase peptone [Becton, Dickinson & Co.], 2% yeast extract [Difco Laboratories], 0.5% glucose, 0.1% cysteine). The culture was grown for 6 h in a 46°C water bath and was in turn used to inoculate a 1,000-ml batch of TGY medium. The batch culture was grown at 46°C until it reached an absorbance at 600 nm of 0.8, equivalent to a density of 10 mg of cells (wet weight) per ml. No special precautions were followed to maintain anaerobiosis. Cells were harvested by centrifugation for 10 min at 10,000 $\times g$, and the cell paste was frozen and stored at -80°C.

Purification of PS synthase. The following steps were carried out at 5°C.

(i) **Preparation of cell-free extracts.** Frozen cells (10 g) were washed once in 50 ml of 50 mM Tris-hydrochloride buffer (pH 7.5) containing 10 mM MgCl₂ and suspended in 50 ml of the same wash buffer. Cells were disrupted by sonic oscillation with a Branson model W-185 sonicator and centrifuged for 10 min at 12,000 $\times g$. The supernatant was further treated as described below.

(ii) **Preparation of cell envelope.** The cell-free extract was centrifuged at 100,000 $\times g$ for 60 min. The resulting pellet was suspended in wash buffer (30 ml) and centrifuged again at 100,000 $\times g$ for 60 min. The pellet was resuspended in 6.0 ml of wash buffer supplemented with 20% glycerol (wt/vol) and stored at -80°C.

(iii) **Preparation of Triton X-100 extract.** The enzyme was extracted from the cell envelope fraction (containing 1,350 U of PS synthase activity) with solubilization buffer (50 mM Tris-hydrochloride [pH 7.5], 2.0 mM MnCl₂, 20% glycerol [wt/vol]) containing 1.0% Triton X-100 in a total volume of 20 ml as described by Cousminer and Carman (8).

(iv) **Affinity chromatography.** CDP-diacylglycerol-Sepharose resin (1.5 ml) was washed with 50 volumes of deionized water and equilibrated with 12 volumes of solubilization buffer containing 1% Triton X-100. Resin was then mixed batchwise with 19.5 ml of the Triton X-100 extract (containing 1,300 U of PS synthase activity), and allowed to react for 2.5 h with shaking. The mixture was then poured into a 20-ml capacity column, and the run-through was collected and assayed for PS synthase activity. The column was washed with 16 column volumes of solubilization buffer containing 0.5% Triton X-100. Solubilization buffer containing 0.5% Triton X-100, 0.8 M hydroxylamine, and 1.6 mM CDP-diacylglycerol was then applied to the column and allowed to react for 2 h. The enzyme was then eluted with 16 column volumes of this buffer at a flow rate of 20 ml/h. Fractions (6 ml) were assayed for PS synthase activity, and protein was monitored by following the absorbance at 280 nm. Fractions containing PS synthase activity were pooled and dialyzed overnight against solubilization buffer containing 0.5% Triton X-100 and 50% glycerol to concentrate the enzyme and remove hydroxylamine. Samples of the partially purified enzyme were stored at -80°C for later studies.

Sephacryl S-300 column chromatography. A sample of the dialyzed peak fractions from the affinity column (containing 60 U of PS synthase activity) was applied to a Sephacryl S-300 column (2.5 by 40.0 cm) equilibrated and eluted with solubilization buffer containing 0.5% Triton X-100. Fractions (3.0 ml) were collected at a flow rate of 20 ml/h and analyzed for PS synthase activity.

Protein determination. Protein was determined by the Coomassie blue dye-binding method of Bradford (2) with bovine serum albumin as the standard.

SDS-polyacrylamide gel electrophoresis. Electrophoresis in the presence of SDS was carried out as described by Laemmli (21) with an acrylamide/*N,N'*-methylenebisacrylamide ratio of 36.5:1. Samples for electrophoresis were first treated at 100°C for 90 s in buffer containing 62.5 mM Tris-hydrochloride (pH 6.8), 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol. Electrophoresis was carried out in a buffer consisting of 0.25 M Tris-hydrochloride (pH 8.3),

0.192 M glycine, and 0.1% SDS at 30 mA for 4 h with model SE-600 vertical slab gel electrophoresis unit with a PS-500 power supply (Hoefer Scientific Instruments, Inc.). Gels were stained in 0.125% Coomassie blue and destained in a 7% acetic acid–5% methanol solution. Protein molecular-weight standards were phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).

Enzyme assay. PS synthase activity was measured in triplicate assays performed at 30°C by following the incorporation of 0.5 mM L-[3-³H]serine (1,000 to 1,500 cpm/nmol) into chloroform-soluble material in the presence of 0.1 M Tris-hydrochloride buffer (pH 8.5)–3.0 mM MnCl₂–0.75 mM CDP-diacylglycerol–2.7 mM Triton X-100–0.6 µg of purified enzyme protein in a final volume of 0.1 ml. The reaction was stopped at 20 min by the addition of 0.5 ml of 0.1 N HCl in methanol. Chloroform (2 ml) and 1 M MgCl₂ (3 ml) were added, the system was mixed, and the phases were separated by a 2-min centrifugation at 100 × g. A 1-ml sample of the chloroform phase was removed and taken to dryness in an 80°C water bath. Insta-Fluor (10 ml) was added to the sample, and radioactivity was determined with a Packard 3255 liquid scintillation counter. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol of product per min under the assay conditions described above. The specific activity was defined as the units of enzyme activity per milligram of protein. The phospholipid product, PS, was identified by chromatography on activated (110°C for 45 min) silica gel thin-layer plates. Phospholipids were visualized by the method of Dittmer and Lester (11). A single spot cochromatographed with standard PS in the solvent system chloroform-methanol-water (65/25/4) with an *R_f* of 0.25 and in the solvent system chloroform-methanol-acetic acid (65/25/8) with an *R_f* of 0.17.

RESULTS

Purification of PS synthase. The purification procedure for PS synthase from *C. perfringens* required the development of a system for solubilizing the enzyme from the membranes and affinity chromatography of the solubilized extract with the CDP-diacylglycerol–Sephadex resin developed by Larson et al. (24). A summary of the purification procedure is given in Table 1. Successful solubilization of the enzyme with Triton X-100 was completely dependent on the

addition of manganese ions (8). Approximately 96% of the PS synthase activity was solubilized from the cell envelope fraction with a 4.5-fold increase in specific activity. The major purification was achieved by affinity chromatography with a CDP-diacylglycerol–Sephadex column. It was essential that the solubilized enzyme be allowed to interact with the resin in the presence of manganese for at least 2 h for effective binding to take place. Under the conditions reported here, 97% of the applied units of PS synthase activity were bound to the resin, whereas over 96% of the applied protein was recovered in the run-through and wash fractions. A typical elution profile for PS synthase and protein from an affinity resin is shown in Fig. 1. Elution of the enzyme required the use of a buffer containing both the substrate CDP-diacylglycerol and hydroxylamine. In the absence of either of these components, enzyme dissociation from the column was significantly reduced. Under the eluting conditions reported here, 88% of the bound PS synthase activity was eluted from the affinity resin. Fractions containing PS synthase activity were pooled and dialyzed against 50% glycerol to remove hydroxylamine and concentrate the enzyme. The affinity chromatography step resulted in a 21-fold increase in specific activity and an overall purification of 337-fold over the cell-free extract, with a yield of 73%. After dialysis, the pooled eluant was stored at –80°C in the presence of 50 mM Tris-hydrochloride (pH 7.5)–3.0 mM CDP-diacylglycerol–0.94% Triton X-100–2.0 mM MnCl₂. Under these conditions, the enzyme was virtually 100% stable for at least 4 months and was stable to one cycle of freezing and thawing. After four cycles of freezing and thawing, approximately 60% of the original activity was retained. When a sample of the enzyme preparation was subjected to polyacrylamide gel electrophoresis in the presence of SDS, two major bands were evident, one which migrated with an apparent minimum subunit molecular weight of 39,000 and a second, less intense band which migrated with an apparent minimum subunit molecular weight of 31,000 (Fig. 2). In addition to these major bands, a few faint bands appeared in the gel. Subsequently,

TABLE 1. Purification of PS synthase^a

Purification step	Vol (ml)	Total U	Total protein (mg)	Sp act	% Yield	Purification (fold)
1. Crude cell-free extract	50.0	1,530	264	5.8	100	1
2. Cell envelope	6.0	1,350	65	21	88	3.6
3. Triton X-100 extract	19.5	1,300	14	93	85	16
4. CDP-diacylglycerol–Sephadex	9.5	1,120	0.57	1,960	73	337

^a Data are based on starting with 10 g (wet weight) of *C. perfringens* cell paste.

FIG. 1. Elution profile of PS synthase and protein from CDP-diacylglycerol-Sepharose. Fractions (6.0 ml) were collected and assayed for PS synthase activity (●) and protein (X) as described in the text. ↓, Point at which elution buffer was applied.

when a sample containing 60 U of the purified PS synthase activity was applied to a calibrated Sephacryl S-300 gel filtration column, the enzyme eluted in the void volume with an apparent native molecular weight of greater than 1.0×10^6 . Presumably, the enzyme was associated with a mixed micelle containing both Triton X-100 and CDP-diacylglycerol (which were present in the purified preparation and were required for enzyme stability). The fractions containing PS synthase activity also contained phosphatidylglycerophosphate (PGP) synthase activity. PGP synthase is a phospholipid biosynthetic enzyme from *C. perfringens* (6) that also catalyzes a reaction which involves CDP-diacylglycerol. The specific activity of PGP synthase in the preparation was 250. The overall purification of PGP synthase was 144-fold over the cell-free extract, with a yield of 77%. Additional purification techniques were attempted to separate the two CDP-diacylglycerol-dependent enzymes, but our efforts either failed to separate the two activities or resulted in considerable loss of PS synthase activity. Characterization studies of PS synthase were performed with the CDP-diacylglycerol-Sepharose-purified enzyme.

Effect of pH, Triton X-100, and manganese on PS synthase activity. PS synthase activity was assayed at various pH values ranging from 5.0 to

9.5. 2-(*N*-Morpholino)ethanesulfonic acid buffer was used for pH 5.0 to 7.0, and Tris buffer was used for pH 7.25 to 9.5. The pH optimum for the purified enzyme was pH 8.5. The identical pH optimum was found when the enzyme was assayed at various pH values with Tris-maleate buffer. The effect of the nonionic detergent Triton X-100 on PS synthase activity is shown in Fig. 3. The addition of Triton X-100 to the assay system stimulated activity to a maximum at a concentration of 2.7 mM, which corresponds to a Triton X-100/CDP-diacylglycerol ratio of 3.6:1. Concentrations of the detergent above this level resulted in an apparent inhibition of PS synthase activity, presumably owing to substrate dilution kinetics (4). PS synthase activity was measured in the presence of various concentrations of its preferred cofactor, manganese (6). Activity was completely dependent on the

FIG. 2. Polyacrylamide gel electrophoresis in the presence of SDS. Six micrograms of standard molecular-weight proteins (lane 1), 12 micrograms of the Triton X-100 extract (lane 2), 12 micrograms of the affinity column run-through (lane 3), and 3 micrograms of the dialyzed CDP-diacylglycerol-Sepharose eluant (lane 4) were applied to the stacking gel and subjected to electrophoresis as described in the text.

FIG. 3. Effect of Triton X-100 concentration on PS synthase activity. PS synthase (0.30 U) was assayed as described in the text with the indicated concentrations of Triton X-100.

addition of manganese ions, with maximum activity obtained at 3.0 mM (Fig. 4). Magnesium ions (50 mM) could replace manganese ions for activity (6); however, no other divalent cations would serve as a cofactor for the enzyme.

Dependence of PS synthase activity on the concentration of CDP-diacylglycerol and L-serine. Purified PS synthase exhibited normal saturation kinetics when the concentration of L-serine was held constant (2.0 mM) and the concentration of CDP-diacylglycerol was varied. The molar ratio of Triton X-100 to CDP-diacylglycerol was maintained at 5:1. The apparent K_m for CDP-diacylglycerol was 0.24 mM. Saturation kinetics were also exhibited when the concentration of CDP-diacylglycerol was held constant (1.2 mM) and the concentration of L-serine was varied. The apparent K_m for L-serine was 0.26 mM. The apparent V_{max} for the reaction was 4,200 U/mg. The kinetic constants were determined from reciprocal plots drawn as the result of a least-squares analysis of the data. The regression coefficients from these plots were 0.98.

Determination of the equilibrium constant for the reaction. To determine the equilibrium constant, a standard assay for PS synthase activity was allowed to continue until equilibrium was reached. Under these conditions, the reaction was linear for the first 90 min, and equilibrium was reached at 5 h with 98% conversion of L-serine to PS. The equilibrium constant for the reaction was determined to be 83, indicating that the synthetic (forward) reaction was favored.

Temperature studies. PS synthase was assayed over a range of temperatures from 5 to 70°C. Maximum activity was observed at 40°C (Fig. 5). Assay temperatures higher than 40°C probably inactivated the enzyme. An Arrhenius plot for PS synthase was constructed from a

least-squares analysis of the temperature values from 5 to 40°C (Fig. 5). The regression coefficient from the plot was 0.98. The energy of activation for the reaction was determined to be 18 kcal/mol (75,362 J/mol). The enzyme was not stable to heating for 10 min above 30°C, with inactivation occurring most rapidly above 40°C (Fig. 6). Activity was negligible above 60°C. In comparison, PS synthase present in the cell envelope fraction was quite stable, retaining virtually 100% of its original activity even after being heated for up to 20 min at temperatures as high as 60°C (6). This difference might be due to a protective effect afforded to the enzyme by its native membrane environment.

DISCUSSION

PS synthase from *C. perfringens* is an integral membrane protein which requires detergent treatment to obtain it in soluble form. In addition to its role in membrane disruption, the nonionic detergent Triton X-100 is required during purification procedures to prevent the solubilized enzyme from undergoing irreversible self-aggregation (presumably through hydrophobic interactions) in aqueous chromatography buffers (23). This complicates further purification procedures because the presence of detergent in the solubilized enzyme extract interferes with many of the standard techniques for enzyme purification. This is due to the tendency of solubilized membrane proteins to bind large quantities of detergent (12) which can mask the hydrodynamic and electrostatic characteristics of the protein, which are essential for the separation of proteins with traditional purification techniques (18).

Affinity chromatography techniques are not hindered by the presence of detergents, as their

FIG. 4. Effect of manganese ions on PS synthase activity. PS synthase activity (0.24 U) was measured as described in the text with the indicated concentrations of $MnCl_2$. The enzyme used for this experiment was desalted in a Sephacryl S-300 column.

FIG. 5. Effect of temperature on catalysis. PS synthase activity (0.40 U) was measured over the indicated range of temperatures under standard assay conditions in a controlled-temperature water bath.

effectiveness depends solely on the specificity of the enzyme for its substrate. CDP-diacylglycerol-Sepharose affinity chromatography has been used to purify Triton X-100-solubilized PGP synthase from *Bacillus licheniformis* (24) and *E. coli* (19). Using the CDP-diacylglycerol-Sepharose affinity resin described by Larson et al. (24), we were able to purify PS synthase from *C. perfringens*. Since the nonionic detergent Triton X-100 has been shown to be required for in vitro activity of this enzyme (6), its presence in the solubilized extract presumably increased purification effectiveness by enhancing the binding of substrate by the enzyme. Under the conditions of our experiments, we were able to get 97% of the applied units of solubilized PS synthase activity to bind to the affinity column, and of this, we were able to recover approximately 88% of the units with a 337-fold purification over the cell-free extract. The purification procedure failed to yield a homogeneous enzyme preparation, based on the results of SDS-polyacrylamide gel electrophoresis, as two major protein bands were evident along with a few minor bands. It is probable that the two major bands represent PS synthase and PGP synthase. Attempts to separate these activities have been unsuccessful.

It has been widely noted that the in vitro

activity of many of the phospholipid biosynthetic enzymes in bacteria is either directly dependent on or greatly enhanced by the presence of small concentrations of nonionic detergents such as Triton X-100 (6, 7, 14, 19, 20, 22, 23, 30, 34). In the *E. coli* assay system for PS synthase, Triton X-100 forms a mixed micelle with the lipid substrate CDP-diacylglycerol, thereby improving the accessibility of the substrate to the enzyme (4). The effect of Triton X-100 on PS synthase activity from *C. perfringens* is similar to that shown for PS synthase from *E. coli*.

Like PS synthase derived from all gram-positive bacteria studied thus far (6, 14, 22, 30, 36), the enzyme purified from *C. perfringens* requires the addition of divalent metal ions for in vitro activity. In contrast, there is no cation requirement for the assay of PS synthase from gram-negative bacteria (13, 20, 23, 34).

Experimental evidence also indicates a probable difference in the reaction mechanism between the PS synthase derived from the two bacterial families. Based on a series of exchange reactions catalyzed by PS synthase from cell extracts of *B. licheniformis*, the enzyme appears to follow a sequential BiBi reaction mechanism

FIG. 6. Thermal stability of PS synthase. Samples (10 μ l) of the purified enzyme (0.21 U) were heated for 10 min at the indicated temperatures in a controlled-temperature water bath. After incubation, samples were cooled on ice and assayed for PS synthase activity at 30°C as described in the text.

(14). On the other hand, PS synthase from *E. coli* follows a "ping-pong" reaction mechanism (23). Owing to presence of CDP-diacylglycerol in our purified preparations, we were unable to perform extensive kinetic or isotopic exchange studies to determine the reaction mechanism for PS synthase from *C. perfringens*. However, the PS synthase from *C. perfringens* would not catalyze an exchange reaction between CMP and CDP-diacylglycerol in the absence of L-serine (data not shown). This would imply that PS synthase from *C. perfringens* does not follow a ping-pong type of reaction mechanism. Another indication of differing reaction mechanisms between PS synthase from *C. perfringens* and from *E. coli* is that the *E. coli*-derived enzyme does not bind to a CDP-diacylglycerol-Sepharose column (23), whereas PS synthase from *C. perfringens* does bind to CDP-diacylglycerol-Sepharose.

Strains of *E. coli* have been constructed with hybrid plasmids carrying the structural gene for PS synthase which directs the 20-fold overproduction of the enzyme (35). These strains have made the isolation of large quantities of pure enzyme for characterization more simple. The development of strains that overproduce PS synthase in *C. perfringens* or in other gram-positive bacteria would be helpful.

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LITERATURE CITED

- Bangham, A. D. 1972. Lipid bilayers and biomembranes. *Annu. Rev. Biochem.* 41:753-776.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* 72:248-254.
- Caplan, E. S., and R. M. Kluge. 1976. Gas gangrene: review of 34 cases. *Arch. Intern. Med.* 136:788-791.
- Carman, G. M., and W. Dowhan. 1979. Phosphatidylserine synthase from *Escherichia coli*: the role of Triton X-100 in catalysis. *J. Biol. Chem.* 254:8391-8397.
- Carman, G. M., and A. S. Fischl. 1980. Modification of the Agranoff-Suomi method for the synthesis of CDP-diacylglycerol. *J. Food Biochem.* 4:53-59.
- Carman, G. M., and D. S. Wiczorek. 1980. Phosphatidylglycerophosphate synthase and phosphatidylserine synthase activities in *Clostridium perfringens*. *J. Bacteriol.* 142:262-267.
- Carman, G. M., R. L. Zaniewski, and J. J. Cousminer. 1981. CDP-diacylglycerol synthase activity in *Clostridium perfringens*. *Appl. Environ. Microbiol.* 43:81-85.
- Cousminer, J. J., and G. M. Carman. 1981. Solubilization of membrane-associated phosphatidylserine synthase from *Clostridium perfringens*. *Can. J. Microbiol.* 27:544-547.
- Craven, S. E. 1980. Growth and sporulation of *Clostridium perfringens* in foods. *Food Technol.* 34:80-87.
- Cronan, J. E., Jr. 1979. Phospholipid synthesis and assembly, p. 35-66. In M. Inouye (ed.), *Bacterial outer membranes*. John Wiley & Sons, Inc., New York.
- Dittmer, J. C., and R. L. Lester. 1964. A simple specific spray for the detection of phospholipids on thin layer chromatograms. *J. Lipid Res.* 5:126-127.
- 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.
- Dutt, A., and W. Dowhan. 1977. Intracellular distribution of enzymes of phospholipid metabolism in several gram-negative bacteria. *J. Bacteriol.* 132:159-165.
- Dutt, A., and W. Dowhan. 1981. Characterization of a membrane-associated cytidine diphosphate-diacylglycerol-dependent phosphatidylserine synthase in bacilli. *J. Bacteriol.* 147:535-542.
- Hanson, R. L., and E. P. Kennedy. 1973. Energy-transducing adenosine triphosphatase from *E. coli*: purification, properties, and inhibition by antibody. *J. Bacteriol.* 114:772-781.
- Hatheway, C. L., D. N. Whaley, and V. R. Dowell, Jr. 1980. Epidemiological aspects of *Clostridium perfringens* foodborne illness. *Food Technol.* 34:77-79.
- Hawrot, E., and E. P. Kennedy. 1975. Biogenesis of membrane lipids: mutants of *Escherichia coli* with temperature-sensitive phosphatidylserine decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 72:1112-1116.
- Helenius, A., and K. Simons. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta* 415:29-79.
- Hirabayashi, T., T. J. Larson, and W. Dowhan. 1976. Membrane-associated phosphatidyl glycerophosphate synthetase from *Escherichia coli*: purification by substrate affinity chromatography on cytidine 5'-diphospho-1,2-diacyl-sn-glycerol Sepharose. *Biochemistry* 15:5205-5211.
- Kanfer, J., and E. P. Kennedy. 1964. Metabolism and function of bacterial lipids. II. Biosynthesis of phospholipids in *Escherichia coli*. *J. Biol. Chem.* 239:1720-1726.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Langley, K. E., M. P. Yaffe, and E. P. Kennedy. 1979. Biosynthesis of phospholipids in *Bacillus megaterium*. *J. Bacteriol.* 140:996-1007.
- Larson, T. J., and W. Dowhan. 1976. Ribosomal-associated phosphatidylserine synthetase from *Escherichia coli*: purification by substrate-specific elution from phosphocellulose using cytidine 5'-diphospho-1,2-diacyl-sn-glycerol. *Biochemistry* 15:5212-5218.
- Larson, T. J., T. Hirabayashi, and W. Dowhan. 1976. Phosphatidylglycerol biosynthesis in *Bacillus licheniformis*: resolution of membrane-bound enzymes by affinity chromatography on cytidinediphospho-sn-1,2-diacylglycerol Sepharose. *Biochemistry* 15:974-979.
- Leive, L. 1973. The cell envelope, p. 107-137. In B. D. Davis, R. Dulbecco, H. N. Eisen, H. S. Ginsberg, W. B. Wood, Jr., and M. McCarty (ed.), *Microbiology*, 2nd ed. Harper & Row, Publishers, New York.
- Louie, K., and W. Dowhan. 1980. Investigations on the association of phosphatidylserine synthase with the ribosomal component from *Escherichia coli*. *J. Biol. Chem.* 255:1124-1127.
- MacLennan, J. D. 1962. The histotoxic clostridial infections of man. *Bacteriol. Rev.* 26:177-274.
- March, S. C., I. Parikh, and P. Cuatrecasas. 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. *Anal. Biochem.* 60:149-152.
- Nalk, H. S., and C. L. Duncan. 1977. Enterotoxin formation in foods by *Clostridium perfringens* type A. *J. Food Safety* 1:7-18.
- Patterson, P. H., and W. J. Lennarz. 1971. Studies on the membranes of bacilli: phospholipid biosynthesis. *J. Biol. Chem.* 246:1062-1072.
- Raetz, C. R. H. 1978. Enzymology, genetics, and regula-

- tion of membrane phospholipid synthesis in *Escherichia coli*. Microbiol. Rev. 42:614-659.
32. Raetz, C. R. H., and J. Foulds. 1977. Envelope composition and antibiotic hypersensitivity of *Escherichia coli* mutants defective in phosphatidylserine synthetase. J. Biol. Chem. 252:5911-5915.
 33. Raetz, C. R. H., and E. P. Kennedy. 1972. The association of phosphatidylserine synthetase with ribosomes in extracts of *Escherichia coli*. J. Biol. Chem. 247:2008-2014.
 34. Raetz, C. R. H., and E. P. Kennedy. 1974. Partial purification and properties of phosphatidylserine synthetase from *Escherichia coli*. J. Biol. Chem. 249:5038-5045.
 35. Raetz, C. R. H., T. J. Larson, and W. Dowhan. 1977. Gene cloning for the isolation of enzymes of membrane lipid synthesis: phosphatidylserine synthetase overproduction in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 74:1412-1416.
 36. Silber, P. R., P. Borie, and H. Goldfine. 1980. The enzymes of phospholipid synthesis in *Clostridium butyricum*. J. Lipid Res. 21:1022-1031.
 37. Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. Science 175:720-731.
 38. Smith, L. DS., and G. Hobbs. 1973. Genus III, *Clostridium*, p. 551-573. In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
 39. Smith, L. DS., and L. V. Holdeman. 1968. The pathogenic anaerobic bacteria, p. 185-200, 201-205, 406-415. Charles C Thomas, Publishers, Springfield, Ill.
 40. Sours, H. E., and D. G. Smith. 1980. Outbreaks of foodborne disease in the United States, 1972-1978. J. Infect. Dis. 142:122-125.
 41. Tanford, C. 1980. The hydrophobic effect: formation of micelles and biological membranes, 2nd ed., p. 181-204. John Wiley & Sons, Inc., New York.