Purification and Properties of Phosphatidylserine Decarboxylase from *Escherichia coli*

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SUMMARY

Phosphatidylserine decarboxylase, a membrane-bound enzyme of *Escherichia coli*, catalyzes the final step in the biosynthesis of phosphatidylethanolamine, the principal phospholipid of that organism. The enzyme has now been extracted from the membranes with the nonionic detergent Triton X-100 and has been purified about 3600-fold to near homogeneity. The detergent was present throughout the purification, which includes steps of solvent fractionation, ion exchange chromatography, gel filtration, and gradient centrifugation. Electrophoresis on disc gels in the presence of sodium dodecyl sulfate revealed the presence of a single polypeptide estimated to be of molecular weight 35,000. The amino acid composition of this purified membrane protein is not strikingly different from that of the total protein of *E. coli*.

The enzyme is completely dependent for activity on added detergent in the test system and is highly sensitive to inhibition by reagents that attack carbonyl groups. Other properties of the enzyme are described.

The pathways for the biosynthesis of membrane phospholipids in *Escherichia coli* have been outlined in previous reports from this laboratory (1-5). The final step in the formation of phosphatidylethanolamine, which constitutes about 70% of the phospholipids of this organism, is the decarboxylation of phosphatidylserine:

\[
\text{Phosphatidylserine} \rightarrow \text{phosphatidylethanolamine} + \text{CO}_2
\]

With the striking exception of phosphatidylserine synthetase which is associated with ribosomes in extracts of *E. coli* (5), the enzymes catalyzing the synthesis of phospholipids are localized in the inner, cytoplasmic membrane. Study of the enzymes of phospholipid biosynthesis is therefore not only important for an understanding of membrane biogenesis and its regulation, but also may shed light on the properties of an important class of membrane proteins. Progress in membrane biochemistry is presently severely limited by the lack of adequate methods for the isolation and characterization of membrane proteins. The work to be described here thus had two objectives: to isolate and characterize an enzyme essential for membrane biogenesis and to test and develop procedures that might have some more general usefulness for purifying membrane proteins.

Some aspects of this work were described in a preliminary report (6).

**Materials and Methods**

Following are the sources of unusual chemicals used in this study: phosphatidylserine from bovine brain (estimated from thin layer chromatography to be about 50% pure) from Mann; DEAE-cellulose (DE52) from Whatman; QAE-Sephadex from Pharmacia; Triton X-100 (isoctyl polyethoxyethanol) from Rohm and Haas; Agarose-4% from Aldrich Chemical Co.; 4-bromo-3-hydroxybenzoxylamine (NSD-1055) from Smith and Nephew Ltd.; Barlox-12 (lauryldimethylamine oxide) from Baird Chemicals; lactate dehydrogenase and pyruvate kinase from Boehringer; and O-phosphatidylserine from Calbiochem. Frozen cells of *E. coli* B used as a source of the enzyme were obtained from Grain Processing Corp. The cells had been grown on rich medium and harvested late in log phase of growth.

Phosphatidyl[1-14C]serine was prepared enzymatically from (dipalmitoyl) CDP-diglyceride by a procedure based on that of Kanfer and Kennedy (2). Glycerophosphoryl[1-14C]serine was prepared from the labeled phospholipid by mild alkaline hydrolysis (2).

**Assay of Phosphatidylserine Decarboxylase**—Activity was measured by release of 14CO2 from phosphatidyl[1-14C]serine at 37° in a 25-ml Erlenmeyer flask stopped with a rubber serum cap and equipped with a center well containing a folded piece of Whatman No. 1 filter paper (2 X 3 cm) wet with 0.05 ml of 2 N KOH. The assay mixture contained 0.2 M potassium phosphate, pH 7.0, 0.2% Triton X 100, 0.1 to 0.2 nmol phosphatidyl[1-14C]serine, and enzyme in a final volume of 0.5 ml. The specific activity of the labeled dipalmitoylphosphatidylserine was adjusted to about 100,000 cpm per nmole by mixing with unlabeled brain phosphatidylserine which, although of different fatty acid composition, was found to be indistinguishable from the dipalmitoyl phospholipid as an isotopic diluent. The reaction was stopped after 30 min by the addition of 0.5 ml of 1 N HCl from a syringe through the rubber cap. After 30 min to allow the radioactive CO2 to be taken up, the paper was removed and counted in a mixture of 10 ml of Patterson-Greene scintillation fluid (7) and 1 ml of water. One unit of activity is defined as the amount of enzyme which forms 1 nmole of product per min under these conditions.

**Amino Acid Analysis**—Enzyme samples containing detergent were precipitated with 90% acetone at 0°. The precipitated samples (0.2 to 0.8 mg) then were hydrolyzed in acid-washed, evacuated tubes containing 1 ml of constant boiling HCl and a crystal of phenol to retard oxidation of tyrosine. The hydrolyzed sam-
Acrylamide Gel Electrophoresis—Acrylamide gel electrophoresis under non-denaturing conditions was carried out using the running buffer of pH 8.2 described by Davis (8). The concentrations of acrylamide and of bisacrylamide normally employed were 4.5 and 0.15%, respectively, in the running gel and 2.5% acrylamide and 0.03% bisacrylamide in the stacking gel. The buffers sometimes contained 0.1% Triton X-100. In order to determine the position of the decarboxylase, the gels were sliced in 2-mm segments which then were incubated at room temperature for 6 hours in 0.2 ml of the assay mixture minus substrate. Substrate then was added and decarboxylase activity determined.

Acrylamide disc gels in the presence of sodium dodecyl sulfate were run by the method of Neville (9) using a running gel of pH 6.1 (3% in acrylamide and 0.2% in bisacrylamide). In order to determine the position of protein bands, gels were first fixed in 10% acetic acid-50% methanol and then stained in the same solvent containing 0.2% Coomassie blue. The gels were destained in 10% acetic acid. Destained gels were scanned at 600 nm using a Gilford recording spectrophotometer equipped with a linear transporter and a No. 2412 cuvette.

Determination of Protein—Protein was determined by the method of Lowry et al. (10); correction for the presence of small amounts of Triton X-100 was made when necessary by adding an identical amount of Triton X-100 to the standard samples. In some experiments, the cells used for fractionation were labeled by the addition of small amounts of cells grown for one generation in the presence of uniformly labeled [14C]leucine. Content of protein in fractions then was estimated from radioactivity, on the assumption that all of the proteins of the cells were uniformly labeled.

RESULTS

Purification of Phosphatidylserine Decarboxylase

Seven batches of cells were fractionated through Step 5 of the procedure described below. All seven batches were then pooled at Step 6 and carried through each succeeding step as described. The combined results of the seven batches are shown in Table 1.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total volume</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Yielda</th>
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<tr>
<td>1. Cell-free extract...</td>
<td>20.5 liters</td>
<td>1150 g</td>
<td>14</td>
<td>100</td>
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<tr>
<td>2. Triton extract...</td>
<td>7.1 liters</td>
<td>147 g</td>
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<td>60</td>
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<tr>
<td>3. Acetone precipitation</td>
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<td>43.6 g</td>
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<td>29</td>
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<tr>
<td>4. DEAE-cellulose...</td>
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<td>392 mg</td>
<td>0.750</td>
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<td>7. Sucrose gradient centrifugation...</td>
<td>6 ml</td>
<td>20 mg</td>
<td>48,000</td>
<td>6</td>
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<tr>
<td>8. Agarose, 4%...</td>
<td>31 ml</td>
<td>14.3 mg</td>
<td>49,000</td>
<td>5</td>
</tr>
</tbody>
</table>

a Cumulative results of seven batches, each of 1.1 kg of Escherichia coli B.

The combined results of the seven batches are shown in Table 1.

Step 1: Preparation of Cell-free Extract—Frozen E. coli B cell paste (1.1 kg) was suspended in 1.6 liters of buffer (25%) containing 0.1 M potassium phosphate, pH 6.8, 5 mM MgSO₄, and 10 mM 2-mercaptoethanol, using a large Waring Blendor. The cell suspension was then passed four times through a Mantin-Gaulin press; the temperature of the suspension usually rose to 30°-35°.

Step 2: Preparation of the Membrane Extract—The cell envelope fraction was collected by centrifugation of the cell-free extract at 45,000 × g for 5 hours at 4°. The supernatant was discarded and the pellet suspended with the aid of a Waring Blendor at 4° in 970 ml of 0.1 M potassium phosphate buffer of pH 7.15, containing 5% Triton X-100 and 10 mM 2-mercaptoethanol. Although no further extraction occurred after 30 min at 4°, the suspension was allowed to stand 12 hours before insoluble material was removed by a 90-min centrifugation at 45,000 × g and 4°.

Step 3: Acetone Precipitation—The membrane extract was brought to 20% in glycerol and the pH lowered to between 5.1 and 5.4 with 0.5 N acetic acid. Acetone (4°) was rapidly added with stirring to a final concentration of 70%. The precipitate was immediately collected by a 10-min centrifugation (4°) at 14,000 × g. The supernatant was discarded. The pellet was suspended with gentle stirring (15 min) in a Waring Blendor in 3 liters (4°) of 10 mM sodium acetate, pH 5.0, containing 1% Triton X-100, 15% glycerol, and 10 mM 2-mercaptoethanol; insoluble material was removed by centrifugation at 4° (14,000 × g, 20 min) and discarded. The supernatant was then adjusted to between pH 7.0 and 7.4 with a saturated solution of Tris free base.

Step 4: Chromatography on DEAE-cellulose—The supernatant from Step 3 (4°) was applied to a column (9 × 28 cm) of DEAE-cellulose at room temperature suspended in Buffer A (10 mM potassium phosphate (pH 7.4), 1% Triton X-100, 10% glycerol, and 10 mM 2-mercaptoethanol) at a flow rate of 1.0 to 1.5 liters per hour; the column was then washed with 500 ml of Buffer A. The enzyme was eluted from the column using a linear gradient of NaCl (0 to 0.6 M in 12 liters) in Buffer A at the rate of 1.0 liters per hour. The peak of activity was recovered in about 1 liter emerging at about 0.17 M NaCl. To concentrate this material, it was diluted with 2.5 volumes of distilled water and applied to a column (2.4 × 4 cm) of DEAE-cellulose suspended in Buffer A. The flow rate was 800 ml per hour. The enzyme was eluted from this column with Buffer B identical with Buffer A, but with only 0.1% Triton) containing 0.6 M NaCl. The activity was recovered in a volume of 10 to 20 ml.

Step 5: Filtration through Sephadex G-150—The enzyme concentrate was applied to a column (3 × 90 cm) of Sephadex G-150 (room temperature) equilibrated with Buffer B containing 50 mM NaCl. The column then was developed at 30 ml per hour by upward flow. The enzyme emerged at the void volume.

Step 6: Chromatography on QAE-Sephadex—The partially purified enzyme from seven lots of cells processed through Step 5 was pooled and adjusted to pH 6.0 with 0.5 N acetic acid. The preparation then was divided and applied to two separate columns (each 2.4 × 40 cm) of QAE-Sephadex (A-25) at room temperature, in Buffer C (20 mM potassium phosphate, pH 6.0, 0.5% Triton X-100, 10% glycerol, and 10 mM 2-mercaptoethanol) which also contained 50 mM NaCl. The columns were washed with 60 ml of Buffer C containing 50 mM NaCl and then developed with a linear NaCl gradient (50 ml to 0.5 M in 1.0 liters) in Buffer C at 50 ml per hour. The pools of decarboxylase from each column were concentrated separately by first diluting the samples 1:3.5 with distilled water followed by adsorption of the enzyme on separate DEAE-columns (1.2 × 27 cm) in Buffer A. Buffer B, containing 0.6 M NaCl and only 1% glycerol, was used to elute the enzyme from the columns.

Step 7: Sucrose Gradient Centrifugation—Sucrose density gradients (5 to 20% in 0.01 M potassium phosphate, pH 7.4, 0.1% Triton X-100, and 10 mM 2-mercaptoethanol) were prepared in 12-ml polycarbonate tubes (9.5 cm in length). The enzyme con-
Centrino (0.45 ml per gradient) was layered on top of each gradient and the samples were centrifuged at 200,000 x g at 20° for 28 hours using an International B-60 preparative ultracentrifuge equipped with an SB 283 rotor. Each gradient was collected by piercing the bottom of the tubes with a short No. 22 needle, which was left in place to assure even flow. The decarboxylase was found by enzymatic assay to migrate about three-quarters of the distance down the gradient. The degree of contamination of each fraction was determined by acrylamide gel electrophoresis in the presence of either sodium dodecyl sulfate or Triton X-100 (see “Materials and Methods”), and the best fractions were pooled.

**Step 8: Agarose Gel Filtration**—The pooled enzyme fractions from Step 7 were filtered in two batches through a 4% Agarose column (1.8 x 40 cm) in Buffer B (also containing 0.6 M NaCl) at a flow rate of 8 ml per hour. The $K_v$ for the decarboxylase was 0.46 and the best fractions, as determined by acrylamide gel electrophoresis in sodium dodecyl sulfate, were pooled.

**Purity of Phosphatidylserine Decarboxylase**

Phosphatidylserine decarboxylase purified by this procedure approaches homogeneity as judged by the coincidence of enzymatic activity (nearly 100% recovery) with a peak representing more than 95% of the protein on acrylamide disc gels (Fig. 1A). Disc gels run in the presence of sodium dodecyl sulfate (Fig. 1B) show a small amount of high molecular weight contaminant (some of which may be undissociated decarboxylase) as well as one peak at lower molecular weight which represents about 3 to 10% of the major protein band in various batches.

**Properties of Phosphatidylserine Decarboxylase**

**Stability**—In solutions containing 10% glycerol and 0.1% Triton X-100 in the pH range from 6.0 to 7.8, the enzyme is stable for 1.5 years at -25°, for several months at 0°, and for at least 1 week at room temperature. At neutral pH the crude extract (Step 1) retains about half its activity after 10 min at 65°. Once the enzyme is extracted from the membrane (Step 2 and after) no enzymatic activity remains after heating for 2 min at 65°. Dilute enzyme at levels used in assay is unstable at 37° outside the pH range from 6.0 to 7.5; a 30 to 50% inactivation of the enzyme occurs in 10 min.

In general, the enzyme is stabilized by low temperature, neutral pH, and glycerol.

**Effect of pH**—When assayed under standard conditions the enzyme exhibits a broad, bell-shaped activity curve from pH 5.5 to 8.5 with a maximum from pH 6.5 to 7.5. An almost identical curve is observed if the enzyme is first incubated at 37° for 10 min at various values of pH and then assayed at pH 7.0, suggesting that the variation with pH may reflect the stability of the enzyme as well as its catalytic activity. Maximal activity was observed at 0.2 M potassium phosphate and higher; at 0.02 M potassium phosphate the activity was about 20% lower. The addition of NaCl up to 1 M caused no stimulation of the activity observed in low phosphate buffer.

**Effect of Detergents**—The enzyme has an absolute requirement for nonionic detergent such as Triton X-100 (Fig. 2) in the assay system. Crude Escherichia coli phospholipids neither substitute for Triton nor stimulate activity in the presence of Triton. The enzyme is inhibited by the addition to the standard assay of ionic detergents (0.1%) such as Barlox-12 and sodium dodecyl sulfate.

**Substrate Specificity**—Half the maximal rate of decarboxylation was observed at a concentration of 23 μM (dipalmitoyl) phosphatidylserine with the purified enzyme, indistinguishable from the value (24 μM) for the crude cell-free extract (Fig. 3). These concentrations represent total amounts of substrate added to the system; it is not known whether the enzyme acts on micellar or monomeric forms of the substrate. Since phosphatidylserine from beef brain is decarboxylated at the same rate as (dipalmitoyl) phosphatidylserine, as revealed by isotope dilution experiments, the decarboxylase from E. coli must have little specificity with regard to fatty acid residues on the substrate.

To test the affinity of the enzyme for O-phospho-D-serine and D-serine, these substances were added to the test system in 10- to 20-fold excess over the concentration of phosphatidyl[1-14C]serine (0.1 mM). No reduction in the rate of decarboxylation was noted, leading to the conclusion that the decarboxylase has no...
As assayed as described under "Materials and Methods" except that the concentration of Triton X-100 was varied as shown.

8 (0.24 unit) was used for Curve A and enzyme from Step 1 (0.32 unit) of the purification scheme was used for Curve B. The slope of the line equals 1/Km, and the intercept on the ordinate equals 1/Vmax.

**Fig. 3.** Effect of concentration of (dipalmitoyl) phosphatidylserine on the activity of phosphatidylserine decarboxylase. Release of 14CO2 was determined under the standard conditions at 37°C except that the (dipalmitoyl) phosphatidylserine concentration was varied. Phosphatidylserine decarboxylase from Step 8 was concentrated by dialysis under pressure of 0.1% dodecyl sulfate. The principal protein fraction emerged from the column at the same volume as the standard proteins with a molecular weight of the order of 36,500.

**Fig. 2.** Effect of Triton X-100 concentration on the activity of phosphatidylserine decarboxylase. The purified enzyme was assayed as described under "Materials and Methods" except that the concentration of Triton X-100 was varied as shown.

> **Amino Acid Composition**—Since electrophoresis on acrylamide gels in the presence of dodecyl sulfate showed a small amount of contaminating protein in the purified decarboxylase preparations, these impurities were removed by chromatography on Sephadex G-150 in buffer containing dodecyl sulfate prior to analysis of the enzyme for amino acids. A portion of the purified enzyme after Step 8 was concentrated by dialysis under pressure (Amicon XM50 filter), heated at 80°C for 10 min in 1% sodium dodecyl sulfate containing 10 mM 2-mercaptoethanol, and chromatographed on a column (1.8 x 40 cm) of Sephadex G-150 in a solution of 0.1% dodecyl sulfate. The principal protein fraction emerged from the column at the same volume as the standard protein pepticase (mol wt 35,000) and deoxyribonuclease (mol wt 31,000) in other runs. The amino acid composition (Table II) of the pooled protein peak was determined as described under "Materials and Methods". The average best whole number values based on 3.0 half-cystine residues per mole of protein yield a minimum molecular weight of 36,500 in good agreement with the value of 35,000 to 36,000 based on electrophoresis on acrylamide gels in the presence of dodecyl sulfate. Estimates based on the dry weight of the protein could not be made because of the presence of detergent in the samples.

**Estimates of Molecular Weight**—The purified decarboxylase was denatured at 80°C in 1% dodecyl sulfate containing 10 mM 2-mercaptoethanol. The minimum or subunit molecular weight was then estimated from electrophoresis on polyacrylamide gels in buffers containing dodecyl sulfate, along with standard proteins of known molecular weight (Fig. 4). Three such determinations yielded values from 35,000 to 36,000 in good agreement with the minimum molecular weight of 36,500 based on 3 half-cystine residues per mole.

The molecular weight of the native enzyme appears to be much larger than 36,500, although reliable determinations of the precise value were prevented by the presence of detergent in the purified sample. The decarboxylase in the presence of 0.1% Triton X-100 was excluded from Sephadex G-200, but in 4% agarose it has a Kav of about 0.46, suggesting a size corresponding to that of standard proteins with a molecular weight of the order of 400,000. Some part of this apparent size, however, must be attributed to the detergent, with which the enzyme strongly interacts, as is shown by the experiment of Fig. 5. When small amounts (10 μg) were centrifuged in a sucrose gradient containing no Triton, the enzyme sedimented in a single peak close to that of lactate dehydrogenase (mol wt 170,000). As the Triton concentration in the gradient was increased, the decarboxylase sedimented more slowly, in the range expected for proteins of molecular weight between 60,000 and 170,000. This behavior...
Fig. 4. Determination of the minimum molecular weight of phosphatidylserine decarboxylase by electrophoresis on acrylamide gel in the presence of 0.1% sodium dodecyl sulfate. Mobilities (Rf) were calculated relative to the bromphenol blue marker dye. The marker proteins hemoglobin (HB), trypsin inhibitor (TI), ovalbumin (OVA), and bovine serum albumin (BSA), as well as the purified decarboxylase, were denatured prior to electrophoresis by heating at 80°C for 10 min in 1% sodium dodecyl sulfate and 10 mM 2-mercaptoethanol at pH 6 (upper gel buffer). See text for details.

Fig. 5. Effect of Triton X-100 on the sedimentation velocity of phosphatidylserine decarboxylase. Sucrose density gradients were prepared as described under Step 7 of the purification scheme from solutions containing no Triton (A), 0.1% Triton (B), or 0.5% Triton (C). Samples (0.4 ml each) applied to the gradients contained 0.1 mg of lactate dehydrogenase (LDH), 0.1 mg of pyruvate kinase (PK), 1 mg of hemoglobin (HB), 10 μg of phosphatidylserine decarboxylase, and either 0.01% Triton (A), 0.1% Triton (B), or 0.5% Triton (C), respectively. The gradients were centrifuged at 200,000 X g for 22 hours at 4°C. The position of lactate dehydrogenase, pyruvate kinase, and decarboxylase were determined by enzymatic assay while the position of hemoglobin was determined by its absorbance at 430 nm.

appears to be dependent on the protein to Triton ratio since, at a higher level of protein, higher levels of Triton are needed to cause such effects (data not shown). The significance of these results is discussed briefly below.

Spectrum—The enzyme at a concentration of 0.4 mg per ml shows no absorption peaks in the visible region; the ultraviolet absorption appears to be unremarkable although the spectrum is complicated by the presence of Triton X-100 which absorbs near the protein aromatic bands.

DISCUSSION

No single experimental approach may be expected to be universally applicable to the purification of membrane proteins since these are very diverse. The procedures described here, however, based on extraction of membrane proteins with nonionic detergent followed by standard methods of purification compatible with the presence of the detergent, appear to have considerable general usefulness. Diglyceride kinase (12) and a hydrolase specific for CDP-diglyceride (13) have been extensively purified in this laboratory by methods essentially similar to that used for purification of the decarboxylase. Indeed, the hydrolase was obtained, purified more than 100-fold, as a by-product from the same batches of cells used for preparation of the decarboxylase, and has subsequently been purified more than 1000-fold using the same general approach. On the other hand, it has not been possible to purify the membrane protein component of lactase permease system using these general methods, since this protein forms aggregates in the Triton extracts that cannot be cleanly fractionated. Independent studies in numerous laboratories have reported success in the purification of other membrane proteins in Triton-containing buffers, notably the insulin receptor (14), the acetylcholine receptor (15), and cytochrome b₅ (16).

The inhibition of phosphatidylserine decarboxylase by reagents that attack carbonyl groups suggests the presence of a cofactor such as pyridoxal phosphate, found in many amino acid decarboxylases, or a keto acid at the active site, as in the decarboxylases for histidine (17) and 8-adenosylmethionine. The inhibited enzyme cannot be reactivated by the addition of pyridoxal phosphate, nor could we detect an absorption spectrum characteristic of pyridoxal-containing enzymes. Further work will be needed to settle the question of a possible cofactor.

The minimum molecular weight of the enzyme is about 36,000 estimated from electrophoresis in the presence of dodecyl sulfate, but the size of the native enzyme is not known. The enzyme sediments in sucrose gradients more slowly than would be expected from its apparent size during chromatography on agarose. This anomaly may result in part from a difference in shape from the standard proteins but may principally result from the binding of Triton by the membrane enzyme. A similar behavior was noted for the cholinergic receptor by Meunier et al. (15) who showed by studies of sedimentation in D₂O and in water that Triton caused an increase in the apparent partial specific volume of the receptor, explained by the formation of a complex with the less dense detergent.

It is striking that the sedimentation of the soluble, standard proteins is affected much less by the detergent than that of the membrane protein. Helenius and Simons (18) have similarly reported that a number of water-soluble proteins failed to bind detectable amounts of Triton X-100 under conditions where certain "lipophilic" proteins, such as the protein moiety of human low density serum lipoprotein, bound Triton and deoxycholate in amounts up to about 70% of their weight.

Membrane proteins appear to vary widely in their content of hydrophobic amino acids. A few such proteins are extremely

1 C. H. R. Raetz, unpublished experiments.
hydrophobic, a most notable example being the C₄₋isoprenoid alcohol phosphokinase described by Sandermann and Strominger (19) which is soluble in butanol at pH 4.2. Using the definition of Hatch and Bruce (20) for the set of apolar amino acids (Val + Ile + Leu + Phe + Met) the phosphokinase has the highest content (46 molar per cent) of any protein yet described. Twelve membrane lipoproteins reported by Hatch and Bruce (20) had an average of 29.9% apolar residues, only slightly different from the average of 31 soluble, oligomeric proteins (27.5% apolar residues). Phosphatidylserine decarboxylase has 31.1% of such residues (Table II) compared with 27.9% for the total protein of the soluble supernatant fraction of E. coli (22). It seems difficult to explain the distinctive properties of membrane proteins such as the decarboxylase on the basis of their relatively slight enrichment in apolar residues. Other general differences in their structures from those of soluble proteins must be considered, such as the clustering of the apolar residues in distinct regions, as has been reported for the membrane-bound cytochrome b₅ by Spatz and Strittmatter (16).

REFERENCES

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