Phosphatidyl glycerophosphate phosphatase

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ABSTRACT An enzyme (phosphatidyl glycerophosphate phosphatase) that catalyzes the formation of phosphatidyl glycerol from phosphatidyl glycerophosphate has been rendered soluble by treatment of the particulate fraction of *E. coli* with Triton X-100 in the presence of EDTA, and has been partially purified. The enzyme is specific for phosphatidyl glycerophosphate and does not catalyze the hydrolysis of other simple phosphomonoesters. It requires Mg^{++} for activity and is inhibited by sulfhydryl agents. Some other properties of the enzyme are also described.

KEY WORDS phosphatidyl glycerophosphate · phosphatidyl glycerol · enzymatic · *E. coli* · particulate fraction · Triton · EDTA · extraction · phosphatidic acid · alkaline phosphatase · acid phosphatase

PHOSPHATIDYL GLYCEROPHOSPHATE does not accumulate in *E. coli* (1) nor in animal tissues (2), but is dephosphorylated with the formation of phosphatidyl glycerol, which is one of the principal phospholipids in these organisms. Kiyasu, Pieringer, Paulus, and Kennedy (2) have shown that an enzyme that catalyzes such a dephosphorylation is present in the mitochondrial fraction of liver, but the enzyme has not been purified or characterized.

In the present study, phosphatidyl glycerophosphate phosphatase from *E. coli* has been rendered soluble by treatment of the particulate fraction with Triton in the presence of EDTA, and has been partially purified. The enzyme appears to be quite specific for phosphatidyl glycerophosphate. It has a high affinity for this substrate, and is much less active when tested with other phosphomonoesters. Some other properties of the enzyme have also been determined.

Abbreviation: CDP, cytidine 5′-diphosphate.

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MATERIALS AND METHODS

Cells of *E. coli* B and *E. coli* ML 308 were grown and stored as described in a previous paper (3).

Whatman DE-32 microgranular cellulose anion exchanger was washed with 0.5 N HCl and 0.5 N NaOH before use. The acetate form of the DEAE-cellulose was prepared according to the method of Hendrickson and Ballou (4).

Solvent systems for paper chromatography were those previously described (3).

Dipalmitoyl-DL, α-glycerophosphate was synthesized by a procedure based on that of Baer (5).

L-Glycerol-2-^{3}H 3-phosphate and L-glycerol 3-phosphate ^{32}P were enzymatically synthesized from glycerol-2-^{3}H and ATP, and glycerol and ATP-γ-^{32}P, respectively, in the presence of glycerol kinase as described previously (3).

Preparation of Phosphatidyl Glycerophosphate-2′-^{3}H-3′-^{32}P

Phosphatidyl glycerophosphate-2′-^{3}H-3′-^{32}P was prepared from CDP-dipalmitin and L-glycerol-2-^{3}H 3-phosphate-^{32}P in a reaction catalyzed by L-glycerol 3-phosphate: CMP-phosphatidyltransferase. The incubation mixture contained Tris–HCl buffer of pH 8.0 (250 mM), mercaptoethanol (5 mM), Triton X-100 (5 mg/ml), $MgCl_2$ (20 mM), serum albumin (1 mg/ml), CDP-dipalmitin Tris salt (1 mM), L-glycerol-2-^{3}H 3-phosphate-^{32}P (1 mM), and enzyme (3) with a specific activity of 120 mU/mg protein (1.4 mg of protein) in a final volume of 30 ml. After incubation at 37°C for 4 hr, methanol (150 ml) was added followed by 300 ml of chloroform. The mixture was filtered through a layer of glass wool and the filtrate was equilibrated with 600 ml of 2 M $MgCl_2$. The upper, aqueous phase was discarded. The chloroform was washed with 300 ml of water,

and taken to dryness in a rotary evaporator. The lipid residue was dissolved in a small volume of chloroform. The yield was 20 μmoles of labeled lipid, or 66% based on CDP-dipalmitin. The ratio of $^{32}P/^{3}H$ was 1.0. The lipid was taken to dryness again in a conical tube in a stream of nitrogen. The residue was washed twice with 5-ml portions of acetone at −15°C to free it of Triton X-100. Phosphatidyl glycerophosphate is insoluble under these conditions. The product was finally dissolved in a small volume of chloroform and stored at 0°C.

Preparation of ^{32}P-Labeled Phosphatidic Acid

^{32}P-labeled phosphatidic acid was prepared from D-α,β-diglyceride and ATP-γ-^{32}P in the presence of diglyceride kinase.

D-α,β-Diglycerides were prepared from egg lecithin by treatment with lecithinase from *Clostridium perfringens* as described by Wilgram and Kennedy (6). The purity of D-α,β-diglyceride was checked by chromatography on thin layers of silicic acid in the solvent system *n*-hexane–ether–acetic acid 70:30:1, which showed a single spot with an R_f of 0.4 corresponding to that of synthetic dipalmitin. The D-α,β-diglycerides were emulsified in Tween 20 in a final concentration of 20 μmoles/ml of 0.1% Tween 20.

ATP-γ-^{32}P was prepared according to the method of Glynn and Chappell (7).

The particulate fraction of *E. coli* B, which contained diglyceride kinase, was prepared as described by Pieringer and Kunnes (8). Frozen *E. coli* B cells were washed once in 0.01 M sodium phosphate buffer of pH 7.0 containing 0.01 M mercaptoethanol, and were disrupted in the same medium by ultrasonication. The suspension was centrifuged at 30,000 *g* for 30 min. The particulate fraction so obtained was washed and finally resuspended in the same buffer in a final concentration of 7.5 mg of protein/ml.

The incubation mixture contained sodium phosphate buffer of pH 6.8 (50 mM), D-α,β-diglyceride (1.2 mM), Triton X-100 (14 mg/ml), ATP-^{32}P (3 mM, 1.5 × 10^6 cpm/μmole), $MgCl_2$ (100 mM), and the particulate fraction (30 mg of protein) in a final volume of 20 ml. After incubation at 37°C for 3 hr, the lipid product was extracted with chloroform as described above for the preparation of phosphatidyl glycerophosphate. The yield, relative to D-α,β-diglyceride, was 28%. The washed chloroform extract was taken to dryness and the residue was washed twice with 5 ml of acetone at −15°C. The radioactive phosphatidic acid so obtained was free from Triton X-100 and diglycerides. The radiochemical purity of the product was checked by paper chromatography after deacylation by mild alkaline hydrolysis. A single radioactive spot was found with an R_f identical with that of authentic DL-glycerol 3-phosphate. The product was readily hydrolyzed by rat liver phosphatidic acid phosphatase (EC 3.1.3.4) under the conditions described by Wilgram and Kennedy (6).

Assay of Phosphatidyl Glycerophosphate Phosphatase

The release of $^{32}P_i$ from phosphatidyl glycerophosphate-3′-^{32}P was used as a measure of enzyme activity. Appropriate amounts of radioactive phosphatidyl glycerophosphate and of Triton X-100, dissolved in chloroform, were first added to each assay tube. After the solvent had been completely removed in a stream of air, other components were added. The incubation mixture contained Tris buffer of pH 7.4 (50 mM), phosphatidyl glycerophosphate-3′-^{32}P (0.1 mM), Triton X-100 (1.2 mg/ml), $MgCl_2$ (2 mM), EDTA (1 mM), and mercaptoethanol (5 mM) in a final volume of 0.2 ml. After incubation at 37°C for 20 min, 0.5 ml of 10% (w/v) trichloroacetic acid was added, followed by 0.1 ml of 5 mM sodium phosphate and 0.2 ml of 1% (w/v) serum albumin. The tubes were chilled in ice for 5 min, and the precipitates were removed by centrifugation. The supernatant solutions were extracted twice with 1.5 ml of chloroform to remove traces of radioactive phosphatidyl glycerophosphate. Aliquots were then counted in a liquid scintillation counter. 1 unit of enzyme activity was defined as the amount that catalyzes the release of 1 μmole of $^{32}P_i$ per min under these conditions. Zero-time control tubes, in which the trichloroacetic acid was added immediately after the enzyme, were included in each experiment.

Phosphatidic acid phosphatase was assayed as described by Wilgram and Kennedy (6), except that ^{32}P-labeled phosphatidic acid was used and the release of $^{32}P_i$ was the measure of the activity.

Alkaline phosphatase of *E. coli* (Sigma Chemical Co.) was assayed as described by Garen and Levinthal (9).

Acid phosphatase of wheat germ (Worthington Biochemical Co.) was assayed as described by Bandenberger and Hanson (10). When phosphatidyl glycerophosphate-3′-^{32}P was used as the substrate of the alkaline or the acid phosphatase, the $^{32}P_i$ released was measured.

Other materials and methods are described in the preceding paper (3).

RESULTS

Properties of the Particulate Enzyme Preparation

When suspensions of cells disrupted sonically were fractionated by differential centrifugation, nearly all of the phosphatidyl glycerophosphatase activity was recovered in the precipitate obtained by centrifugation at 40,000 *g* for 1 hr (Table 1). The reaction was dependent on added Mg^{++} ions and surfactant. The pH optimum of the reaction was approximately 7.0.

TABLE 1 INTRACELLULAR LOCALIZATION OF PHOSPHATIDYL GLYCEROPHOSPHATE PHOSPHATASE

Cell Fraction	Specific Activity
	mU/mg of protein
Whole sonicate	1.9
3,000 *g* Supernatant fraction	2.0
40,000 *g* Supernatant fraction	0.65
40,000 *g* Residue	8.1

Cells of *E. coli* ML 308 were harvested in log-phase and disrupted in a Branson Sonifier in 0.1 M Tris buffer of pH 7.4 containing 0.01 M mercaptoethanol. The suspension was centrifuged at 3,000 *g* for 15 min and the residue was discarded. The 3,000 *g* supernatant fraction was then centrifuged at 40,000 *g* for 1 hr. Each fraction was assayed essentially as described under Materials and Methods except that the final Triton concentration was 1 mg/ml and the time of incubation was 15 min.

The requirement for surfactant was nearly absolute. Among various surfactants tried, only Triton X-100 and sodium dodecyl sulfate stimulated the enzymatic activity. Others exhibited either no effect or inhibitory effect on the enzyme (Table 2).

Extraction of Enzyme

Several enzymes that catalyze reactions of lipid biosynthesis can be readily extracted from the particulate fraction of *E. coli* (which contains cell envelope fragments) by Triton-containing buffers. In contrast, phosphatidyl glycerophosphatase resists extraction with Triton alone, but can be readily extracted with Triton in the presence of EDTA (Table 3). Solutions containing EDTA but no Triton will not extract the enzyme (data not shown).

Since a considerable amount of protein is extracted by Triton in the absence of EDTA, an appreciable purification of the enzyme can be achieved by the procedure described in Table 3.

The ratio of extraction medium to particulate fraction appears to have an important influence on the degree of extraction. For best results, the amount of particulate protein should not exceed 10 mg/ml of extraction medium.

TABLE 2 EFFECT OF VARIOUS SURFACTANTS

Surfactant Added	$^{32}P_i$ Released
	mμmoles
None	4.9
Trimethyloctadecyl ammonium chloride	0
Tri-*n*-octylamine	4.6
Octadecylamine	0
Sodium dodecyl sulfate	23
Triton X-100	73

An unfractionated suspension of sonically disrupted cells was used as enzyme. The method of assay was essentially that described under Materials and Methods, except that the various surfactants were added as indicated in a final concentration of 1 mg/ml and the final volume was 1.0 ml.

TABLE 3 EXTRACTION OF PHOSPHATIDYL GLYCEROPHOSPHATE PHOSPHATASE FROM THE PARTICULATE FRACTION

	Specific Activity
	mU/mg of protein
1st Extract (0.5% Triton X-100)	3.3
2nd Extract (0.5% Triton X-100 + 5 mM EDTA)	19
Residue after 2nd extraction	2.9

Frozen *E. coli* B cells were disrupted sonically in 0.1 M Tris buffer of pH 7.4 containing 0.01 M mercaptoethanol and fractionated by differential centrifugation as described in Table 1. The 40,000 *g* particulate fraction (56 mg of protein), with a specific activity of 4.6 mU/mg of protein, was extracted with 6 ml of 0.1 M Tris buffer of pH 8.0 containing 0.01 M mercaptoethanol and 0.5% (w/v) Triton X-100, by stirring at 20°C for 15 min. The suspension was chilled and centrifuged at 0°C for 1 hr at 40,000 *g*. The supernate constituted the first extract. The residue was similarly extracted with 0.1 M Tris of pH 8.0, and 0.5% Triton to which EDTA was added to a final concentration of 5 mM (second extraction).

Fractionation on DEAE-Cellulose

Extracts obtained by treatment of the particulate fraction with Tris buffer of pH 8.0 containing 0.5% Triton X-100 and 5 mM EDTA, essentially as described in Table 3, were further fractionated on columns of DEAE-cellulose in a cold-room at 4°C.

The DEAE-cellulose suspended in 0.02 M Tris buffer of pH 7.4 containing 2 mM mercaptoethanol was packed into a column 8 cm in length and 1.2 cm in diameter. The extract (10 ml containing 9.2 mg of protein) was adjusted to pH 7.4 with acetic acid and diluted to 50 ml with ice-cold distilled water, and passed over the column, which was then eluted first with 8 ml of 0.1 M Tris buffer of pH 7.4 containing 0.01 M mercaptoethanol and 0.5% Triton X-100. This fraction contained no enzymatic activity and was discarded. The column was next eluted with 20 ml of the same buffer containing 0.1 M NaCl in four fractions of 5 ml each. The enzyme appeared in the second fraction which was immediately stored in liquid nitrogen.

The purification of the enzyme is summarized in Table 4. The enzyme was purified only 10-fold after the DEAE-cellulose column chromatography, but further purification was made difficult by the lability of the enzyme.

Stability of the Enzyme

The enzyme in the 40,000 *g* particulate fraction was stable for a period of weeks when stored at −20°C but was much less stable at 0°C. Addition of Triton X-100 accelerated the inactivation of the enzyme.

After extraction with EDTA–Triton, the enzyme lost 66% of its activity in 24 hr at 0°C. Addition of salts, such as $MgCl_2$, NaCl, or Na_2HPO_4, or of DL-glycerol 3-phosphate, did not protect the enzyme, nor did glycerol or ethylene glycol (20% v/v). The activity was lost more

TABLE 4 PURIFICATION OF PHOSPHATIDYL GLYCEROPHOSPHATE PHOSPHATASE

Purification Steps	Total Activity*	Specific Activity
		mU/mg of protein
Whole sonicate	100	2.7
40,000 *g* Particulate fraction	70	5.4
2nd Extract	22	19
DEAE-cellulose column fraction 2	17	28

* Total activity of the whole sonicate is arbitrarily set as 100.

quickly at room temperature than at 0°C. (Certain "cold-labile" enzymes exhibit the opposite behavior.)

Triton was removed from the enzyme preparation by fractionation with ethanol, but the resultant ethanol fractions were likewise unstable.

It was finally found that if the pH of the enzyme fractions was adjusted to 7.4, and the enzyme stored in liquid nitrogen, about 95% of the activity could be retained for several weeks.

Characteristics of the Enzyme

Time Course of Reaction. The hydrolysis of phosphatidyl glycerophosphate catalyzed by the partially purified enzyme preparation proceeded linearly for at least 90 min. After longer periods of incubation, the rate was reduced.

Effect of Enzyme Concentration. The rate of the reaction was a linear function of the amount of added enzyme up to levels of about 8 μg of purified enzyme. Above this level, the rate was no longer linear.

Effect of Triton X-100 on Enzyme Activity. The effect of Triton X-100 on the activity of the enzyme is shown in Fig. 1. The optimal concentration was 1–1.5 mg/ml. Higher concentrations were inhibitory, but when the concentration of substrate was increased from 0.1 mM to 0.2 mM, the inhibition was less pronounced.

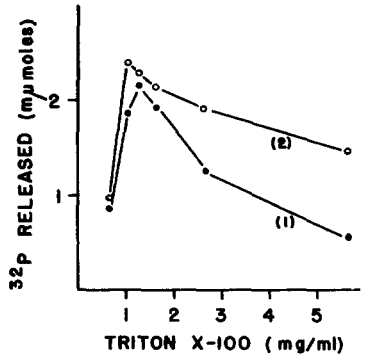

FIG. 1. Effect of Triton X-100 on the activity of phosphatidyl glycerophosphate phosphatase. The standard assay was used, except that the concentration of Triton X-100 was varied as shown. The enzyme preparation used was DEAE-cellulose fraction 2 (5 μg of protein). The concentration of phosphatidyl glycerophosphate-3'-^{32}P was 0.1 mM in curve (*1*) and 0.2 mM in curve (*2*) (4.0×10^6 cpm/μmole).

Effect of EDTA. When the concentration of Mg^{++} was kept constant at 2 mM, a distinct stimulatory effect of EDTA at a concentration of 1 mM was observed (Fig. 2). As might be expected, higher concentrations of EDTA, sufficient to bind the available magnesium, were inhibitory.

Requirement for Divalent Cation. Enzymatic activity was dependent on the presence of Mg^{++} ions. Fig. 3 shows the effect of increasing concentration of $MgCl_2$ in the presence of 1 mM EDTA. Ca^{++} or Zn^{++} ions could not substitute for Mg^{++} in the reaction. Only one-tenth of the enzymatic activity was observed when 2 mM $MnCl_2$ was substituted for 2 mM $MgCl_2$.

pH Optimum. The pH optimum was near 7.5 when Tris-HCl buffer was used (Fig. 4). Little activity was observed when pH was above 8.0 or below 7.0.

Affinity for Substrate. When other components in the incubation mixture were kept constant and the concentration of phosphatidyl glycerophosphate was varied, the K_m for phosphatidyl glycerophosphate was 8.3×10^{-5} M (Fig. 5).

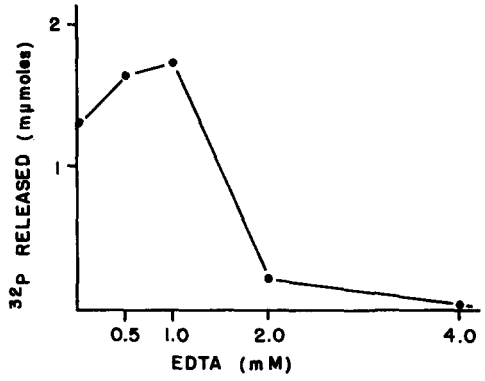

FIG. 2. Effect of EDTA. The standard assay was used, except that the concentration of EDTA was varied as shown. The enzyme was DEAE-cellulose fraction 2 (5 μg of protein).

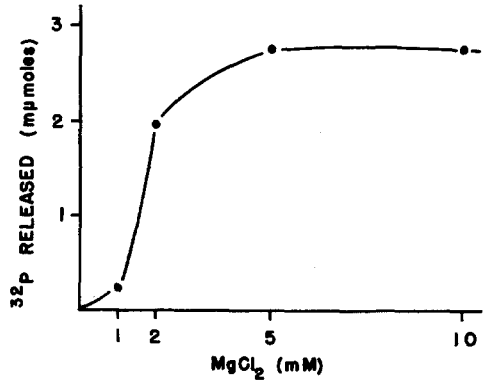

FIG. 3. Effect of $MgCl_2$. The standard assay was used, except that the concentration of $MgCl_2$ was varied as shown. The enzyme was DEAE-cellulose fraction 2 (5 μg of protein).

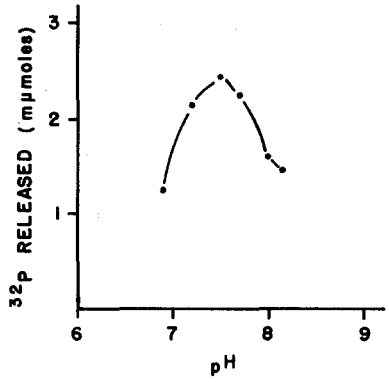

FIG. 4. Effect of pH. The standard assay was used, except that the pH of the system was varied with Tris–HCl buffer (50 mM) of various pH values. The pH of the incubation mixture was measured with a glass electrode.

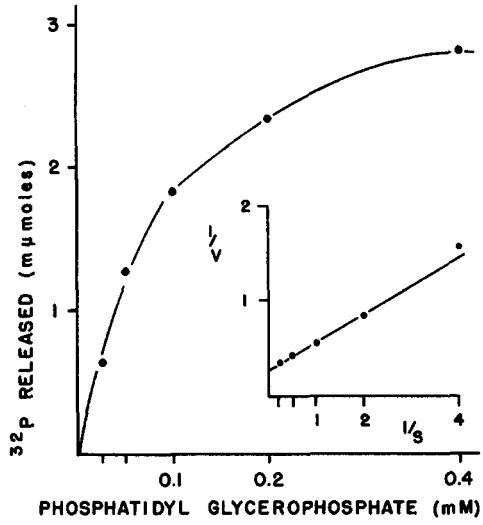

FIG. 5. Affinity of phosphatidyl glycerophosphate phosphatase for phosphatidyl glycerophosphate. The concentration of phosphatidyl glycerophosphate in the standard assay system was varied as shown. The enzyme was DEAE-cellulose fraction 2 (5 μg of protein).

Effect of Inhibitors. The enzyme was inhibited by sulfhydryl reagents such as $HgCl_2$ and *N*-ethylmaleimide as shown in Table 5. Fluoride ion also inhibited the enzyme. Orthophosphate up to a concentration of 10 mM had little effect. The inhibition observed at higher levels of orthophosphate was probably due to the binding of Mg^{++} ion.

Specificity of Enzyme

The partially purified enzyme appeared to be quite specific for the hydrolysis of phosphatidyl glycerophosphate. It catalyzed no detectable hydrolysis of L-glycerol 3-phosphate-^{32}P (Table 6). When unlabeled glycerophosphate was added to radioactive phosphatidyl glycerophosphate, no inhibition was observed, indicating that the enzyme has no detectable affinity for glycerophosphate itself. Radioactive phosphatidic acid was cleaved at rates less than one-tenth of those observed for phosphatidyl glycerophosphate. Increasing the concentration of phosphatidic acid from 0.1 mM to 1 mM did not increase the rate of cleavage, which suggests that the phosphatidic acid is being cleaved by small amounts of phosphatidic acid phosphatase or a similar enzyme with relatively high affinity for phosphatidic acid. When both phosphatidic acid and phosphatidyl glycerophosphate were incubated together, the release of radioactivity was roughly additive. This supports the view that two separate enzymes may be involved in attacking these substrates.

TABLE 5 EFFECT OF INHIBITORS

Inhibitor	Concentration	$^{32}P_i$ Released
	mM	*mμmoles*
None (with mercaptoethanol)		2.4
None (without mercaptoethanol)		2.1
$HgCl_2$	5	0
N-Ethylmaleimide	2	0.80
NaF	5	0.55
Sodium phosphate	5	2.5
	10	2.2
	25	1.3
	50	0.8

The incubation mixture and the assay were the same as those described in Materials and Methods, except that various inhibitors were added as shown. When $HgCl_2$ or *N*-ethylmaleimide was added, mercaptoethanol was omitted from the incubation mixture.

TABLE 6 SUBSTRATE SPECIFICITY OF PHOSPHATIDYL GLYCEROPHOSPHATE PHOSPHATASE

Substrate and Concn	$^{32}P_i$ Released	
	total cpm	*mμmoles*
(*a*) Phosphatidyl glycerophosphate-2′-^{3}H-3′-^{32}P (0.1 mM)	2712	3.94
(*b*) Phosphatidyl glycerophosphate-2′-^{3}H-3′-^{32}P (0.1 mM) plus DL-glycerol 3-phosphate (1 mM)	2990	4.35
(*c*) Phosphatidyl glycerophosphate-2′-^{3}H-3′-^{32}P (0.1 mM) plus phosphatidic acid-^{32}P (0.1 mM)	3334	—
(*d*) L-Glycerol 3-phosphate-^{32}P (0.9 mM)	0	0
(*e*) Phosphatidic acid-^{32}P (0.1 mM)	302	0.307

The standard incubation mixture was used, except that the substrate(s) was varied as shown. The enzyme was DEAE-cellulose fraction 2 (8 μg of protein). After incubation for 20 min at 37 °C, the release of $^{32}P_i$ was measured as described in Materials and Methods in *a–c* and *e*. In (*d*), $^{32}P_i$ released in the reaction was measured by the method of Ernster et al. (11).

Identification of Reaction Products

The water-soluble radioactive product derived from phosphatidyl glycerophosphate-2′-^{3}H-3′-^{32}P in the standard assay procedure was identified as $^{32}P_i$ by the method of Ernster, Zetterström, and Lindberg (11). All of the radioactivity could be accounted for as $^{32}P_i$, which ex-

cludes the possibility that L-glycerol 3-phosphate is also released during the course of the reaction.

To identify the lipid product, we carried out an experiment under conditions similar to those of the standard assay method, except that the final volume was 14 ml and 4.2 μmoles of phosphatidyl glycerophosphate-2′-^{3}H-3′-^{32}P was used as substrate. The enzyme used in this experiment had been purified by DEAE chromatography (Table 4). After incubation for 5 hr at 37°C, the lipid was extracted with chloroform and converted to the sodium salt (12). It was then chromatographed on DEAE-cellulose in the acetate form (4) on a column 25 cm in length and 1.3 cm in diameter made in chloroform–methanol–water 20:9:1. The column was first washed with 30 ml of the solvent mixture, and then eluted with a linear gradient of ammonium acetate. Fractions of 5 ml each were collected.

The results are shown in Fig. 6. Two principal radioactive peaks were found. The first to be eluted contained only tritium (phosphatidyl glycerol). The second contained both ^{32}P and tritium with a ratio of isotopes identical with that in the substrate (phosphatidyl glycerophosphate).

The fractions containing only tritium were pooled (2.2 μmoles). Aliquots were subjected to mild alkaline hydrolysis and the products chromatographed on paper in solvent system A (13). A single radioactive spot was detected with an R_f of 0.64, identical with that of authentic glycerolphosphoryl glycerol. Hydrolysis with 90% acetic acid in a boiling water bath for 20 min yielded glycerophosphate as the principal water-soluble product, identified by chromatography in solvent system A (R_f = 0.27).

Hydrolysis of Phosphatidyl Glycerophosphate with Other Enzymes

Phosphatidyl glycerophosphate was not appreciably hydrolyzed by *E. coli* alkaline phosphatase, but it was hydrolyzed by acid phosphatase from wheat germ, with the formation of P_i. The rate of the hydrolysis by acid phosphatase was increased if Triton X-100 (1 mg/ml) was also included in the incubation mixture.

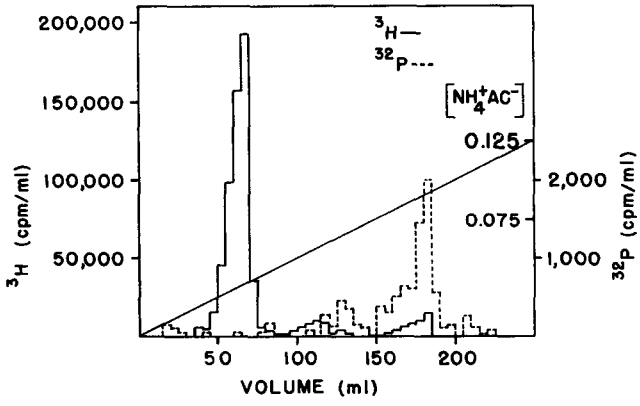

FIG. 6. Chromatography on DEAE-cellulose of the lipid product of the reaction catalyzed by phosphatidyl glycerophosphate phosphatase. Details are given in the text. Solid line: ^{3}H; broken line: ^{32}P.

DISCUSSION

Phosphatidyl glycerophosphatase can be readily distinguished from the nonspecific alkaline phosphatase of *E. coli*. The latter enzyme is recovered in the soluble supernatant fraction of sonically disrupted or osmotically shocked (14) cells. It has a pH optimum of 8–9 and is inhibited by low concentrations of P_i. In contrast, the phosphatidyl glycerophosphatase is rather firmly bound to the particulate, membrane-containing fraction, has a pH optimum of 7.4, and is unaffected by moderate concentrations of orthophosphate.

Phosphatidyl glycerophosphatase appears to be quite specific. It has relatively high affinity for phosphatidyl glycerophosphate (Fig. 5) and partially purified fractions do not hydrolyze L-glycerol 3-phosphate at a significant rate. Although some activity towards phosphatidic acid remains, it seems likely that this should be attributed to contamination by some other enzyme. Phosphatidic acid phosphatase of liver is inhibited by magnesium ions (15), while phosphatidyl glycerophosphatase of *E. coli* exhibits an absolute requirement for added magnesium.

Phosphatidyl glycerophosphatase of *E. coli* is similar to the corresponding enzyme from liver in its sensitivity to sulfhydryl reagents (2). Since L-glycerol 3-phosphate: CMP phosphatidyltransferase is relatively unaffected by sulfhydryl reagents, the synthesis of phosphatidyl glycerol, catalyzed by crude particulate fractions from either liver or *E. coli*, may be blocked at the stage of formation of phosphatidyl glycerophosphate by the addition of sulfhydryl poisons. In nature, however, the activity of phosphatidyl glycerophosphatase appears not to be limiting, since accumulation of phosphatidyl glycerophosphate is not observed (1).

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