Stimulation and inhibition of the activity of rat liver cytosolic phosphatidate phosphohydrolase by various phospholipids

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Summary The influence of phospholipids on the activity of the soluble phosphatidate phosphohydrolase from rat liver was studied. Phosphatidylethanolamine stimulated the enzyme activity whereas phosphatidylglycerol, phosphatidylserine, and phosphatidylinositol were inhibitory. At a phospholipid concentration of 0.7 mg/ml, phosphatidylglycerol inhibited phosphatidate phosphohydrolase activity by 75%, while the enzyme activity was stimulated twofold in the presence of phosphatidylethanolamine. Both lysophosphatidylglycerol and lysophosphatidylethanolamine inhibited phosphatidate phosphohydrolase activity as did octylglucoside, sodium cholate, and Tween 20. The finding that phospholipids influence hepatic phosphatidate phosphohydrolase activity indicates that changes in the lipid environment may modulate the enzyme activity.—**Humble, E., and L. Berglund.** Stimulation and inhibition of the activity of rat liver cytosolic phosphatidate phosphohydrolase by various phospholipids. *J. Lipid Res.* 1991. **32:** 1869–1872.

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Data have accumulated suggesting a role for hepatic phosphatidate phosphohydrolase (EC 3.1.3.4) in the regulation of the rate of triacylglycerol biosynthesis (1–3). Several enzymes influence the generation of phosphatidic acid, both from phospholipids as well as by de novo formation from glycerol-3-phosphate (4). The conversion of phosphatidic acid to diacylglycerol, catalyzed by phosphatidate phosphohydrolase, occurs at a branchpoint in glycerolipid metabolism (1). Furthermore, both phosphatidic acid and diacylglycerol are involved in hormonal regulation (5–8). Therefore it seems likely that factors that influence the activity of phosphatidate phosphohydrolase may affect several important pathways.

Phosphatidate phosphohydrolase is present in the cytosol as well as in the microsomal fraction, and previous studies indicate that the enzyme activity might shift between those two compartments and that this transition may have a regulatory role (9, 10). In order for this type of translocation to occur, the enzyme must express both hydrophobic and hydrophilic properties. Indeed, such properties could be of importance for the activity as well as the subcellular localization. Therefore, we investigated the effect of phospholipids on the enzyme activity, as these substances are amphipathic and are also important membrane constituents. In the present paper we report a stimulation of the enzyme activity by phosphatidylethanolamine. In contrast, several other phospholipids were found to inhibit the enzyme activity.

EXPERIMENTAL PROCEDURES

Materials

Unlabeled dipalmitoyl phosphatidic acid, phenylmethylsulfonyl fluoride, dithiothreitol, octylglucoside, Tween 20, and phospholipids were purchased from Sigma Chemicals, St. Louis, MO. Sodium cholate and thin-layer chromatography plates, precoated with silica gel, were bought from Merck AG, Darmstadt, Germany. Hydroxylapatite was a product of Bio-Rad, Richmond, CA. All solvents used were of the highest analytical grade commercially available.

Radioactive phosphatidic acid was prepared by incubation of [1-^{14}C]palmitic acid (Amersham International, Amersham, U.K.) with rat liver microsomes as described (11). The labeled phosphatidic acid was extracted and isolated using thin-layer chromatography (11).

Preparation of phosphatidate phosphohydrolase

Male Sprague-Dawley rats weighing 200–250 g were used. In order to induce phosphatidate phosphohydrolase activity, the rats were injected intraperitoneally with ethanol (2 mg/kg body weight) 16–18 h prior to being killed. The livers were excised and homogenized with 4 volumes of ice-cold 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA, and 0.2 mM dithiothreitol. All subsequent steps were performed at 4°C unless otherwise stated. The homogenate was centrifuged at 20,000 *g* for 15 min and, after addition of phenylmethylsulfonyl fluoride to 1 mM final concentration, the supernatant was recentrifuged at 100,000 *g* for 60 min. The resulting supernatant fraction was filtered through glass wool and mixed with hydroxylapatite (4 ml of sedimented gel per g wet weight), equilibrated with 10 mM potassium phosphate buffer, pH 7.4. The suspension was left for 2 h with occasional gentle shaking. Thereafter, it was transferred to a glass column. When the gel had sedimented, the column was washed with five column volumes of 100 mM potassium phosphate buffer (pH 7.4), containing 30% (v/v) glycerol, 1 mM EDTA, and 0.1 mM dithiothreitol. The enzyme was eluted with 400 mM potassium phosphate buffer, pH 7.4, containing the same additions as above. The enzyme-containing fractions were pooled and could be stored for several months at −20°C without any detectable loss of activity. The specific activity of phosphatidate phosphohydrolase was at

Abbreviations: PE, phosphatidylethanolamine; PAP, phosphatidate phosphohydrolase; PG, phosphatidylglycerol; PS, phosphatidylserine, PI, phosphatidylinositol; PC, phosphatidylcholine.

least sixfold higher in this pool than in the 100,000 *g* supernatant fraction. This procedure is a modification of one used in our previous studies (11, 12) as it was found that omission of the ammonium sulfate fractionation step and stepwise rather than gradient elution from the hydroxylapatite resulted in a more reproducible chromatographic behavior and higher yield of the enzyme.

Assay of phosphatidate phosphohydrolase activity

Phosphatidate phosphohydrolase was assayed at 37°C for 15 min by a modification of the previously described procedure (11). The assay mixture used in the present study contained 75 mM Tris-maleate buffer (pH 6.9), 100 mM potassium phosphate (pH 7.4), 3 mM $MgCl_2$, 0.25 mM EDTA, 7.5% (v/v) glycerol, 0.1 mM dithiothreitol, and free, micellar phosphatidic acid, 500 nmol, with a specific radioactivity of 2×10^6 cpm/μmol. The final volume was 0.8 ml. In some experiments various phospholipids were also included. The phospholipids were stored as chloroform solutions at −20°C. Prior to use the solvent was evaporated under a stream of nitrogen or argon at 30°C, whereafter 10 mM Tris-HCl buffer, pH 7.4, was added and the material was emulsified by sonication for 2 min in 30-sec bursts. The enzyme was preincubated for 5 min at 37°C with phospholipids and the reaction was started by the addition of substrate. The enzyme reaction was terminated by the addition of 4 ml of chloroform–methanol 2:1 (v/v). The chloroform phase was evaporated and the residue was subjected to thin-layer chromatography using two solvent systems as previously described (11). The conversion of labeled phosphatidic acid into diacylglycerol was measured by scanning the chromatoplates with a computerized radioscanner (automatic TLC linear analyzer model Tracemaster 20, Berthold, Wildbad, Germany). The radioactivity in the diacylglycerol spot was measured as the percentage of the total radioactivity present in the chromatogram, and the amount of diacylglycerol formed was calculated from these figures. The experiments described were performed several times using different batches of both enzyme and substrate. On all occasions similar results were obtained. However, as the absolute enzyme activity varied somewhat between different series of experiments due to the micellar nature of the substrate, the results are given as relative values.

RESULTS

The effect of various phospholipids on phosphatidate phosphohydrolase (PAP) activity was studied. As shown in **Table 1,** phosphatidylglycerol (PG), phosphatidylserine (PS), and phosphatidylinositol (PI) were inhibitory, while phosphatidylethanolamine (PE) had a stimulatory effect. PE from different sources (egg yolk, bovine brain, porcine liver), which might differ somewhat in fatty acid content, stimulated PAP activity to a similar extent (data not shown).

Phosphatidylcholine (PC) inhibited PAP activity to some extent (Table 1). This latter finding was in contrast to previous reports by Butterwith, Hopewell, and Brindley (13) who observed a stimulation of PAP activity in the presence of PC. In their experiments, the reaction was started by the addition of a mixed emulsion of PC and phosphatidate. Also, in the present study, when PC was emulsified in the presence of the substrate and the enzyme reaction was initiated by addition of this mixture, a slight stimulation (20% at 0.2 mg PC/ml) was seen. Thus, the mode of presentation of PC influenced the effect on the enzyme activity. In contrast, the degree of stimulation by PE was similar irrespective of whether PE (from egg yolk) was added separately or as an emulsion with the substrate.

The influence of two phospholipids, one stimulatory (PE) and one inhibitory (PG), on PAP at different concentrations of phosphatidic acid was examined (**Fig. 1**). It was found that PE stimulated and PG inhibited the activity of PAP over the substrate range tested. The inhibition

TABLE 1. Effect of phospholipids on phosphatidate phosphohydrolase (PAP) activity

Phospholipid Added	PAP Activity[a]
	% of control
PG	
0.14 mg/ml	53 (50–55)
0.70 mg/ml	25 (23–27)
PS	
0.14 mg/ml	76 (69–84)
0.70 mg/ml	43 (40–45)
PI	
0.14 mg/ml	83 (80–85)
0.70 mg/ml	67 (63–71)
PE	
0.14 mg/ml	135 (121–150)
0.70 mg/ml	194 (186–207)
PC	
0.14 mg/ml	81 (77–88)
0.70 mg/ml	80 (75–89)
LysoPG	
0.14 mg/ml	10 (9–12)
0.70 mg/ml	7 (5–10)
LysoPE	
0.14 mg/ml	83 (69–91)
0.70 mg/ml	73 (64–82)

The abbreviations used and the sources of phospholipids were: PE, phosphatidylethanolamine (egg yolk), PG, phosphatidylglycerol (egg yolk), PC, phosphatidylcholine (egg yolk), PI, phosphatidylinositol (soy bean), PS, phosphatidylserine (bovine brain), lysoPG, lysophosphatidylglycerol (egg yolk), and lysoPE, lysophosphatidylethanolamine (egg yolk).

[a]Numbers represent mean values and range (in parentheses) of triplicate determinations and are expressed as percentage of control experiments without addition of phospholipids. The range in the control determinations was 95–105%.

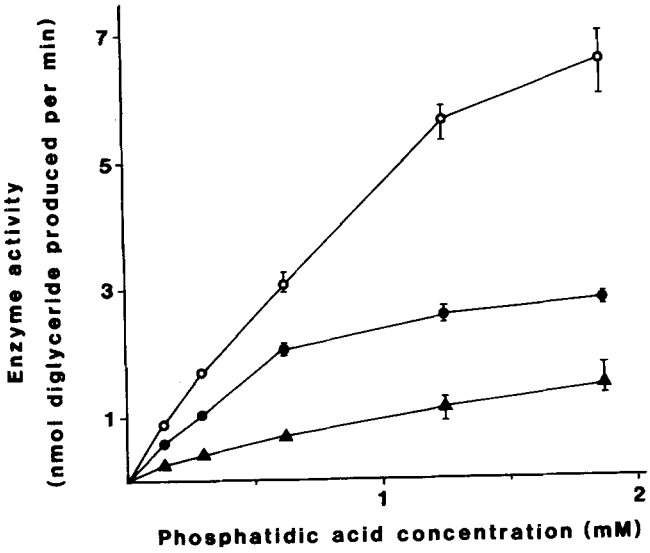

Fig. 1. Influence of phospholipids on the activity of phosphatidate phosphohydrolase at different concentrations of phosphatidic acid. Enzyme activity in the absence (●) and in the presence of 0.7 mg phosphatidylethanolamine per ml (○) or 0.7 mg phosphatidylglycerol per ml (▲). The values represent means and range in triplicate determinations. At low concentrations of phosphatidic acid, the variation between the triplicate values was small enough to be contained within the area of the symbol.

of PAP by PG could thus not be overcome even at concentrations of phosphatidic acid more than threefold higher than the one used under standard conditions.

The effect of the lysophospholipids lysoPG and lysoPE was also investigated (Table 1). LysoPG inhibited PAP activity almost completely at a concentration of 0.14 mg/ml and the degree of inhibition was considerably more pronounced than that seen with PG. The possibility that the inhibition by PG and lysoPG was caused by depletion of magnesium from the assay system was considered. However, this did not seem to be the case since the inhibition was evident at all magnesium concentrations tested (0.5–5 mM; not shown) and did not decrease with increasing magnesium concentration. Also lysoPE inhibited PAP activity but to a far less extent than lysoPG (Table 1). As lysophospholipids have detergent-like properties, it was of interest to compare the effect of the lyso-derivatives to that of other detergents on PAP activity. As seen in **Fig. 2,** different types of detergents inhibited the enzyme activity except at very low concentrations.

DISCUSSION

In the present study both stimulatory as well as inhibitory effects were found depending on the type of phospholipid added (Table 1). Our results suggest that the polar part of the phospholipid is of importance in determining the effect on phosphatidate phosphohydrolase. However, addition of ethanolamine (0.2–5 mM) did not influence the enzyme activity (data not shown), indicating that the polar part is not sufficient in itself to affect the enzyme activity. The nature of the inhibitory and stimulatory effects of the various phospholipids seem to be complex. The phospholipids could, in theory, interact with PAP as well as with the phosphatidate substrate or both. In the presence of PE, both the apparent K_m for phosphatidate and the apparent V_{max} values increased (Fig. 1), while addition of PG resulted in an increase in the apparent K_m and also a small but consistent decrease of the apparent V_{max}. Thus, PG does not seem to be a true competitive inhibitor with respect to the substrate phosphatidic acid.

The interaction between the enzyme and its substrate, i.e., the phosphate group in phosphatidic acid, as well as the process of translocation of the enzyme between the cytosol and the membraneous microsomal fraction (9, 10) should require that both hydrophobic and hydrophilic properties of the enzyme protein are involved. This is supported by the previous findings that oleic or palmitic acid promoted the translocation of phosphatidate phosphohydrolase to the microsomal fraction (9).

Also in other systems, e.g., lung surfactants, interaction between hydrophobic proteins and fatty acids occurs, enabling interaction with phospholipids (14). Interaction with cellular phospholipids could therefore be of importance both for the enzyme activity and for directing the transport of the enzyme protein between subcellular compartments. The finding that several types of detergents inhibit the enzyme activity is also well in line with the observed inhibitory action of several phospholipids. In addition, a strong inhibition of phosphatidate phosphohydrolase activity was seen in the presence of the lyso-derivatives of PG and PE, which have detergent-like properties.

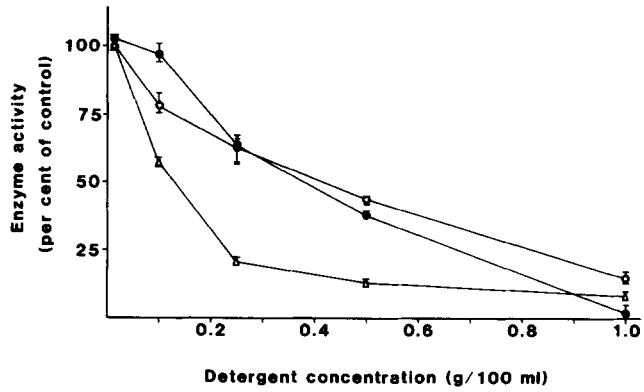

Fig. 2. Influence of detergents on the activity of phosphatidate phosphohydrolase. Octylglucoside (●), sodium cholate (○), and Tween 20 (△). The values represent means and range in triplicate determinations.

In conclusion, the present findings demonstrate that phospholipids might modulate phosphatidate phosphohydrolase activity, and further illustrate the regulatory potential of this enzyme. It is of interest in this context that the activator phosphatidylethanolamine is the second most abundant phospholipid in rat liver tissue (15, 16). The interaction between phosphatidate phosphohydrolase and amphiphilic agents may also be of importance for the stability of the enzyme during isolation, and this could partly explain the difficulties encountered so far in the purification of the mammalian enzyme.

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REFERENCES

1. Bell, R. M., and R. A. Coleman. 1980. Enzymes of glycerolipid synthesis in eukaryotes. *Annu. Rev. Biochem.* **49:** 459–487.
2. Fallon, H. J., R. G. Lamb, and S. C. Jamdar. 1977. Phosphatidate phosphohydrolase and the regulation of glycerolipid biosynthesis. *Biochem. Soc. Trans.* **5:** 37–40.
3. Skorve, J., D. Asiedu, A. C. Rustan, C. A. Drevon, A. Al-Shurbaji, and R. K. Berge. 1990. Regulation of fatty acid oxidation and triglyceride and phospholipid metabolism by hypolipidemic sulfur-substituted fatty acid analogues. *J. Lipid Res.* **31:** 1627–1635.
4. Tijburg, L. B. M., M. J. H. Geelen, and L. M. G. van Golde. 1989. Regulation of the biosynthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine in the liver. *Biochim. Biophys. Acta.* **1004:** 1–19.
5. Moolenaar, W. H., W. Kruijer, B. C. Tilly, I. Verlaan, A. J. Bierman, and S. W. de Laat. 1986. Growth factor-like action of phosphatidic acid. *Nature.* **323:** 171–173.
6. Billah, M. M., S. Eckel, T. J. Mullmann, R. W. Egan, and M. I. Siegel. 1989. Phosphatidylcholine hydrolysis by phospholipase D determines phosphatidate and diglyceride levels in chemotactic peptide-stimulated human neutrophils. Involvement of phosphatidate phosphohydrolase in signal transduction. *J. Biol. Chem.* **264:** 17069–17077.
7. Augert, G., S. B. Bocckino, P. F. Blackmore, and J. H. Exton. 1989. Hormonal stimulation of diacylglycerol formation in hepatocytes. Evidence for phosphatidylcholine breakdown. *J. Biol. Chem.* **264:** 21689–21698.
8. Bocckino, S. B., P. B. Wilson, and J. H. Exton. 1989. An early elevation of diacylglycerol and phosphatidate in regenerating liver. *Biochem. Biophys. Res. Commun.* **164:** 290–294.
9. Brindley, D. N. 1984. Intracellular translocation of phosphatidate phosphohydrolase and its possible role in the control of glycerolipid synthesis. *Prog. Lipid Res.* **23:** 115–133.
10. Hopewell, R., P. Martin-Sanz, A. Martin, J. Saxton, and D. N. Brindley. 1985. Regulation of the translocation of phosphatidate phosphohydrolase between the cytosol and the endoplasmic reticulum of rat liver. *Biochem. J.* **232:** 485–491.
11. Berglund, L., I. Björkhem, and K. Einarsson. 1982. Apparent phosphorylation-dephosphorylation of soluble phosphatidic acid phosphatase in rat liver. *Biochem. Biophys. Res. Commun.* **105:** 288–295.
12. Berglund, L., I. Björkhem. B. Angelin, and K. Einarsson. 1989. Activation of rat liver cytosolic phosphatidic acid phosphatase by nucleoside diphosphates. *Biochim. Biophys. Acta.* **1002:** 382–387.
13. Butterwith, S. C., R. Hopewell, and D. N. Brindley. 1984. Partial purification and characterization of the soluble phosphatidate phosphohydrolase of rat liver. *Biochem. J.* **220:** 825–833.
14. Curstedt, T., J. Johansson, P. Persson, A. Eklund, B. Robertson, B. Löwenadler, and H. Jörnvall. 1990. Hydrophobic surfactant-associated polypeptides: SP-C is a lipopeptide with two palmitoylated cysteine residues, whereas SP-B lacks covalently linked fatty acyl groups. *Proc. Natl. Acad. Sci. USA.* **87:** 2985–2989.
15. Marinetti, G. V., J. Erbland, and E. Stotz. 1958. The phosphatide composition of rat tissues. *Biochim. Biophys. Acta.* **30:** 642–643.
16. Wuthie, R. E. 1966. Two-dimensional chromatography on silica gel-loaded paper for the microanalysis of polar lipids. *J. Lipid Res.* **7:** 544–550.