Phosphatidate Phosphohydrolase Catalyzes the Hydrolysis of Ceramide 1-Phosphate, Lysophosphatidate, and Sphingosine 1-Phosphate*

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A Mg²⁺-independent phosphatidate phosphohydrolase was purified from rat liver plasma membranes in two distinct forms, an anionic protein and a cationic protein. Both forms of the enzyme dephosphorylated phosphatidate, ceramide 1-phosphate, lysophosphatidate, and sphingosine 1-phosphate. When assayed at a constant molar ratio of lipid to Triton X-100 of 1:500, the apparent Kₘ values of the anionic phosphohydrolase for the lipid substrates were 3.5, 1.9, 0.4, and 4.0 μM, respectively. The relative catalytic efficiency of the enzyme for phosphatidate, ceramide 1-phosphate, lysophosphatidate, and sphingosine 1-phosphate was 0.16, 0.14, 0.48, and 0.04 liter (min·mg)⁻¹, respectively. The hydrolysis of phosphatidate was inhibited competitively by ceramide 1-phosphate, lysophosphatidate, and sphingosine 1-phosphate. The Kᵯ(app) values were 5.5, 5.9, and 4.0 μM, respectively. The hydrolysis of phosphatidate by the phosphohydrolase conformed to a surface dilution kinetic model. It is concluded that the enzyme is a lipid phosphomonoesterase that could modify the balance of phosphatidate, ceramide 1-phosphate, lysophosphatidate, and sphingosine 1-phosphate relative to diacylglycerol, ceramide, monoaicylglycerol, and sphingosine, respectively. The enzyme could thus play an important role in regulating cell activation and signal transduction.

Phosphatidate D and PAP (1, 2). Such changes in DAG are involved in the induction of DNA synthesis (1), oocyte maturation (3), and morphological changes in fibroblasts (4). PA has potent mitogenic effects in several cell lines (2, 5–7); it stimulates the respiratory burst in neutrophils independently of DAG (8, 9), and it activates monooacylglycerol acyltransferase (10), phospholipase C-γ (11), p21ras (12), and phosphatidylinositol 4-phosphate kinase (13). PA can also serve as a precursor for LPA, which is released from activated platelets, and this process is thought to be involved in local wound repair (14). LPA is a potent mitogen for fibroblasts (2, 14). Production of LPA by secretory phospholipase A₂ action on membrane microvesicles may represent a novel pro-inflammatory pathway (15). When added externally to cells LPA activates tyrosine kinases, the Ras-Raf-mitogen-activated protein kinase pathway, focal adhesion kinase, arachidonate production, Ca²⁺ mobilization, and phospholipase D activity, and it decreases cAMP concentrations (14, 16). Many of these effects are similar to those of PA (2, 7, 14).

Agoïn-stimulated stimulation of s根据不同 agonists including neurotransmitters and hormones activates phospholipases that generate glycerolipids and sphingolipids that are putative second messengers in signal transduction. Diacylglycerol (DAG) formed by agonist-stimulated hydrolysis of phosphatidylcholine is converted to DAG by phospholipase D or decreasing cAMP concentrations (36).

1 The abbreviations used are: DAG, sn-1,2-diacylglycerol; CerP, ceramide 1-phosphate; LPA, lysophosphatidate; PA, phosphatidate; PAP, phosphatidate phosphohydrolase; SPP, sphingosine 1-phosphate.
the dephosphorylation of SPP is catalyzed by an enzyme that has properties similar to that of Mg²⁺-independent and N-ethylmaleimide-insensitive PAP that is located in plasma membranes (23, 24). The present work employed PAP purified from rat liver and establishes that a single enzyme hydrolyzes PA, LPA, CerP, and SPP. This enzyme could therefore regulate signaling by these four bioactive lipids and putative second messengers.

**EXPERIMENTAL PROCEDURES**

Preparation of PA, LPA, CerP, and SPP—The sources of most materials have been described previously (7, 23, 36, 37). Dolichol, dolichol monophosphate, and dioleoylglycerol were purchased from Sigma. Monodioleoylglycerol was purchased from NuChek Prep. Long-chain ceramide, sn-1,2-dioleoylglycerol, or monodioleoylglycerol (approximately 2 mg) was added in a 1:500 molar ratio of lipid to Triton X-100. Analysis of assays were performed with mixed micelles consisting of a typical Michaelis-Menten kinetics (Fig. 1, and by using a surface dilution kinetic (41, 42) were evaluated at 100 µM PA, a Hanes-Woolf plot of the results was linear (Fig. 3B) and yielded a K_{i(app)} of 0.024 mol% and V_{max} of 0.813 µmol (min-ng)^{-1}. These results demonstrate a relatively high affinity of PAP for

**RESULTS AND DISCUSSION**

An N-ethylmaleimide-insensitive phosphohydrolase was purified from rat liver and characterized on the basis of its ability to hydrolyze PA (37). Using a similar assay, the anionic form of PAP was also shown to hydrolyze CerP, LPA, and SPP with typical Michaelis-Menten kinetics (Fig. 1, A and B). These assays were performed with mixed micelles consisting of a constant 1:500 molar ratio of lipid to Triton X-100. Analysis of the results using a Hanes-Woolf plot (Fig. 1, C and D) yielded K_{i(app)} for the bulk concentrations of PA, CerP, LPA, and SPP of 3.5, 1.9, 0.4, and 4.0, µM respectively. V_{max} values under these conditions were 0.55, 0.26, 0.19, and 0.15 µmol (min-ng)^{-1}, and the relative catalytic efficiency (V_{max}/K_{m}) of the enzyme to the various substrates was 0.16, 0.14, 0.48, and 0.04 liter (min-ng)^{-1}, respectively. These results indicate that LPA may be a preferred substrate since it is dephosphorylated three times more efficiently than PA and CerP. Although the maximal rate of SPP hydrolysis was similar to the other lipids, the relative efficiency of SPP hydrolysis was 3.5-4-fold less than that of PA or CerP. This might be explained partly because the hydrolysis of SPP gives rise to sphingosine which can inhibit PAP (24). Sphingosine is a mixed type inhibitor of PAP. 2

![Fig. 1. Phosphatidate phosphohydrolase hydrolyzes phosphatidate, ceramide 1-phosphate, lysophosphatidate, and sphingosine 1-phosphate. The ability of purified anionic PAP to hydrolyze PA (C), LPA (D), CerP (E), and SPP (F) was determined at the concentrations of substrate indicated. Triton X-100 was present at 500 times the molar concentration of the lipid substrates. Reaction velocities were determined in triplicate.](image-url)

2 D. W. Waggoner, J. Dewald, and D. N. Brindley, unpublished work.
lines with the abscissa in the secondary plots equals shown

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**FIG. 3. Phosphatidate hydrolysis conforms to surface dilution kinetics.** A, anionic PAP activity was determined at bulk concentrations of 30 (○), 50 (●), 100 (●), and 200 (●) μM PA. The surface concentration of PA in the micelles was varied from 0.5 to 15 μM of total lipid by the addition of appropriate amounts of Triton X-100. Assays were performed in triplicate, and no more than 7% of total substrate was hydrolyzed under any condition. B, Hanes-Woolf analysis of PA hydrolysis at a bulk concentration of 100 μM PA. The surface concentration of PA was varied as described above. 

\[ \frac{S}{V} = \frac{[PA]}{mol\%} \times \frac{1}{[PA] \text{ mol} \% / \text{mol} \text{ (min-mg)}^{-1}} \]

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PA and CerP. By contrast, the PAP that we purified from rat liver plasma membranes showed competitive inhibitory kinetics toward these two substrates.

It has also been reported that a dolichol monophosphate phosphatase found in the plasma membrane of cells is inhibited by PA and LPA (45, 46). To evaluate whether the hydrolysis of PA by anionic PAP was inhibited by dolichol monophosphate, increasing concentrations of the latter lipid were included in an assay of \[^{32}P\]PA hydrolysis. Dolichol monophosphate inhibited PA hydrolysis but much less so than did an equivalent amount of CerP (Fig. 4A). Dolichol monophosphate was hydrolyzed by anionic PAP but much less efficiently than the other phospholipids. No detectable hydrolysis of dolichol monophosphate was seen in 1-4 h incubations with anionic PAP (results not shown). Hydrolysis by anionic PAP did occur (Fig. 4B) when dolichol monophosphate was incubated for 16 h with 250 times more protein than was used in the kinetic assays. Furthermore, the activity of dolichol monophosphate phosphatase is decreased 70% by 8 mM Pi (47), whereas the activity of PAP, which was purified using a variety of buffers including 10 mM sodium phosphate, is not affected by Pi. Therefore, we conclude that the PAP that we purified is unlikely to be the dolichol monophosphate phosphatase and that dolichol monophosphate is a poor substrate and inhibitor of anionic PAP. Rat liver PAP does not hydrolyze glycerol 3-phosphate, phosphatidylcholine, phosphatidylethanolamine, phosphatidylycerine, phosphatidylserine, phosphatidylinositol, nor diphasatidylglycerol (24). Additionally, glycerol 3-phosphate (24, 44), phosphatidylcholine, phosphatidylycerine, phosphatidylinositol, and diphas-
which there may be “cross-talk” between the glycerolipid and sphingolipid signaling pathways.

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REFERENCES

1. Billah, M. M., and Athens, J. C. (1990) Biochem. J. 269, 281–291
2. Martin, A., Gómez-Muñoz, A., Duffy, P. A., and Brindley, D. N. (1994) in Signal-activated Phospholipases (Liscovitch, M., ed) pp. 139–164, Landes Co., Austin, TX
3. Carrero, A., and Laca, J. C. (1993) J. Cell. Biochem. 52, 440–448
4. Ha, K. S., and Exton, J. H. (1993) J. Cell Biol. 123, 1789–1796
5. Yu, C.-L., Tsai, M.-H., and Stacey, D. W. (1988) Cell 52, 63–71
6. van Corven, E. J., van Rijswijk, A., Jalink, K., van der Bend, R. L., van Blitterswijk, W. J., and Moedenaar, W. H. (1992) Biochem. J. 281, 163–169
7. Gómez-Muñoz, A., Martin, A., O’Brien, L., and Brindley, D. N. (1994) J. Biol. Chem. 269, 8937–8943
8. Rossi, F., Grzeszowiak, M., Della Bianca, V., Calzetti, F., and Gandini, G. (1995) Biochem. Biophys. Res. Commun. 168, 320–327
9. Agwu, D. E., McPhail, L. C., Sozzani, S., Bass, D. A., and McCall, C. E. (1991) J. Clin. Invest. 88, 531–539
10. Bhat, B. G., Wang, P., and Coleman, R. A. (1994) J. Biol. Chem. 269, 13172–13178
11. Jones, G. A., and Carpenter, G. (1993) J. Biol. Chem. 268, 20845–20850
12. Tsai, M.-H., Yu, C.-L., and Stacey, D. W. (1992) Science 250, 982–985
13. mating, A., De Graan, P. N. E., Gispen, W. H., and Wirtz, K. W. A. (1992) J. Biol. Chem. 267, 7207–7210
14. Moedenaar, W. H. (1993) Trends Cell Biol. 4, 213–219
15. Fourcade, O., Simon, M.-F., Violet, C., Rugani, N., Leballe, F., Ragab, A., Fournié, B., Sarda, L., and Chap, H. (1995) Cell 80, 919–927
16. van Corven, E. J., Groenink, A., Jalink, K., Eichholz, T., and Moedenaar, W. H. (1995) Cell 80, 45–54
17. Hanusin, Y. A. (1994) J. Biol. Chem. 269, 3125–3128
18. Kolev, R., and Golde, D. W. (1994) Cell 77, 325–328
19. Helfer, R. A., and Krönke, M. (1994) J. Cell Biol. 126, 5–9
20. Merrill, A. H. J. (1992) Nutr. Rev. 50, 78–80
21. Ballou, L. R., Chao, C. P., Holmes, M. A., Barker, S. C., and Raghow, R. (1992) J. Biol. Chem. 267, 20044–20050
22. Mathias, S., Younes, A., Kan, C. C., Orlov, I., Joseph, C., and Kolesnick, R. N. (1993) Science 260, 519–521
23. Gómez-Muñoz, A., Waggoner, D. W., O’Brien, L., and Brindley, D. N. (1995) J. Biol. Chem. 270, 26318–26325
24. Jamal, Z., Martin, A., Gómez-Muñoz, A., and Brindley, D. N. (1991) J. Biol. Chem. 266, 2988–2996
25. Gómez-Muñoz, A., Hamza, E. H., and Brindley, D. N. (1992) Biochim. Biophys. Acta 1127, 49–56
26. Lavio, Y., and Liscovitch, M. (1990) J. Biol. Chem. 265, 3868–3872
27. Sakane, F., Yamada, K., and Kanoh, H. (1989) FEBS Lett. 255, 409–413
28. Zhang, H., Buckley, N. E., Gibson, K., and Spiegel, S. (1990) J. Biol. Chem. 265, 76–81
29. Spiegel, S., Olvera, A., and Carlson, R. O. (1993) Adv. Lipid Res. 25, 105–127
30. Chao, C.-P., Launderkerd, S. J. F., and Ballou, L. R. (1994) Biochem. J. 269, 5849–5856
31. Desai, N. N., Zhang, H., Olvera, A., Mattie, M. E., and Spiegel, S. (1992) J. Biol. Chem. 267, 23212–23218
32. Mattie, M., Brooker, G., and Spiegel, S. (1994) J. Biol. Chem. 269, 3181–3188
33. Spiegel, A., and Spiegel, S. (1993) Nature 365, 557–560
34. Dressler, K. A., and Kolesnick, R. N. (1990) J. Biol. Chem. 265, 14917–14921
35. Boudker, O., and Futerman, A. H. (1993) J. Biol. Chem. 268, 22150–22155
36. Gómez-Muñoz, A., Duffy, P. A., Martin, A., O’Brien, L., Byun, H.-S., Bittman, R., and Brindley, D. N. (1995) Mol Pharmacol. 47, 883–899
37. Waggoner, D. W., Martin, A., Dewald, J., Gómez-Muñoz, A., and Brindley, D. N. (1995) J. Biol. Chem. 270, 19422–19429
38. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. 37, 917–917
39. Fiske, C. H., and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400
40. Segel, I. H. (1973) Enzyme Kinetics, pp. 108–109, John Wiley & Sons Inc., New York
41. Dunn, H. N. Eaton, R. D., and Dunn, E. A. (1975) J. Biol. Chem. 250, 9013–9020
42. Carman, G. M., Deems, R. A., and Dennis, E. A. (1975) J. Biol. Chem. 250, 1071–1074
43. Kanan, H., Imai, S., Yamada, K., and Sakane, F. (1992) J. Biol. Chem. 267, 25309–25314
44. Fleming, I. N., and Yeaman, S. J. (1995) Biochem. J. 318, 983–989
45. Rook, W. J., Rupar, A., Chaudhury, N., and Carroll, K. K. (1981) J. Biol. Chem. 256, 1929–1934
46. Beloço, E., and Bosco, D. B. (1982) Eur. J. Biochem. 125, 167–173
47. Weddow, J. F., and Strominger, J. L. (1980) J. Biol. Chem. 255, 1120–1123