A Specific Acetylhydrolase for 1-Alkyl-2-acetyl-sn-glycero-3-phosphocholine (a Hypotensive and Platelet-activating Lipid)*

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1-Alkyl-2-acetyl-sn-glycero-3-phosphocholine, a phospholipid with platelet activating and hypotensive properties, has an extremely labile acetate grouping. The acetate group is obviously important in the expression of the biological properties of this unique derivative of plasmanic acid since once it is hydrolyzed from the parent compound to form the lyso product, all biological activity is lost. Our studies show that the enzyme responsible for the hydrolysis of the acetate moiety, 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine: acetylhydrolase, occurs in the cytosolic fraction of a variety of tissues and has a pH optimum of 7.5 to 8.5. Effects of calcium, magnesium, EDTA, dithiothreitol, deoxycholate, and diisopropylfluorophosphate on the enzyme activity and the fact that egg phosphatidylycholine was not inhibitory indicate the acetylhydrolase enzyme responsible for the removal of the acetate group. Until now, synthetic enzymes are of microsomal origin. The relatively short term antihypertensive effect in rats (lasting <10 min) after administration of 1 μg or less of alkylacyl-GPC (1) suggests that the active phospholipid is rapidly degraded in the body. Our earlier experiments have demonstrated that an active alkylacyl-GPC acetylhydrolase exists in the cytosolic fraction of rat liver (4). The product of this reaction, alkyllyso-GPC, does not affect blood pressure (1) or platelet aggregation (2) and, therefore, emphasizes the important role of the acetylhydrolase in the metabolism of alkylacyl-GPC. Perhaps the transfer of the acetate group to other molecules, such as proteins, is the crucial step for expression of the biological properties of alkylacyl-GPC.

In view of the importance of the removal of acetate from alkylacyl-GPC, we have investigated the details of the tissue distribution and kinetic characteristics of the acetylhydrolase responsible for the removal of the acetate group. Until now, acetylhydrolases that utilize phospholipids as substrates have not been described.

MATERIALS AND METHODS

Racemic hexadecyl octadecenoyl-GPC (R. Berchtold, Biochemisches Labor, Bern, Switzerland) was treated with phospholipase A1 (Glycophagus hannah venom, Sigma Chemical Co.) by the method of Okuyama and Nogima (6). Phosphorus analysis (7) of the reaction products that had been separated on Silica Gel HR layers in chloroform/methanol/glacial acetic acid/water (50:25:8.4, v/v) revealed the presence of equal quantities of 1-hexadecyl-2-lyso-GPC and 3-hexadecyl-2-octadecenoyl-sn-glycero-1-phosphocholine. These results demonstrated that complete hydrolysis of the natural isomer had occurred. 1-Hexadecyl-2-lyso-GPC and the unreacted 3-hexadecyl-2-octadecenoyl-sn-glycero-1-phosphocholine were isolated from this reaction mixture by preparative thin layer chromatography (1). Unreacted 3-hexadecyl-2-octadecenoyl-sn-glycero-1-phosphocholine was subjected to mild alkaline hydrolysis, acetylation, and purification, as previously described (1), to obtain the unnatural isomer 3-hexadecyl-2-acetyl-sn-glycero-1-phosphocholine. 1-Hexadecyl-2[H]acetate-GPC was produced by reacting the 1-hexadecyl-2-lyso-GPC (2 μmol), formed by phospholipase A1 hydrolysis, with [H]acetate anhydride (50 μmol, 500 Ci/mol, Amersham Corp., Arlington Heights, IL) as described by Gupta et al. (8). The entire reaction mixture was transferred in chloroform to a 0.5-cm diameter glass column packed to a height of 2 cm with silicic acid (Unislit from Clarkson Chemical Co., Inc., Williamport, PA). After eluting the excess acetic anhydride with 25 ml of chloroform containing 2% glacial acetic acid followed by 25 ml of 10% methanol in chloroform (v/v), the 1-hexadecyl-2[H]acetate-GPC (250 Ci/mol) was eluted with 30 ml of methanol. Thin layer chromatographic analysis showed that the labeled acetylated substrate co-chromatographed with alkylacyl-GPC prepared from beef heart (1) and had a purity of >99%. A portion of the 1-hexadecyl-2-lyso-GPC was also acetylated with unlabeled acetic anhydride and the resulting 1-hexadecyl-2-acetyl-GPC isolated (1). 1-Hexadecyl-2-acetyl-sn-glycero-3-phosphoethanolamine was prepared clearly shown that a variety of rat tissues can actively synthesize alkylacyl-GPC by direct acetylation of alkyllyso-GPC (4, 5) or by a selective cholinephosphotransferase that utilizes 1-alkyl-2-acetyl-sn-glycero as the substrate (4). Both of these synthetic enzymes are of microsomal origin.

An ether-linked phospholipid, 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine, has been shown to possess marked hypotensive activity when given orally or intravenously to "Goldblatt" hypertensive rats (1) or SHR genetic hypertensive rats. The relatively short term antihypertensive effect in rats (lasting <10 min) after administration of 1 μg or less of alkylacyl-GPC (1) suggests that the active phospholipid is rapidly degraded in the body. Our earlier experiments have demonstrated that an active alkylacyl-GPC acetylhydrolase exists in the cytosolic fraction of rat liver (4). The product of this reaction, alkyllyso-GPC, does not affect blood pressure (1) or platelet aggregation (2) and, therefore, emphasizes the important role of the acetylhydrolase in the metabolism of alkylacyl-GPC. Perhaps the transfer of the acetate group to other molecules, such as proteins, is the crucial step for expression of the biological properties of alkylacyl-GPC.

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2. "Acetylation" is defined as the reaction of alkylacyl-GPC with [H]acetate anhydride (50 μmol, 500 Ci/mol, Amersham Corp., Arlington Heights, IL) as described by Gupta et al. (8). The entire reaction mixture was transferred in chloroform to a 0.5-cm diameter glass column packed to a height of 2 cm with silicic acid (Unislit from Clarkson Chemical Co., Inc., Williamport, PA). After eluting the excess acetic anhydride with 25 ml of chloroform containing 2% glacial acetic acid followed by 25 ml of 10% methanol in chloroform (v/v), the 1-hexadecyl-2[H]acetate-GPC (250 Ci/mol) was eluted with 30 ml of methanol. Thin layer chromatographic analysis showed that the labeled acetylated substrate co-chromatographed with alkylacyl-GPC prepared from beef heart (1) and had a purity of >99%. A portion of the 1-hexadecyl-2-lyso-GPC was also acetylated with unlabeled acetic anhydride and the resulting 1-hexadecyl-2-acetyl-GPC isolated (1). 1-Hexadecyl-2-acetyl-sn-glycero-3-phosphoethanolamine was prepared.

3. W. Renouf and F. Snyder (1980) Biochim. Biophys. Acta, in press.
from 1-hexadecyl-2-acetyl-GPC by a transphosphatidylidation reaction (9). The purified product had the same relative thin layer chromatographic migration described by Wykle and Schremmer (10) and could be completely converted to the dinitrobenzene derivative (11). A phosphatidylcholine fraction containing plasmalogens was isolated from beef heart lipids by the silicic acid column elution sequence of Frieden et al. (12) plus a final step with chloroform/methanol (2:1, v/v). After mild saponification of this fraction and neutralization with acetic acid, the 1-alk-1-ethyl-2-lyso-GPC was isolated by preparative thin layer chromatography and acetylated (1). The purified 1-alk-1-ethyl-2-acetyl-GPC was titrated (Amersham Corp.) to yield 1-[1-2-3H]alkyl-2-acetyl-GPC which was then purified in the same manner as described for 1-hexadecyl-2-[3H]acetate-GPC; radioactivity of the product (48 Ci/mmol) as determined by thin layer chromatography was >98%. Acylacetate-GPC was prepared from egg phosphatidylcholine (Sigma Chemical Co.) as previously described (1). Other compounds used in our experiments included dithiobisretiol, deoxycholate, disopropylfluorophosphate (all from Sigma Chemical Co.), EDTA (Fisher Scientific Co.), pinacyanol chloride (Eastman Organic Chemicals), and selachylidiacetate (1-alkyl-2,3-diacyl-sn-glycerol, Western Chemical Industries, Vancouver, Canada).

Livers from adult, male CDF rats were homogenized in 0.25 M sucrose in the form of a glass pasteur pipette in a loose fitting glass homogenizer tube; various other tissues were homogenized with five strokes of a motor-driven Potter-Elvehjem homogenizer. We prepared subcellular fractions by conventional centrifugation methods. For tissues other than liver, only the postnuclear (600 × g × 10 min supernatant) and cytosolic fractions (100,000 × g × 10 min supernatant) were used. Protein was determined by the method of Lowry et al. (13).

Initial attempts to dissolve the 1-hexadecyl-2-[3H]acetate-GPC in water (10 nmol/ml) using glass tubes resulted in low recoveries of tritium in the aqeous phase (48 ± 1%). However, when polypropylene tubes (Vanguard International, Inc., Neptune, NJ) were substituted for glass tubes, we could recover 90.8 ± 4.9% of the radioactivity in solution. Using a dye technique (14), the critical micellar concentration of 1-hexadecyl-2-acetyl-GPC in 0.1 M phosphate buffer, pH 8.0, was estimated to be 2.5 to 3.0 μmol.

Incubations were done in screw-capped polypropylene tubes containing protein and 1-hexadecyl-2-[3H]acetate-GPC in a final volume of 0.5 ml of 0.1 M phosphate buffer (pH 8.0) at 37°C for 5 min. Substrate and protein concentrations are stated in the legends of the tables and figure. Incubations were terminated by adding 1 ml of chloroform and 0.5 ml of a 10% sodium bicarbonate solution. This mixture was shaken and then centrifuged at 1900 rpm; the upper layer was washed three times with 1-ml aliquots of fresh chloroform. [3H]Acetate in the aqueous layer (0.5-ml aliquot) was radioassayed by liquid scintillation spectrophotometry. Results obtained with the boiled enzyme preparations containing added internal standards of [1-14C]-acetate and [1-2-3H]acetate-2-[H] were corrected to 100% of the 14C and only <1% of 3H were extracted into the aqueous layer under these conditions. These percentages were used to correct the acetate calculations for the amount of H-labeled 1-hexadecyl-2-acetyl-GPC found in the aqueous layer after each incubation. Incubations using the 1-12.7-1-Halkyl-2-acetyl-GPC were terminated by the addition of 1 ml of methanol containing 2% acetic acid. The entire contents were evaporated to dryness with N2, redissolved in 1 ml of chloroform/methanol (1:1, v/v), and the products were analyzed for radioactivity after separation by thin layer chromatography (1).

In an experiment to verify that the tritium released by the acetylhydrolase activity was in the form of a glass pasteur pipette, we incubated 40 pg of protein from the soluble fraction of kidney cortex with 10 nmol of 1-hexadecyl-2-[3H]acetate-GPC for 10 min and then terminated the reaction by adding 50 μl of glacial acetic acid. The samples were immediately frozen and then lyophilized. Volatile components were collected in a trap submerged in liquid nitrogen. Acetic acid was converted to the ammonium salt by exposing the trapped solution to an excess of ammonia vapor generated from NH4OH. An aliquot of the ammonium acetate solution was then chromatographed on Silica Gel G layers (250 μm thick) in chloroform/methanol/ammonium hydroxide (65:35:8, v/v/v) for staining the developed plates with iodine vapor to locate the ammonium acetate band (identified by co-chromatography with a standard having an Rf of 0.2 to 0.3), various areas of the plate were scraped into vials and the tritium assayed in a liquid scintillation spectrophotometer. When sodium [1-14C]acetate was subjected to this same procedure, the overall recovery of radioactivity was 72%.

RESULTS AND DISCUSSION

The activity of alkylacetate-GPC:acylhydrolase in the soluble fraction of rat liver was linear with protein concentrations up to at least 50 μg and with the time of incubation through at least 10 min. A broad pH optimum existed between 7.5 and 8.5.

Results obtained for the subcellular distribution of alkylacetate-GPC:acylhydrolase in rat liver (Table I) demonstrate that the hydrolase activity is highest in the cytosolic fraction. Therefore, it seems likely that once alkylacetate-GPC enters the cell, it is first hydrolyzed to the 2-lyso-compound by the cytosolic acylhydrolase. The product produced by the hydrolase, 1-hexadecyl-2-lyso-GPC, could then be subsequently metabolized in microsomes by several enzymes, e.g. alkylsophospholipase D (15) and alkyllysoglycerophosphocholine monoxygenase, and/or resynthesized to alkylacetate-GPC by an acetyl-CoA transferase (4, 5).

In order to determine whether the alkylacetate-GPC:acylhydrolase activity had properties similar to the phospholipase A2 that utilizes phospholipids containing two long chain acyl groups, we added various compounds to the incubations that have been reported to influence the activity of the typically encountered phospholipase A2. Results obtained with the liver cytosolic fraction (Table II) show that the acylhydrolase activity was not greatly affected by additions of Ca2+ or Mg2+, and that EDTA and dithiothreitol had slight stimulating effects. However, the reaction was severely inhibited by disopropylfluorophosphate (0.11 to 11 μM) and, to some extent, by deoxycholate. The lack of stimulation by Ca2+ or Mg2+ and the slight stimulation by EDTA indicate that the acylhydrolase activity in the cytosolic fraction of rat liver does not behave like the typical phospholipase A2 activity of rat liver (16) since the latter exhibits completely different properties. Moreover, the severe inhibition of the acylhydrolase by disopropylfluorophosphate, which does not inhibit phospholipase A2 (17), suggests that the acylhydrolase may be similar to the short chain acylhydrolase activities encountered by Wykle and Schremmer (10) in their studies of plasmalogen biosynthesis with microsomal fractions of Fischer sarcomas.

Table III presents the acylhydrolase activities found in the soluble fraction of several rat tissues. Specific activities of acylhydrolase were also measured in the postnuclear fraction (600 × g × 10 min supernatant) from each tissue and were found to be two-thirds to one-half less than that in the cytosolic fraction (100,000 × g × 60 min supernatant). The soluble fraction from kidney had the highest acylhydrolase activity of all tissues examined, although the lung and brain also had relatively high activities. The specific activity of acylhydrolase found in the soluble fraction of the kidney medulla was two-thirds to one-half that of the kidney cortex. In view of the high activities present, the cytosolic fraction of the kidney cortex was used to obtain the kinetic data depicted in the double reciprocal plot shown in Fig. 1. Acylhydrolase had apparent Km and Vmax values of 3.1 μM and 17.8 nmol/min/mg of protein, respectively.

After formation of the ammonium salt, the tritium released from 1-hexadecyl-2-[3H]acetate-GPC by the kidney cortex soluble fraction was shown to co-chromatograph on thin layer chromatograms with an acetate standard. The overall yield was equal to that found using the collection technique for sodium [1-14C]acetate described under "Materials and Methods." Kidney cortex soluble fraction was also used to determine the stoichiometry of the acylhydrolase reaction by...
Distribution of alkylacyl-1-GPC:acyethylhydrolase in subcellular fractions of rat liver

All samples (40 to 50 μg of protein and 1 nmol of 1-hexadecyl-2-[1-1,2-3H]jakyl-2-acyethyl-GPC) were incubated for 5 min at 37°C. Values are averages of duplicate incubations that agreed within ±10% of each other.

| Fraction                      | nmol/min/mg protein |
|-------------------------------|---------------------|
| 600 × g × 10 min supernatant  | 0.48                |
| 15,000 × g × 10 min pellet     | 0.64                |
| 100,000 × g × 60 min pellet    | 0.32                |
| 100,000 × g × 60 min supernatant | 1.36              |

Effect of various compounds on alkylacyl-1-GPC:acyethylhydrolase activity in the cytosolic fraction of rat liver

Incubations were performed as described under “Materials and Methods”; each incubation contained 30.4 μg of protein from the cytosolic fraction and 1 nmol of 1-hexadecyl-2-[1,2-3H]jakyl-2-acyethyl-GPC. Distribution of alkylacyl-1-GPC:acyethylhydrolase was preincubated with the protein for 20 min at 0°C. Values are averages of duplicate incubations that agreed within ±10% of each other.

| Addition                        | % of control |
|--------------------------------|--------------|
| None                           | 100*         |
| Ca** (10 mM)                   | 95           |
| Mg** (10 mM)                   | 104          |
| EDTA (10 mM)                   | 118          |
| Dithiothreitol (1 mM)          | 115          |
| Deoxycholate (0.1 mM)          | 80           |
| Diisopropylfluorophosphate      |              |
| 11 mM                          | 0            |
| 1.1 mM                         | 9            |
| 0.11 mM                        | 48           |

* Control activity in this experiment was 1.30 nmol/min/mg of protein.

Alkylacyl-1-GPC:acyethylhydrolase activities in the cytosolic fractions of various rat tissues

Incubations were performed as described under “Materials and Methods”, each sample contained 10 nmol of 1-alkyl-2-[1,2-3H]jakyl-2-acyethyl-GPC. Values are averages of duplicate incubations at two different times (5 min and 10 min) that agreed within ±10% of each other.

| Tissue  | nmol/min/mg protein |
|---------|---------------------|
| Kidney  | 13.1                |
| Lung    | 9.7                 |
| Spleen  | 2.6                 |
| Brain   | 4.7                 |
| Heart   | 1.3                 |
| Liver** | 1.5                 |
| Plasma  | 1.4                 |

** The cytosolic fraction was prepared from a liver homogenate with a Potter-Elvehjem homogenizer.

Table II

FIG. 1. Double reciprocal plot for alkylacyl-1-GPC:acyethylhydrolase activity in the cytosolic fraction of kidney cortex from rats. All incubations (5 min at 37°C) contained 13.3 μg of protein. Values are averages from duplicate incubations that agreed within ±10% of each other.

Table III

A Phospholipid Acylhydrolase

Effects of various substrate analogs on alkylacyl-1-GPC:acyethylhydrolase activity from the cytosolic fraction of the kidney cortex of rats

Incubations were performed as described under “Materials and Methods” with 13.3 μg of protein and 1 nmol of 1-hexadecyl-2-[1,2-3H]jakyl-2-acyethyl-GPC per tube, except the mixed substrate was added in 10 μl of ethanol. This amount of ethanol had no effect on the acetylhydrolase activity in control incubations. Values are averages of duplicate incubations that agreed within ±10% of each other.

| Addition                        | % of control |
|--------------------------------|--------------|
| Egg phosphatidylcholine         | 2            |
| Acylacyl-GPC                    | 15           |
| 3-Hexadecyl-2-acetyl-sn-glycerol | 3            |
| 1-Hexadecyl-2-acetyl-sn-glycerol | 3            |
| 1-Alkyl-2,3-diacetyl-sn-glycerol | 3            |

* Control activity in these experiments was 8.2 nmol of [3H]acetate released/min/mg of protein.

Using 1-hexadecyl-2-[1,2-3H]jakyl-2-acyethyl-GPC or 1-[1,2-3H]jakyl-2-acyethyl-GPC as the substrates in separate but parallel incubations. Ratios of the nanomoles of 1-[1,2-3H]jakyl-2-lyso-GPC formed to nanomoles of [3H]acetate released were 0.90 ± 0.14 (mean ± S.D.) when duplicate incubations were done at both high (19.8 μg of protein, 2 nmol of alkylacyl-GPC) and low (13.3 μg of protein, 10 nmol of alkylacyl-GPC) protein to substrate concentrations. Furthermore, no radioactive lipids other than 1-[1,2-3H]jakyl-2-lyso-GPC (the product) and 1-[1,2-3H]jakyl-2-acyethyl-GPC (the substrate) were found in these incubations.

Only a slight inhibition of alkylacyl-1-GPC:acyethylhydrolase activity was observed when egg phosphatidylcholine (long chain diacyl type) was added to the incubations containing the cytosolic enzyme from rat kidney cortex (Table IV). In contrast to the data obtained with egg phosphatidylcholine, the phosphatides having sn-2 acetyl groups significantly inhibited the hydrolysis of the alkylacyl-GPC (Table IV). However, 1-alkyl-2,3-diacetyl-sn-glycerol caused only a slight decrease in acetylhydrolase activity. These findings are consistent with our results obtained with the inhibitor studies (Table II) and further support the premise that the acetylhydrolase for alkylacyl-GPC differs from the phospholipase A2 that utilizes long chain diacylphospholipids as substrates. Our results might also be explained on the basis of phosphatidylcholine activating and hypotensive properties; therefore, the alkyl moiety must be a critical determinant of its profound biological activity. Perhaps it is the alkyl portion of the...
A molecule that determines the specificity of the transfer of the acetate to other compounds that modulate the biological responses involved. Regardless, the acetylhydrolase that removes the acetate moiety of alkylacetyl-GPC appears to be a very important enzyme in the metabolism of this biologically active phospholipid.

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REFERENCES

1. Blank, M. L., Snyder, F., Byers, L. W., Brooks, B., and Muirhead, E. E. (1979) Biochem. Biophys. Res. Commun. 90, 1194-1200
2. Demopoulos, C. A., Pinckard, R. N., and Hanahan, D. J. (1979) J. Biol. Chem. 254, 9355-9358
3. Hanahan, D. J., Demopoulos, C. A., Liehr, J., and Pinckard, R. N. (1980) J. Biol. Chem. 255, 5514-5516
4. Renooij, W., Wykle, R. L., Blank, M. L., Lee, T-c., Malone, B., Fitzgerald, V. L., and Snyder, F. (1980) Fed. Proc. 39, 2187, Abstract 3059
5. Wykle, R. L., Malone, B., and Snyder, F. (1980) J. Biol. Chem. 255, in press
6. Okuyama, H., and Nojima, S. (1965) J. Biochem. 57, 529-538
7. Rouser, G., Siakotos, A. N., and Fleischer, S. (1966) Lipids 1, 85-86
8. Gupta, C. M., Radhakrishnan, R., and Khorana, H. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4315-4319
9. Yang, S. F., Freer, S., and Benson, A. A. (1967) J. Biol. Chem. 242, 477-484
10. Wykle, R. L., and Schremmer, J. M. (1979) Biochemistry 18, 3512-3517
11. Renkonen, O. (1968) J. Lipid Res. 9, 34-39
12. Frosolono, M. F., and Marsh, M. (1973) Chem. Phys. Lipids 10, 203-214
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
14. Barden, R. E., and Cleland, W. W. (1969) J. Biol. Chem. 244, 3677-3684
15. Wykle, R. L., Kraemer, W. F., and Schremmer, J. M. (1977) Arch. Biochem. Biophys. 184, 149-155
16. Waite, M., and Van Deenen, L. L. M. (1967) Biochim. Biophys. Acta 137, 408-517
17. Brokerhoff, H., and Jensen, R. G. (1974) in: Lipolytic Enzymes Chapter VI, pp. 194-265, Academic Press, New York