Biochimica et Biophysica Acta, 1042 (1990) 153–158
Elsevier

BBALIP 53307

Characterization of the enzymatic hydrolysis of acetate from alkylacetylglycerols in the de novo pathway of PAF biosynthesis

Merle L. Blank, Zigrida L. Smith, Edgar A. Cress and Fred Snyder

Medical Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN (U.S.A.)

(Received 19 June 1989)

Key words: PAF; Acetate hydrolysis; Glycerolipid; Lipoprotein lipase; (De novo synthesis)

This report describes the partial characterization of the enzymatic activity responsible for the hydrolysis of acetate from 1-alkyl-2-acetyl-*sn*-glycerol, the immediate precursor in the de novo synthesis of PAF (platelet-activating factor or 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) by Ehrlich ascites cells. The highest acetylhydrolase activity for this neutral lipid was associated with the membrane fractions from Ehrlich ascites cells (> 90% of total activity); only a minimal level of activity (< 10%) was observed in the cytosol which contrasts with the cytosolic site of PAF acetylhydrolase in normal cells. Hydrolysis of 1-[^{3}H[hexadecyl-2-acetyl-*sn*-glycerol by the membrane fraction at pH 7.5 and 37°C gave apparent values for K_m and V_{max} of 45 μM and 179 nmol/min per mg protein, respectively. Hydrolysis of acetate from 1-[^{3}H]hexadecyl-2-acetyl-*sn*-glycerol by the membrane fraction was not affected by 5 mM concentrations of Ca^{+2}, Mg^{+2} or EDTA, but was significantly inhibited (80% reduction) by 10 mM NaF. Based on differences in both the subcellular distribution and response to inhibition by NaF, the neutral lipid acetylhydrolase does not appear to be the same enzyme that hydrolyzes acetate from platelet-activating factor. In contrast to inhibition of diacylglycerol lipase by *p*-chloromercuribenzoate and *N*-ethylmaleimide, we found no significant inhibition of acetate hydrolysis from 1-[^{3}H[hexadecyl-2-acetyl-*sn*-glycerol by either of these compounds. Also, *p*-nitrophenyl acetate (a nonspecific esterase substrate) failed to inhibit acetate hydrolysis of 1-[^{3}H]hexadecyl-2-acetyl-*sn*-glycerol. Our studies of this enzyme would indicate that it may play an important role in regulating the levels of platelet-activating factor synthesized by the de novo pathway via hydrolysis of the immediate precursor of PAF.

Introduction

1-Alkyl-2-acetyl-*sn*-glycerol (1-alkyl-2-acetyl-Gro) can induce rabbit platelets to aggregate [1] and produce a hypotensive response in rats [2]. These biological responses can be explained by metabolic conversion of the neutral lipid to platelet-activating factor (PAF; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine or alkylacetyl-GroPCho), a potent biologically active phospholipid, via a dithiothreitol-insensitive cholinephosphotransferase [3,4]. Significant amounts of PAF can be generated from 1-alkyl-2-acetyl-Gro by both rabbit platelets [1,5] and Ehrlich ascites cells (EAC) [6]. However, alkyl-Gro is the major metabolic product formed when 1-alkyl-2-acetyl-Gro is incubated with rabbit platelets [5], human endothelial cells [7], and EAC [6]. Hydrolysis of the acetate group from 1-alkyl-2-acetyl-Gro produced by the de novo pathway of PAF biosynthesis [8,9] could be an important mechanism for regulating the cellular levels of PAF.

We have previously shown that EAC not only contain the enzymes necessary for the biosynthesis of alkylglycerolipids [10] but that they can also be induced (by treatment with oleic acid) to produce PAF from 1-alkyl-2-acetyl-Gro [6]. Therefore, in this report we have used the same cell line to characterize some of the properties of the enzyme(s) responsible for hydrolysis of the acetate group from 1-alkyl-2-acetyl-Gro, the immediate precursor of PAF in the de novo route.

Abbreviations: PAF, platelet-activating factor; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; EAC, Ehrlich ascites cells; TLC, thin-layer chromatography; Gro, *sn*-glycerol; GroPCho, *sn*-glycero-3-phosphocholine.

Correspondence: F. Snyder, Medical Sciences Division, Oak Ridge Associated Universities, Post Office Box 117, Oak Ridge, TN 37831-0117, U.S.A.

Materials and Methods

1-[9,10-^{3}H]Hexadecyl-2-acetyl-*sn*-glycerol (1-[^{3}H]hexadecyl-2-acetyl-Gro) was produced by phospholipase C hydrolysis of 1-[9,10-^{3}H]hexadecyl-2-acetyl-*sn*-

glycero-3-phosphocholine (46 Ci/mmol) as previously described [6]. A portion of the 1-[^{3}H]hexadecyl-2-acetyl-Gro was incubated for 30 min at 37° in 0.02 M Tris-maleate buffer (pH 8.5) to isomerize some of the substrate (50%) to 1-[^{3}H]hexadecyl-3-acetyl-Gro, which was then isolated by thin-layer chromatography (TLC). Unlabeled 1-hexadecyl-2-acetyl-Gro and 1-hexadecyl-3-acetyl-Gro were isolated by preparative TLC from the pancreatic lipase products derived from 1-hexadecyl-2,3-diacetyl-*sn*-glycerol [3]. 1,2-[1-^{14}C]Dioleoyl-Gro was prepared by phospholipase C treatment [6] of 1,2-[1-^{14}C]dioleoyl-*sn*-glycero-3-phosphocholine (105 Ci/mol, from DuPont/New England Nuclear Boston, MA) and unlabeled 1,2-dioleoyl-Gro was purchased from Sigma, St. Louis, MO.

A lipase inhibitor, RHC 80267, was a generous gift from Dr. M.C. Cabot, W. Alton Jones Cell Science Center, Lake Placid, NY. *p*-Chloromercuribenzoate, *N*-ethylmaleimide, and *p*-bromophenacyl bromide were purchased from Sigma. RHC 80267, *p*-chloromercuribenzoate or *p*-bromophenacyl bromide were added to the incubations in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in all incubations never exceeded 1% by volume and, although dimethyl sulfoxide had little effect on the enzymatic activities, the vehicle alone was always included in the controls.

Maintenance, harvest, and washing of the EAC were carried out as previously described [6]. 1 ml of washed, packed EAC were suspended in 10 ml of 0.145 M NaCl containing 0.02 M Tris-maleate (pH 7.5). The cells were then disrupted by two 1 min pulses of sonication [6], which usually caused over 95% cell breakage. Disrupted cells where then centrifuged at $1000 \times g$ for 10 min to produce a cell-free homogenate. The cell-free homogenates were centrifuged at $100\,000 \times g$ for 90 min and these membranes (total membrane fraction) were resuspended in 0.145 M NaCl containing 0.02 M Tris-maleate (pH 7.5) as the enzyme source for most of the experiments. In some experiments, the resulting cell-free homogenate was centrifuged at $13\,000 \times g$ for 15 min to obtain a membrane pellet and the supernatant was centrifuged at $100\,000 \times g$ for 90 min to yield a second membrane fraction and the cytosolic proteins. Only freshly isolated membrane fractions were used in all experiments. Protein was determined by the method of Lowry et al. [11] with BSA as the standard.

Assay of the enzymatic hydrolysis of the acetate group from the 1-[^{3}H]hexadecyl-2-acetyl-Gro (also the 1,3-isomer) was carried out by addition of the substrate (0.15 μCi containing the indicated amounts of unlabeled substrate, dissolved in 10 μl of ethanol) to 1 ml of 0.02 M Tris-maleate buffer (pH 7.5) in 0.145 M NaCl containing an appropriate amount of EAC protein. Samples were incubated for 5 min (unless stated otherwise) at 37°C and the lipids were extracted (on ice) by the method of Bligh and Dyer [12]. To prevent additional isomerization of the remaining substrate during the lipid extraction procedure, care was taken to exclude any upper phase contamination of the lower chloroform layer. The extent of hydrolysis of the 1-[^{3}H]hexadecyl-2-acetyl-Gro (and its 1,3-isomer) was determined by the amount of the substrate that was converted to 1-[^{3}H]hexadecyl-*sn*-glycerol as measured by TLC. We used boric acid impregnated layers of Silica Gel G [3] and a solvent system [13] of chloroform/acetone (19 : 3, v/v) for TLC analysis of the reaction products. Authentic standards were cochromatographed with the samples. Developed TLC plates were exposed to I_2 vapor to visualize the separated compounds and, after allowing the I_2 to evaporate, the bands of silica gel were scraped into scintillation vials for radioassay. Opti-Fluor scintillation fluid (Packard, Downers Grove, IL) containing 6.25% water (v/v) was then added to the scintillation vials, the vials were subjected to sonication in an ultrasonic cleaning bath (Mettler Electronics, Anaheim, CA) for 10 min and the amounts of radioactivity determined by liquid scintillation spectroscopy. Incubations in buffer alone or those containing EAC protein that had been boiled for 5 min gave the same results; thus, these samples were routinely used for subtraction of small amounts of background radioactivity (1–2%) from the experimental samples. Radioactive material migrating on TLC with alkylglycerols was recovered from extracts of pooled incubations and further identified as [^{3}H]alkylglycerol by formation of benzoate derivatives that had the same TLC R_F values and high-performance liquid chromatography retention times as authentic standards [14].

Diglyceride lipase activity of EAC membranes was assayed under the same conditions used for monitoring the hydrolysis of alkylacetyl-Gro except that the final concentration of substrate (1,2-[1-^{14}C]dioleoyl-Gro) was 10 μM, the amount of membrane protein was increased ($\approx$ 500 μg per sample), and the incubation time was 20 min. The extent of 1,2-[1-^{14}C]dioleoyl-Gro hydrolysis by the EAC membrane fraction was determined from the amount of [1-^{14}C]oleic acid released, as measured by radioassay of silica-gel G TLC plates developed in a solvent of hexane/diethyl ether/glacial acetic acid (80 : 20 : 1, v/v), plus the amount of labeled monoacylglycerol produced, as measured by TLC on silica gel G coated plates developed in diethyl ether/glacial acetic acid (100 : 1, v/v).

PAF: acetylhydrolase activity was measured as previously described [15] using 1-hexadecyl-2-[^{3}H]acetyl-GroPCho (Du Pont/New England Nuclear, Boston, MA), adjusted to a specific activity of 22 μCi/μmol with unlabeled 1-hexadecyl-2-acetyl-GroPCho (Sigma) as the substrate. A nonspecific esterase activity was measured essentially as described by Huggins and Lapides [16]. Briefly, a fresh stock solution of *p*-nitrophenyl acetate (Sigma) was prepared for each ex-

periment by dissolving 150 μmol of substrate per ml of methanol and then diluting this solution 1 : 99 with water. Cuvettes used for enzymatic assays contained 150 μM *p*-nitrophenyl acetate and an appropriate amount of EAC protein (200–300 μg) in a final volume of 4 ml of 0.145 M NaCl and 0.02 M Tris-maleate (pH 7.5). 1-Hexadecyl-2-acetyl-Gro (final concentration, 100 μM) was added to some incubations to check for its possible inhibition of the nonspecific esterase. Cuvettes were placed in a temperature controlled (37° C) holder (Model DU-70 spectrophotometer, Beckman Instruments, Fullerton, CA) and, after allowing 6 min for temperature equilibration, the linear increase in absorbance was measured at 400 nm over a period of 5 min. The amount of *p*-nitrophenyl acetate hydrolyzed was calculated using a molar absorptivity of $1.2 \cdot 10^4$ for *p*-nitrophenol.

Results

Preliminary experiments indicated that the membrane fraction (100 000 × *g* for 90 min pellet) obtained from the EAC cell-free homogenate was much more active (> 90% of the total activity) than the cytosolic fraction in hydrolyzing the acetate group from 1-[^{3}H]hexadecyl-2-acetyl-Gro. We therefore used the total membrane fraction, isolated from EAC as described in the Materials and Methods section, to establish optimum conditions for hydrolysis of the acetate moiety from 1-alkyl-2-acetyl-Gro. We found that the 1-[^{3}H]hexadecyl-2-acetyl-Gro was extensively isomerized during 5 min incubations in 0.145 M NaCl containing 0.02 M Tris-maleate buffer at higher pH values (e.g., ≈ 3% isomerized at pH 7.5 and ≈ 11% at pH 8.5). Because the added factor of isomerization makes interpretation of data at higher pH levels more equivocal, we decided to do the enzyme assays at a near physiological pH of 7.5. At this pH, hydrolysis of the acetate group from 1-[^{3}H]hexadecyl-2-acetyl-Gro occurs primarily from the 1,2-isomer as demonstrated in Table I. However, the EAC membrane fraction is also capable of removing the acetate group from the *sn*-3 position of 1-[^{3}H]hexadecyl-3-acetyl-Gro (Table I). In fact when the extent of acetate hydrolysis is adjusted for the amount of protein (Table I), the rate of hydrolysis for the 1,2- and 1,3-isomers is virtually identical.

The rate of hydrolysis of 1-[^{3}H]hexadecyl-2-acetyl-Gro was reasonably linear both with membrane protein concentrations up to at least 50 μg and incubation times within 4–6 min (Fig. 1, A and B). The effect of substrate concentration on the hydrolysis of 1-[^{3}H]hexadecyl-2-acetyl-Gro by the membrane fraction of EAC is shown in Fig. 2A. A double-reciprocal plot of the data (Fig. 2B) gave apparent values for V_{max} and K_m of 179 nmol/min per mg protein and 45.3 μM, respectively; however, these values must be viewed with an under-

TABLE I

Hydrolysis of acetate from 1-[^{3}H]hexadecyl-2-acetyl- and 1-[^{3}H]hexadecyl-3-acetyl-Gro by a total membrane fraction of EAC

Data represent the average percentage of total tritium found in each lipid class ± S.E. from two separate experiments with duplicate samples ($n = 4$). The average amount of protein per incubation tube was 32 μg in the experiments with the 1,2-isomer as substrate and 27 μg with the 1,3-isomer as substrate. Substrates (120 μM) were incubated for 5 min at 37° C in 0.145 M NaCl, 0.02 M Tris-maleate (pH 7.5)

Compound	% tritium			
	Substrate: 1,2-isomer		Substrate: 1,3-isomer	
	− enzyme [a]	+ enzyme	− enzyme [a]	+ enzyme
1,3-isomer	2.4 ± 0.1	3.0 ± 0.4	98.2 ± 0.2	86.2 ± 0.6
1,2-isomer	97.6 ± 0.1	81.5 ± 1.0	1.8 ± 0.2	1.0 ± 0.1
Alkyl-Gro	–	15.5 ± 1.2	–	12.8 ± 0.5

[a] Incubations (− enzyme) done in buffer only.

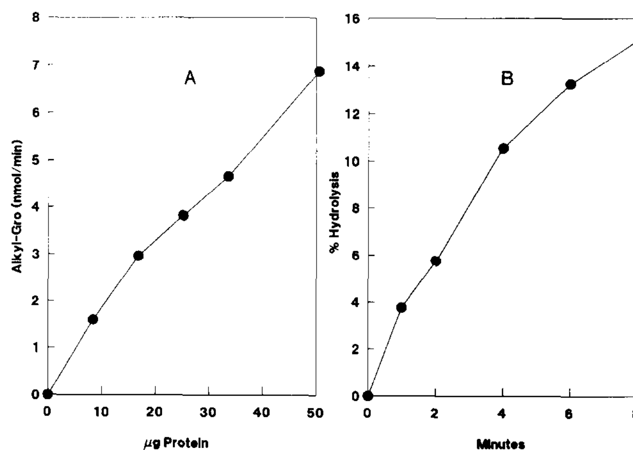

Fig. 1. Effect of protein concentration (A) and incubation time (B) on the hydrolysis of 1-[^{3}H]alkyl-2-acetyl-Gro (200 μM) by the total membrane fraction from EAC. Samples in (A) were incubated for 5 min at 37° C and samples in (B) contained 29 μg of protein. Values represent means from duplicate incubations that varied by less than 10% from the mean. Data in each panel are representative of two separate experiments.

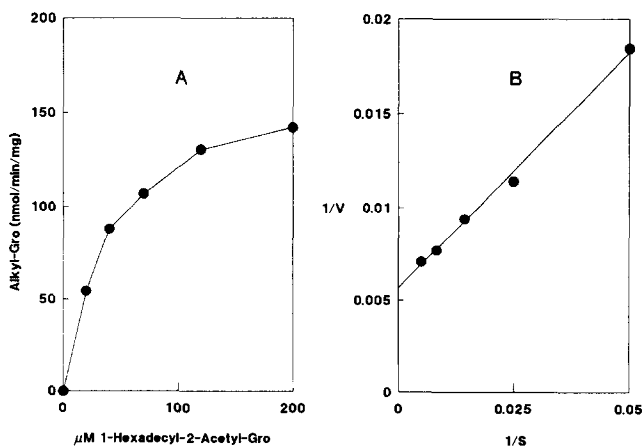

Fig. 2. Effect of substrate concentration on the hydrolysis of 1-[^{3}H]alkyl-2-acetyl-Gro by the total membrane fraction from EAC (A). (B) is a double-reciprocal plot of the data in (A). Values represent the means from two separate experiments done in duplicate ($n = 4$) with a standard error of less than 10%.

TABLE II

Effect of diglyceride lipase inhibitors on the hydrolysis of 1-[³H]hexadecyl-2-acetyl-Gro, p-nitrophenyl acetate and 1,2-[1-¹⁴C]dioleoyl-Gro by a total membrane fraction of EAC

Data represent the average percent of control values (± S.E.) from two separate experiments with duplicate samples ($n = 4$). Controls with 200 μM 1-[^{3}H]hexadecyl-2-acetyl-Gro had a hydrolysis rate of 143 ± 4 nmol/min per mg protein; incubations were for 5 min at 37 °C. Controls with 150 μM *p*-nitrophenyl acetate had a hydrolysis rate of 29.6 ± 1.2 nmol/min per mg protein; incubations were carried out as described in Materials and Methods. Controls with 10 μM 1,2-[1-^{14}C]dioleoyl-Gro had a hydrolysis rate of 0.229 ± 0.006 nmol/min per mg protein; incubations were carried out as described in Materials and Methods. Based on Student's *t*-test, the following values are significantly lower than controls: * $P < 0.01$; and ** $P < 0.001$.

Inhibitor (concn.)	% of control		
Substrate:	1-[^{3}H]hexadecyl-2-acetyl-Gro	*p*-ntirophenyl acetate	1,2-[1-^{14}C]dioleoyl-Gro
p-Chloromercuribenzoate (0.07 mM)	93.9 ± 3.7	103.2 ± 2.4	21.0 ± 1.5 **
N-Ethylmaleimide (0.70 mM)	97.4 ± 3.1	89.0 ± 1.6	30.9 ± 1.6 **
p-Bromaphenacyl bromide (0.10 mM)	79.7 ± 3.7*	33.1 ± 1.6 **	29.6 ± 3.8 **
RHC 80267 (4 μM)	42.0 ± 5.9 **	5.1 ± 0.6 **	35.1 ± 3.7 **

standing of the inherent problems encountered with water-insoluble substrates and membrane-bound enzymes. Most of the subsequent experiments were conducted at a substrate concentration of 200 μM.

In an attempt to differentiate the alkylacetylglycerol hydrolase activity from that of a diglyceride lipase, we tested the effect of three compounds that have been reported to inhibit diglyceride lipase activity in membranes from human platelets [17]. At the concentrations tested, *p*-chloromercuribenzoate, *N*-ethylmaleimide, and *p*-bromophenacyl bromide inhibit diglyceride lipase activity from platelets by 75%, 85% and 50%, respectively [17]. In agreement with these results, the diglyceride lipase activity in the EAC membrane fraction was also significantly inhibited by these three compounds (Table II). However, we found no significant inhibition of acetate hydrolysis from 1-[^{3}H]hexadecyl-2-acetyl-Gro by either *p*-chloromercuribenzoate or *N*-ethylmaleimide and only a 20% inhibition by *p*-bromophenacyl bromide (Table II). It should be noted that *N*-ethylmaleimide is also an inhibitor of human platelet monoglyceride lipase [18] and therefore monoglyceride lipase does not appear to be capable of utilizing alkylacetylglycerol as a substrate. In addition, when 1,2-[1-^{14}C]dioleoyl-Gro was incubated for 20 min with the EAC membrane fraction, the percent of ^{14}C released as oleic acid (per mg protein) was only slightly higher than the percent of ^{14}C found in the monoacylglycerols (24.1 ± 0.8 and 18.9 ± 0.4%, respectively) suggesting that monoglyceride lipase activity is low in the EAC membranes. RHC 80267, a compound that has been shown to inhibit diglyceride lipase [19], inhibited the hydrolysis of acetate from both 1-[^{3}H]hexadecyl-2-acetyl-Gro and the nonspecific esterase substrate *p*-nitrophenyl acetate (Table II). At all levels of RHC 80267 that were tested (2 μM to 20 μM), the inhibition of *p*-nitrophenyl acetate hydrolysis was significantly greater than the inhibition of acetate hydrolysis from 1-[^{3}H]hexadecyl-2-acetyl-Gro (data not shown). Although these results suggest that different enzymes are involved, it is also possible that the increased inhibition of acetate hydrolysis from *p*-nitrophenyl acetate by RHC 80267, compared to the other two substrates, could be due to differences in their affinity, relative to the inhibitor, for the same enzyme.

Additional comparisons were made between the enzymatic activities responsible for hydrolysis of the acetate groups from 1-alkyl-2-acetyl-Gro and *p*-nitrophenyl acetate, a nonspecific esterase substrate. The hydrolysis rate for 150 μM *p*-nitrophenyl acetate by the EAC membrane fraction was 29.9 ± 0.6 nmol/min per mg protein (average ± S.E., $n = 8$) and addition of 100 μM 1-hexadecyl-2-acetyl-Gro to the

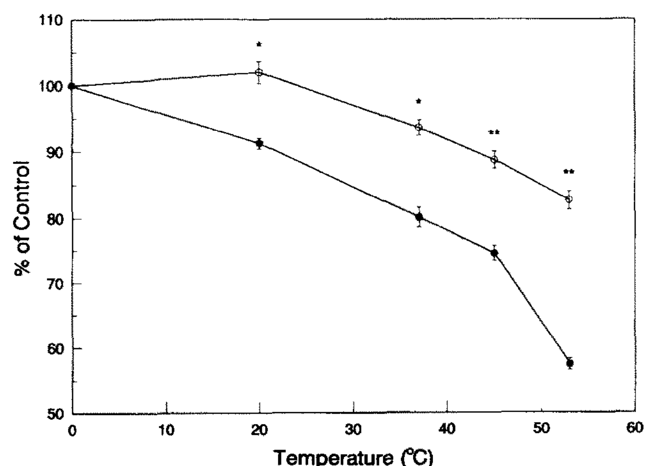

Fig. 3. Effect of preincubation temperature on the enzymatic activity of the total EAC membrane fraction. The EAC membrane fraction was preincubated for 15 min at the indicated temperatures and then assayed for acetylhydrolase with either *p*-nitrophenyl acetate (closed circles) or 1-[^{3}H]hexadecyl-2-acetyl-Gro (open circles) as substrates. Values represent the means ± S.E. from two separate experiments done in duplicate ($n = 4$). Based on Student's *t*-test, the indicated values are significantly higher than the corresponding values for *p*-nitrophenyl acetate, $P < 0.01$ and $P < 0.001$.

incubations caused only a small but significant ($P < 0.01$) reduction in this rate of hydrolysis (83.2 ± 2.5% of control, $n = 8$). Conversely, addition of 150 μM *p*-nitrophenyl acetate failed to reduce ($P > 0.6$) the rate of hydrolysis of 50 μM 1-[^{3}H]hexadecyl-2-acetyl-Gro (97.5 ± 3.6% of control, $n = 4$). Pretreatment of the EAC membrane fraction for 15 min at different temperatures showed that the hydrolytic activity towards *p*-nitrophenyl acetate was somewhat more heat-labile than the activity that hydrolyzes 1-[^{3}H]hexadecyl-2-acetyl-Gro (Fig. 3). The hydrolysis of acetate from either 1-[^{3}H]hexadecyl-2-acetyl-Gro or *p*-nitrophenyl acetate by the membrane fraction of EAC was not affected by 5 mM concentrations of EDTA, Ca^{2+}, or Mg^{2+} (data not shown).

Based on both specific activity and total activity, the two membrane fractions isolated from EAC by differential centrifugation (13000 × *g* and 100000 × *g* pellets) contained most of the enzymatic activity for hydrolysis of the acetate group from 1-[^{3}H]hexadecyl-2-acetyl-Gro (Fig. 4). In contrast to the minor contribution of the cytosolic fraction toward the total acetate hydrolytic activity when 1-[^{3}H]hexadecyl-2-acetyl-Gro was used as a substrate, the majority of the total cellular PAF acetylhydrolase activity was located in the EAC cytosolic fraction (Fig. 4). However, the microsomal fraction from these tumor cells did contain a higher proportion of PAF acetylhydrolase activity than was previously observed in membrane fractions from normal tissues [15].

Hydrolysis of acetate from 1-[^{3}H]hexadecyl-2-acetyl-Gro was significantly inhibited by addition of NaF to the incubation mixture (Table III). It is doubtful that

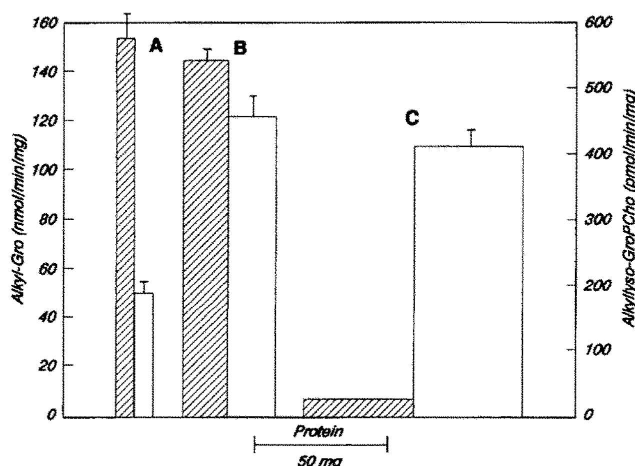

Fig. 4. Subcellular distribution of acetylhydrolase activity toward 1-[^{3}H]alkyl-2-acetyl-Gro (hatched bars) and 1-alkyl-2-[^{3}H]acetyl-GroPCho (open bars). Subcellular fractions were obtained as described in Materials and Methods and represent the 13000 × *g* membrane pellet (A), the 100000 × *g* pellet (B), and the 100000 × *g* supernatant (C). The indicated amounts of protein are equivalent to that present in 1 ml of packed EAC. Data represent the means ± S.E. from two separate experiments done in duplicate ($n = 4$).

TABLE III

Effect of NaF on the hydrolysis of acetate from 1-[^{3}H]hexadecyl-2-acetyl-Gro and 1-hexadecyl-2-[^{3}H]acetyl-GroPCho by a total membrane fraction of EAC

Data represent the average percent of controls (± S.E.) from two separate experiments with duplicate samples ($n = 4$). Controls with 200 μM 1-[^{3}H]hexadecyl-2-acetyl-Gro had a hydrolysis rate of 137 ± 1 nmol/min per mg protein and controls with 10 μM 1-hexadecyl-2-[^{3}H]acetyl-GroPCho had a hydrolysis rate of 406 ± 16 pmol/min per mg protein. Incubations were for 5 min at 37 °C.

Substrate	% of control	
	+5 mM NaF	+10 mM NaF
1-[^{3}H]Hexadecyl-2-acetyl-Gro	39.2 ± 3.4	20.2 ± 1.2
1-Hexadecyl-2-[^{3}H]acetyl-GroPCho	110 ± 8	111 ± 7

the higher enzymatic activity found in the absence of NaF was caused by dephosphorylation of the enzyme to produce an active form, because experiments in which the samples were preincubated without NaF for 5 min at 37 °C (before the addition of the substrate and 10 mM NaF), produced essentially the same extent of inhibition (24% of controls) as shown in Table III. Hydrolysis of acetate from *p*-nitrophenyl acetate was inhibited to 28.4 ± 1.0% ($n = 4$) of control values by 5 mM NaF. As shown in Table III, NaF did not inhibit the PAF acetylhydrolase activity associated with the EAC membrane fraction.

Discussion

The membrane fractions from EAC, cells that can be stimulated by oleic acid to synthesize platelet activating factor from exogenously added 1-alkyl-2-acetyl-Gro [6], were found to contain a membrane-associated enzyme activity that readily hydrolyzes the acetate group from 1-alkyl-2-acetyl-Gro (the PAF precursor in the de novo pathway). Based on differences in both the subcellular distribution (Fig. 4) and the response to inhibition by NaF (Table III), hydrolysis of acetate from 1-alkyl-2-acetyl-Gro and from PAF obviously does not occur by the same enzyme. Although the enzyme activities responsible for hydrolysis of acetate from 1-[^{3}H]hexadecyl-2-acetyl-Gro and *p*-nitrophenyl acetate demonstrated some qualitative similarities in their response to inhibitors, we found quantitative differences in their response to both *p*-bromophenacyl bromide and RHC 80267 (Table II). These quantitative differences in inhibitor response, together with the greater thermal stability of the activity that hydrolyzes 1-[^{3}H]hexadecyl-2-acetyl-Gro compared to the more heat labile nonspecific esterase (Fig. 3), and the fact that *p*-nitrophenyl acetate failed to inhibit the hydrolysis of 1-[^{3}H]hexadecyl-2-acetyl-Gro, suggest that an enzyme other than a nonspecific esterase is involved in the hydrolysis of 1-alkyl-

2-acetyl-Gro by the EAC membranes. Also, based on the lack of inhibition by *p*-chloromercuribenzoate and *n*-ethylmaleimide (Table II), the neutral lipid acetylhydrolase does not appear to be similar to the enzyme activity that hydrolyzes long-chain acyl group from diacylglycerols (diglyceride lipase) or monoacylglycerols (monoglyceride lipase).

Although the enzyme activity in the EAC membrane fraction has a rather high apparent V_{max} (179 nmol/min per mg protein), the apparent K_m for 1-alkyl-2-acetyl-Gro (45 μM) is much higher than the concentration of 1-alkyl-2-acetyl-Gro expected to be generated via the rates in the de novo synthesis of PAF [8]. Therefore, the exact extent that this neutral lipid acetylhydrolase contributes to controlling levels of PAF synthesized by the de novo pathway is difficult to assess. However, significant amounts of alkylglycerols ($\approx 10\%$ of total products) were formed in studies of the 1-alkyl-2-acetyl-*sn*-glycero-3-phosphate phosphohydrolase activity present in rat spleen microsomes [9]. Although alternative biological functions for this enzyme can be envisioned, enzymatic hydrolysis of the acetate group from 1-alkyl-2-acetyl-Gro, the immediate precursor of PAF in the de novo pathway [3,4,9], would appear to provide another important regulatory step in this biosynthetic route. The neutral lipid acetylhydrolase described in this report very likely contributes to the high levels of alkylglycerols found in intact cells when they are incubated with exogenous 1-alkyl-2-acetyl-Gro [5–7].

Acknowledgements

This work was supported by the Office of Energy Research, U.S. Department of Energy (Contract No. DE-AC05-76OR00033), the American Cancer Society (Grant BC-70S) and the National Heart, Lung and Blood Institute (Grant HL-21709-08). The authors express their appreciation to Dr. T-c. Lee for her helpful suggestions during the course of this work.

References

1 Satouchi, K., Oda, M., Saito, K. and Hanahan, D.J. (1984) Arch. Biochem. Biophys. 234, 318–321.
2 Blank, M.L., Cress, E.A. and Snyder, F. (1984) Biochem. Biophys. Res. Commun. 118, 344–350.
3 Renooij, W. and Snyder, F. (1981) Biochim. Biophys. Acta 663, 545–556.
4 Woodard, D.S., Lee, T-c. and Snyder, F. (1987) J. Biol. Chem. 262, 2520–2527.
5 Blank, M.L., Lee, T-c., Cress, E.A., Malone, B., Fitzgerald, V. and Sndyer, F. (1984) Biochem. Biophys. Res. Commun. 124, 156–163.
6 Blank, M.L., Lee, Y.J., Cress, E.A. and Snyder, F. (1988) J. Biol. Chem. 263, 5656–5661.
7 Blank, M.L., Spector, A.A., Kaduce, T.L., Lee, T-c. and Snyder, F. (1986) Biochim. Biophys. Acta 876, 373–378.
8 Lee, T-c., Malone, B. and Snyder, F. (1986) J. Biol. Chem. 261, 5373–5377.
9 Lee, T-c., Malone, B. and Snyder, F. (1988) J. Biol. Chem. 263, 1755–1760.
10 Snyder, F., Lee, T-c. and Wykle, R.L. (1985) in Enzymes of Biological Membranes (Martonosi, A.N., ed.), Vol. 2, pp. 1–58, Plenum, New York.
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
12 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
13 Thomas, III, A.E., Scharoun, J.E. and Ralston, H. (1965) J. Am. Oil Chem. Soc. 42, 789–792.
14 Blank, M.L., Cress, E.A., Lee, T-c., Stephens, N., Piantadosi, C. and Snyder, F. (1983) Anal. Biochem. 133, 430–436.
15 Blank, M.L., Lee, T-c., Fitzgerald, V. and Snyder, F. (1981) J. Biol. Chem. 256, 175–178.
16 Huggins, C. and Lapides, J. (1947) J. Biol. Chem. 170, 467–482.
17 Majerus, P.W. and Prescott, S.M. (1982) Methods Enzymol. 86, 11–17.
18 Prescott, S.M and Majerus, P.W. (1983) J. Biol. Chem. 258, 764–769.
19 Sutherland, C.A. and Amin, D. (1982) J. Biol. Chem. 257, 14006–14010.