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A PAF-acetylhydrolase activity in *Tetrahymena pyriformis* cells

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Our study provides evidence for the existence of an acylhydrolase activity in *Tetrahymena pyriformis* cells, capable of hydrolizing the *sn-2* ester bond of the PAF molecule. This activity is mainly distributed in the microsomal fraction (76.5% of total) and has properties similar to the mammalian PAF-acetylhydrolase since it is Ca^{2+}-independent, acid-labile, is inhibited by DFP and PMSF but it is not affected by egg yolk phosphatidylcholine. This microsomal acylhydrolase has apparent K_m and V_{max} values of 1.56 μM and 373 pmols · mg · min respectively. This is the first report of the existence of a PAF-acetylhydrolase activity in a non-mammalian cell.

Platelet-activating factor; PAF; Phospholipase A_2; PAF-acetylhydrolase; Lipid metabolism; (*Tetrahymena pyriformis*)

1. INTRODUCTION

Platelet Activating Factor (PAF) identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [1–3], is one of the most potent mediators in inflammatory and allergic reactions. PAF is synthesized and released by a variety of cells and tissues and displays an impressive spectrum of biological actions in vivo and in vitro (see reviews [4,5]).

The intracellular or extracellular PAF levels are mainly regulated by a specific hydrolase which degrades PAF into the biologically inactive metabolite lyso-PAF, removing the acetyl group from the *sn-2* position. This enzyme named as PAF-acetylhydrolase (PAF-AH) (EC 3.1.1.48), is an acid-labile enzyme with properties distinct from the classic phospholipase A_2 (PLA_2) and is present in a variety of mammalian cells and tissues as well as in plasma and serum [6–11].

Studies in our laboratory have shown that PAF is a minor lipid component in *Tetrahymena pyriformis* cells, this being the first report for the natural occurence of PAF in a non-mammalian cell [12]. Moreover, exogenously added PAF is taken up rapidly by *T. pyriformis* and stimulates Ca^{2+} influx into the cells [13,14]. Recently we have shown that the intact cells of this protozoan can rapidly metabolize exogenous PAF to 1-*O*-alkyl-2-acyl(long chain)-*sn*-glycero-3-phosphocholine, suggesting a PAF deacetylation reaction as the intermediate step in this metabolic route [15]. In the present study we describe the existence of an acylhydrolase activity in *T. pyriformis* capable of hydrolyzing the *sn-2* acetyl moiety of PAF. The properties of this enzyme, similar to those of mammalian PAF-AH, are also discussed.

Abbreviations: PAF, platelet-activating factor; PAF-AH, PAF-acetylhydrolase; PLA_2, phospholipase A_2; [^{3}H]PAF, 1-*O*-hexadecyl-2-[^{3}H]acetyl-*sn*-glycero-3-phosphocholine; [^{3}H]alkyl-PAF, 1-*O*-[1′,2′-^{3}H]hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; BSA, bovine serum albumin; TCA, trichloroacetic acid; DFP, diisopropylfluorophosphate; PMSF, phenylmethylsulfonylfluoride.

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2. EXPERIMENTAL

2.1. *Materials*

Diisopropylfluorophosphate (DFP), phenylmethylsulfonylfluoride (PMSF), essentially fatty acid-free bovine serum albumin (BSA), Tris-HCl, standard lipids and other chemicals (analytical grade), were purchased from Sigma (St. Louis, USA). 1-*O*-hexadecyl 2-[^{3}H]acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]PAF), (10 Ci/mmol) and 1-*O*-[1′,2′-^{3}H]hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]alkyl-PAF), (59.5 Ci/mmol) were from New England Nuclear (Boston, MA), and solvents (analytical grade) were from Fluka (Switzerland). [^{3}H]PAF or [^{3}H]alkyl-PAF was dissolved in a solution of BSA in 10 mM Tris-HCl, 1 mg/ml, pH 7.4 (BSA/Tris-HCl). Egg phosphatidylcholine was prepared from egg yolk after total lipid extraction by the Bligh and Dyer method [16] followed by TLC with chloroform/methanol/water (65:35:6, by volume) as a solvent system. Lipid phosphorous was determined using the method of Bartlett [17], as modified by Marinetti [18].

2.2. *Culture conditions and cell fractionation*

T. pyriformis strain W was grown axenically at 25°C in a culture medium consisting of 2% (w/v) proteose-peptone, 0.5% (w/v) D(+)glucose and 0.2% (w/v) yeast extract. Cells were fractionated into pellicles, mitochondria, microsomes and cytosol at 4°C essentially according to the procedure of Nozawa and Thompson [19] with the following exceptions. The fractionation buffer consisted of 0.25 M sucrose and 10 mM Tris-HCl, pH 7.4, and cells were homogenized by nitrogen cavitation using a pressure of 40 bar after 1 min equilibration. Light microscopic observation indicated that almost all cells were disrupted by this procedure. Total protein concentration in each fraction was determined by the method of Lowry et al. [20]. All fractions were stored in small aliquots at −20°C. PAF-AH activity was stable for at least 1 month under these conditions.

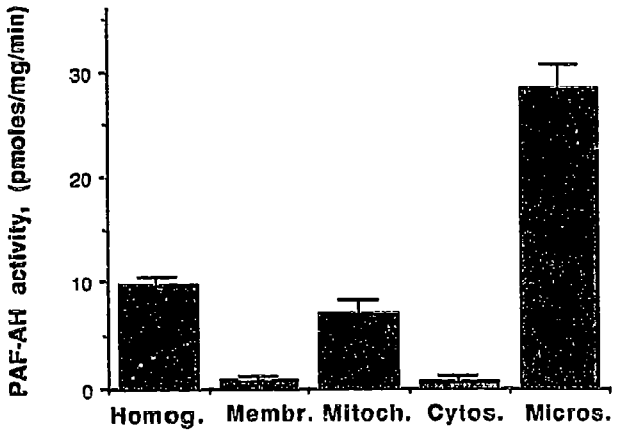

Fig. 1. PAF-AH activity in homogenate and various cell fractions of *T. pyriformis*. Incubations were performed with 125 μg of cell fraction protein and 72 nM [^{3}H]PAF at 37°C, pH 7.4. Values represent the mean ± SD for 4 different cell preparations.

2.3. *Enzyme assay*

PAF-AH activity was determined by the trichloroacetic acid (TCA) precipitation method [21,22], using [^{3}H]PAF as a substrate and the various cell fractions suspended in 10 mM Tris-HCl, pH 7.4, as the source of the enzyme. For the routine assay, 0.5 ml of [^{3}H]PAF solution in BSA/Tris-HCl, was incubated in a polypropylene tube with an equal volume of each cell fraction suspension, at 37°C. The final BSA and [^{3}H]PAF concentrations in the reaction mixture were 0.5 mg/ml and 72 nM respectively while the final cell fraction protein concentration was 125 μg/ml, unless otherwise indicated. Aliquots of 50 μl of the reaction mixture were taken at 1 min time intervals up to 10 min and mixed with 50 μl of 20% TCA solution in microcentrifuge tubes at 4°C. The mixture was allowed to stand for 15 min at 4°C and the denatured protein was separated by centrifugation for 2 min in an Eppendorf microcentrifuge. 50 μl of the supernatant were mixed with 5 ml of the scintillation fluid (Ready Protein cocktail, Beckman, Fullerton, CA) and the radioactivity determined in a liquid scintillation counter (Tri-Carb 3255, Packard). The [^{3}H] released in the supernatant was identified as [^{3}H]acetic acid by the method of Blank et al. [6]. Moreover, when [^{3}H]alkyl-PAF was used as a substrate, the [^{3}H] found in the supernatant after incubation for 10 min with the microsomal fraction, was less than 0.2% of total added [^{3}H]alkyl-PAF. The control values of the released [^{3}H]acetic acid were obtained by mixing 25 μl from the [^{3}H]PAF solution and 25 μl from the cell fraction suspension with 50 μl of 20% TCA in a microcentrifuge tube at 4°C. The [^{3}H] found in the supernatant after the centrifugation (tritiated acetate) was less than 2.5% of total added [^{3}H]PAF. The detection limit of this method is 0.3 pmol·mg·min.

3. RESULTS

PAF-AH activity was determined in all cell fractions and cell homogenate. As shown in Fig. 1, PAF-AH activity is mainly distributed in the microsomal fraction (enzymatic activity, 28.3 ± 2.3 pmols·mg·min, 76.5% of total). Much lower activity is present in mitochondria (7.1 ± 1.2 pmols·mg·min, 19.2% of total) while the pellicle and cytosolic fractions are almost inactive (0.9 ± 0.3 pmols·mg·min and 0.7 ± 0.4 pmols·mg·min respectively). PAF-AH activity in all fractions was not markedly altered in the presence of 10 mM $CaCl_2$ or 10 mM K_2EDTA but was completely and irreversibly inhibited after incubation of microsomes with 2 mM PMSF for 1 h at 37°C (Table I). Further studies with this enzyme were performed using the microsomal fraction. PAF-AH activity in this fraction was linear with protein concentration up to 250 μg/ml (range of protein concentration tested, 50–1000 μg/ml) and with the time of incubation up to at least 10 min. The activity profile of the enzyme as a function of pH, showed a broad pH optimum ranging between 7.4 and 8 (Fig. 2). PAF-AH activity was slightly decreased (about 20%) when the temperature of incubation was 25°C, conditions under which *T. pyriformis* is normally grown, but was completely and irreversibly inhibited after incubation of microsomes with 10 mM DFP for 30 min at 37°C (Table I). Treatment of microsomes with 1 N HCl, pH 3.5 for 1 h at 37°C, followed by pH adjustment to 7.4 with 1 N NaOH, resulted also in a complete loss of the enzyme

Table I

Effect of various compounds on *T. pyriformis* microsomal PAF-AH activity

Compound added	Concentration	% of control [^{3}H]acetate released
None, 37°C	-	100[b]
None, 25°C	-	79 ± 5
Ca^{2+}	10 mM	68 ± 9[c]
EDTA	10 mM	113 ± 2[c]
DFP	10 mM	0
PMSF	2 mM	0[c]
1 N HCl	-	0
Ethanol	5 μl in 1 ml react. mixt.	92 ± 2
Egg phosphatidylcholine[a]	64 nM	95 ± 4
"	640 nM	83 ± 1

Incubations were performed with 125 μg of microsomal protein and 72 nM [^{3}H]PAF at pH 7.4.
[a]5 μl of the egg phosphatidylcholine solutions in ethanol were added to 1 ml of the reaction mixture.
[b]Control activity in these experiments was 26.5 pmols·mg·min.
[c]Similar results were obtained when other cell fractions or cell homogenate were used as the source of the enzyme.
Values represent the mean ± SD for 4 different microsomal preparations.

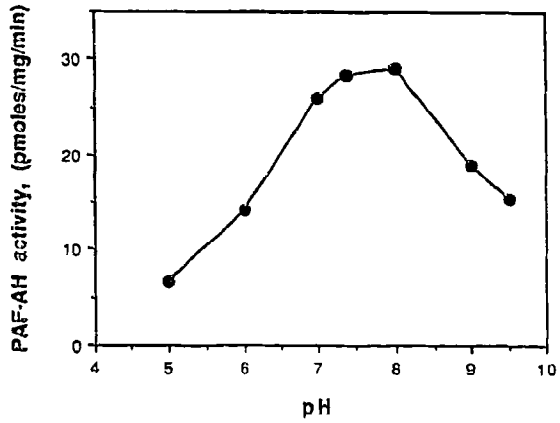

Fig. 2. pH-activity profile of *T. pyriformis* microsomal PAF-AH. Incubations were performed with 125 μg of microsomal protein and 72 nM [^{3}H]PAF at 37°C, pH 7.4. Values represent the mean ± SD for 4 different microsomal preparations.

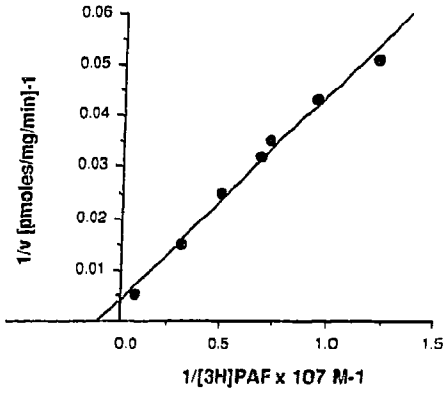

Fig. 3. Lineweaver-Burke double-reciprocal plot of *T. pyriformis* microsomal PAF-AH. Each point represents the mean value of duplicate experiments.

activity (Table I). Finally, this microsomal PAF-AH activity was not affected or was slightly inhibited when 5 μl of egg yolk phosphatidylcholine solution in ethanol were added to the reaction mixture in final concentration of 64 nM or 640 nM respectively (Table I). Kinetic data depicted in the Lineweaver–Burke double reciprocal plot showed that this microsomal hydrolase has apparent K_m and V_{max} values of 1.56 μM and 373 pmols·mg·min respectively (Fig. 3).

4. DISCUSSION

T. pyriformis is considered to be a favorable organism for studying membrane structure, function and metabolism within the eukariotic cell [23]. The microsomal fraction of this protozoan plays an important role in the phospholipid metabolism and a series of different enzymatic activities has been reported in the subcellular fraction. Some of these are: an alkylglycerol monooxygenase that catalyzes the cleavage of the ether bond in alkylglycerols [24,25]; a direct phospholipid desaturation system that catalyzes the conversion of oleoylphosphatidylcholine to linoleoylphosphatidylcholine [26]; and various acyltransferase activities that catalyze the acylation of glycerophosphate, monoacylglycerophosphate and 1-acylglycerophosphocholine [27].

Our study provides evidence for the existence in *T. pyriformis* cells of an acylhydrolase activity capable of hydrolizing the *sn-2* acetyl moiety of the PAF molecule. It is mainly distributed in microsomes and has properties similar to those of mammalian PAF-AH, an enzyme totally different to the typical PLA_2 [6–11]. This acylhydrolase activity is acid-labile, Ca^{2+}-independent and is completely inhibited by DFP and PMSF. Also it seems to be specific for phosphatidates having *sn-2* short chain acyl groups since egg yolk phosphatidylcholine (long chain diacyl type) fails to inhibit PAF hydrolysis. This inhibitory study further supports the premise that this acylhydrolase activity differs from the typical PLA_2 that utilizes long chain diacylphospholipids as substrates. This observation is in accordance with the results of other investigators who reported the absence of any PLA_2 activity capable of hydrolizing phosphatidylcholine or phosphatidylethanolamine (long chain diacyl types) in the microsomal fraction of *T. pyriformis* [28].

The distribution of *T. pyriformis* PAF-AH activity, mainly in microsomes, is in contrast to the distribution of PAF-AH in mammalian cells, which is mainly found in the cytosolic fraction [6]. On the other hand, the profound hydrophobicity of the protozoan PAF-AH resembles that of the plasma PAF-AH which is associated with lipoproteins [11]. Also, the properties of *T. pyriformis* PAF-AH activity seem to be similar to the short chain specific microsomal acylhydrolase reported by Wykle and Schremmer in Fischer R-3259 sarcoma cells [29].

The existence of a PAF-AH activity in the protozoan cells further supports our previous observation that the first step of the metabolism of exogenous PAF by *T. pyriformis* is a rapid deacetylation reaction. Moreover, the enzyme distribution in the internal cell compartments and the absence from the pellicle fraction, could explain the small unmetabolized portion of the exogenous PAF that remains even after 1 h incubation with *T. pyriformis* cells [15].

This is the first report of the existence of a PAF-AH activity in a non-mammalian cell. It is also the first study that provides evidence for the existence of a short chain specific acylhydrolase in *T. pyriformis* cells. The biological role of this acylhydrolase could be the regulation of the endogenous PAF levels in this protozoan. The enzyme purification as well as the substrate specificity are in further investigation in our laboratory.

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