1-*O*-Alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (PAF) is a minor lipid component in *Tetrahymena pyriformis* cells

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The protozoan *Tetrahymena pyriformis* contains 4.2 ± 2.2 ng PAF/10^7 cells. Only 1–3% of this lipid is released in the cell free medium. PAF production is not influenced by different extracellular Ca^{2+} concentrations. Cell stimulation with calcium ionophore A23187 or zymosan particles does not affect the amount of PAF either. This is the first report of a natural occurrence of PAF in a protozoan.

Platelet-activating factor (Tetrahymena pyriformis) *Lipid metabolism* *Ether lipid* Ca^{2+} *Phospholipid*

1. INTRODUCTION

Platelet-activating factor, the potent mediator in inflammatory and allergic reactions, is known to be released from a variety of tissues and cell types under stimulation [1–6]. Its presence in physiological fluids without immunologic challenge has also been reported although its cellular origin is not well documented [7–9].

1-*O*-Alkyl-2-acyl-*sn*-glyceryl-3-phosphorylcholine, the effective precursor of PAF [10–12], is a major component of *Tetrahymena pyriformis* lipids. Therefore we investigated the possibility of PAF production by the protozoan under various incubation conditions or stimulation.

2. EXPERIMENTAL

2.1. *Materials*

Buffer A, pH 7: 50 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM D(+)-glucose, 0.1 mM KH_2PO_4, 0.4 mM K_2HPO_4 and 0.2 mM EGTA. Buffer B, pH 7.2: same as buffer A without EGTA but containing 1.7 mM $CaCl_2$ and 0.5 mM $NaHCO_3$. Buffer C, pH 7.2: same as buffer B, containing 0.9 mM $CaCl_2$.

Aggregation buffers: tyrode-gelatine-EGTA (TG-EGTA) buffer solution, pH 6.5, was used for washing the rabbit platelets and tyrode-gelatine-Ca^{2+} (TG-Ca^{2+}) buffer solution, pH 7.4, was used for aggregation buffer [2].

Platelet aggregation was measured on a Chronolog Co. aggregometer [2].

2.2. *Incubation procedures*

T. pyriformis strain W cells were harvested at the late log-phase by centrifugation, washed 3 times with the buffer A, fractionated into 3 parts and suspended, under agitation at 22°C, in the buffer solutions A, B and C, respectively. Cell density counted in a hemocytometer was found to be 3×10^6 cells/ml.

The cells suspended in buffer B were stimulated with an ionophore A23187 solution in DMSO (final concentration 5.2 μM) or with zymosan particles (final concentration 1 mg/ml).

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Abbreviations: PAF, platelet-activating factor; PLA_2, phospholipase A_2; BSA, bovine serum albumin; CP/CPK, creatine phosphate/creatine phosphokinase

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2.3. *Lipid isolation and purification*

At indicated time intervals, the lipids of 2.5 ml cell suspension were extracted according to Bligh and Dyer [13]. The cell free supernatant was also extracted and examined for PAF activity. The lipid samples were subjected to preparative TLC using chloroform/methanol/water (65:35:6) as a solvent system. The region between the standard lipids sphingomyelin and lyso-phosphatidylcholine was scraped and the lipid residue was extracted [14]. After total elimination of the solvents, the lipid residue was dissolved in BSA/saline, 2.5 mg/ml, and tested for biological activity on washed rabbit platelets. The biologically active lipid was further purified by HPLC using a Micropak Si-10 column, and [^{3}H]PAF as a standard [15].

2.4. *PAF determination*

The biologically active phospholipid was identified as PAF through its chromatographic behaviour on TLC and HPLC, its physicochemical properties with respect to Pinckard tests [16] and its behaviour under treatment with PLA_2 and lipase from *Rhizopus arrhizus* [17]. It was also characterized and quantitatively determined by its ability to aggregate aspirin-treated washed rabbit platelets, in the presence of CP/CPK [2]. The aggregation curves were compared to those of standard solutions of beef-heart derived PAF.

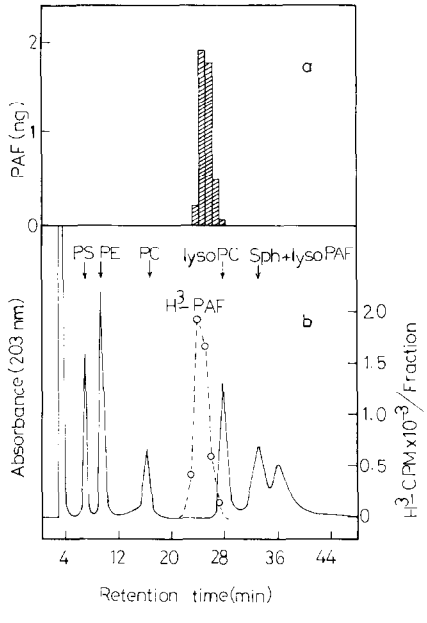

Fig.1. (a) Purification of *T. pyriformis* derived PAF by silica HPLC using acetonitrile/methanol/75% phosphoric acid (130:5:1.5). (b) Separation of phospholipid standards and ^{3}H-labeled PAF by silica HPLC, with the same solvent system as above.

3. RESULTS

T. pyriformis suspended in buffer B produced without any stimulation 4.2 ± 2.2 ng/10^7 cells of a biologically active phospholipid. This production was time independent and only 1–3% of this compound was determined in the cell free medium

Table 1

PAF determination in *T. pyriformis* cell suspension in buffer B as well as in the cell free medium, without stimulation

Time (min)	1	10	30	60
PAF (ng/10^7 cells)	4.2 ± 2.2	3.8 ± 1.8	5.6 ± 2.5	4.1 ± 1.5
PAF in the medium (%)	2.1	1.5	2.8	1.3

Values represent the mean ± SD, n = 8

Table 2

PAF determination in *T. pyriformis* cell suspension in buffers A, B and C without stimulation and in buffer B after treatment with Ca^{2+} ionophore A23187 and zymosan particles

Solutions	Amount of PAF, ng/10^7 cells (60 min incubation)
Buffer A	3.0 ± 1.8
Buffer B	4.1 ± 1.5
Buffer C	3.8 ± 1.0
A23187 in buffer B, 5.2 μM	3.7 ± 1.2
Zymosan particles in buffer B, 1 mg/ml	3.6 ± 1.9

Values represent the mean ± SD, n = 8. PAF was also determined at time intervals of 1, 10 and 30 min in the above conditions. No significant value fluctuations were observed. DMSO was used as control for the PAF determination after treatment with ionophore A23187. It did not affect the PAF content in the cells

(table 1). The phospholipid migrated on TLC at an R_f 0.35, and on HPLC it was eluted at 24 min, identically to [^{3}H]PAF (fig.1a,b).

The aggregation activity on washed rabbit platelets was maintained after treatment with lipase, but it was completely lost after incubation with PLA_2. The phospholipid exhibited the same physicochemical behaviour as beef-heart derived PAF with respect to the Pinckard tests [16].

No significant modification of the phospholipid content in the cells was observed after treatment with calcium ionophore A23187 or zymosan particles. The absence as well as the presence of different extracellular Ca^{2+} concentrations do not affect the phospholipid amount in the cell. The PAF value appears also to be time independent (table 2).

4. DISCUSSION

T. pyriformis contains a variety of biologically active compounds such as serotonin, porphyrins and catecholamines [18], compounds which occur and play an important role in higher organisms. The protozoan is considered to be a good model for studies of membrane behaviour and of the biochemistry of its unusual lipids [19].

Our results show that the isolated biologically active phospholipid exhibits identical properties to beef-heart derived PAF. This is the first report which concludes that PAF is a natural minor component in the protozoan lipids. The fact that PAF biosynthesis is not influenced by Ca^{2+} ionophore A23187 or different extracellular Ca^{2+} concentrations, strongly suggests that it is not affected by Ca^{2+}-dependent PLA_2. PAF production is not influenced by zymosan particles either.

The pathway for PAF formation in mammalian cells studied most, involves Ca^{2+}-dependent PLA_2 and acetyltransferase. 1-*O*-Alkyl-2-acyl-*sn*-glyceryl-3-phosphorylcholine is used as substrate [20–24]. Another pathway involving phosphocholinetransferase and 1-*O*-alkyl-2-acetyl-*sn*-glycerol as a substrate has been proposed. This pathway requires Mg^{2+} and is inhibited by Ca^{2+} [25,26].

Our results do not clearly demonstrate the way of PAF formation in *T. pyriformis*. Nevertheless this protozoan could be proposed as a model for in vivo studies of PAF metabolism and its physiological role in the cell.

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