Phospholipase A_2 activity in T-lymphocytes

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Phospholipase A_2 activity was shown indirectly in T-lymphocytes from rat thymus and a permanent T-cell line by the liberation of arachidonic acid from phospholipids. In addition, phospholipase A_2 activity was measured directly with two different substrates, phosphatidylcholine and labelled *E. coli*.

Phospholipase A_2 *T-lymphocyte*

1. INTRODUCTION

In cellular membranes the composition of phospholipids is in a dynamic equilibrium. Long-chain fatty acyl moieties of membrane phospholipids are rapidly turned over by a metabolic pathway first described by Lands and Merkl [1]. In this pathway a fatty acid is cleaved from the glycerol backbone to generate lysophospholipids, which are then reacylated by the enzyme lysophosphatide acyltransferase. Due to the properties of the lysophosphatide acyltransferase polyenoic fatty acids can be introduced into phospholipids [2]. In addition, deacylation-reacylation reactions can alter the composition of phospholipids. The first step in this cycle, the cleavage of the fatty acyl moiety, is thought to be brought about by the activity of a phospholipase A_2, an enzyme that has been shown to be active in many different cell types [3]. T-lymphocytes rapidly incorporate exogenous long-chain fatty acids, including polyunsaturated ones, indicating that the deacylation-reacylation cycle is operative [4]. Accordingly, lysophosphatide acyltransferase has been shown to be present in lymphocytes [5]. Up to now, however, attempts to show phospholipase A_2 failed [6], although a release of fatty acids could be observed, when prelabelled intact cells were investigated [7,8]. These results were explained by another mechanism of fatty acid exchange, a CoA-mediated transfer of fatty acids catalyzed by the back reaction of the lysophosphatide acyltransferase [6,7]. Free fatty acids could then be generated by hydrolysis of the intermediate fatty acyl-CoA derivative. In a recent paper, Etienne and Polonovski [9] could directly demonstrate phospholipase A_2 activity in lymphocytes from human and rat peripheral blood. Their preparations, however, contain mononuclear phagocytes, which contain considerable phospholipase activity. Therefore, we investigated whether phospholipase A_2 activity could also be demonstrated in pure T-lymphocytes.

2. EXPERIMENTAL

2.1. *Cells*

T-lymphocytes from the thymus of Lewis rats were prepared as described [10]. Pure T-lymphocytes were obtained from the permanent interleukin 2-dependent T-cell line G2 from BH rats (kindly provided by Dr K. Wonigeit, Hannover).

2.2. *Release of arachidonic acid from prelabelled cells*

Thymocytes from rats or G2 cells were incubated in Hepes-buffered RPMI 1640 (Seromed),

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cell density 5×10^7 cells/ml, with 1 nmol/10^7 cells arachidonic acid (Nu Chek Prep) and an appropriate amount of radioactive arachidonic acid (0.05 μCi/assay, Amersham). After 1 h the cells were washed with medium containing 0.5% bovine serum albumin (BSA, essentially fatty acid-free, Sigma) to remove remaining free fatty acid. The cells were then resuspended at the same density and further incubated with or without an excess of oleic acid (35 nmol/10^7 cells) in medium containing 0.5% BSA. The incubation was terminated by the addition of methanol and subsequent lipid extraction as in [11]. Lipids were separated by thin-layer chromatography. The amount of radioactivity in the individual lipids was determined by liquid scintillation counting.

2.3. *Preparation of cell homogenates*

The cells were resuspended in phosphate-buffered saline (PBS) and frozen. Subsequently they were homogenized by brief sonication (10 s, 50 W). Protein was determined by the method of Bradford [12].

2.4. *Assay of phospholipase A_2 activity*

Phospholipase A_2 was measured using two different assay systems. (i) Mixed lipid vesicles were prepared according to [13]: 0.5 μmol 1-palmitoyl-2-oleoylphosphatidylcholine (Sigma), 0.14 μmol oleic acid (Sigma), 0.14 μmol 1-palmitoyl-*sn*-glycerophosphatidylcholine and an appropriate amount of (^{14}C) 1-palmitoyl-2-oleoyl-[1-^{14}C]phosphatidylcholine (about 20000 cpm, NEN) were dried under nitrogen, resuspended in 500 μl bidistilled water and sonicated for 1 h in a sonicating bath (50 W, 60°C). The vesicles used as substrate could be kept at room temperature for several days. 150–200 μg cell protein were incubated with 50 μl substrate (final concentration of phosphatidylcholine: 25 nmol/ml) in a total volume of 500 μl buffer (25 mM Tris-HCl, pH 8, 0.5 mM $CaCl_2$) for 3–5 h. The reaction was terminated by the addition of methanol and subsequent lipid extraction [11] and separation of the liberated free fatty acid from unreacted substrate by thin-layer chromatography. (ii) *E. coli* (JM 103) were grown in Mueller Hinton broth (Mast Diagnostica) and labelled with [^{3}H]oleic acid in the logarithmic phase of growth. The cells were autoclaved for 30 min and subsequently washed several times with 0.2 M Tris-HCl, pH 8, with 1% BSA to remove free oleic acid. The amount of *E. coli* used in the assay was determined by the amount of radioactivity needed (about 50000 cpm). The corresponding amount of phospholipid was determined after extraction and quantitative phosphorus determination [11] to be about 15 nmol/ml in the assay. This quantity, however, could not be used for the exact determination of phospholipase activity as the label was not distributed evenly among the different phospholipid species, but acylated predominantly to position 2 of phosphatidylethanolamine ([14] and unpublished). Therefore, activity was expressed as percent cleavage corrected to controls, i.e. incubations without homogenates. (For detailed assay conditions see section 3.) The reaction was terminated by the addition of 500 μl of 10 mM EDTA and 1% BSA to trap the liberated fatty acid [15]. After centrifugation in an Eppendorf centrifuge aliquots of the supernatant were counted to determine the percentage of liberated fatty acid.

3. RESULTS

3.1. *Release of arachidonic acid*

We could show earlier [4] that T-lymphocytes from mice, prelabelled with arachidonic acid, released arachidonic acid into an incubation medium supplemented with defatted albumin. However, the source of the liberated arachidonic acid could not be determined definitely, as radioactivity was lost from phospholipids as well as from triacylglycerols. In rat thymocytes the triacylglycerol pool was only marginally affected. As can be seen from fig.1a the increase of arachidonic acid in the medium corresponded to a decrease of label in the cellular phospholipid pool. Addition of exogenous oleic acid further increased the amount of liberated fatty acid: 8.8 ± 1.3% arachidonic acid released compared to 5.7 ± 0.9% in control cells after 120 min (n = 3 independent experiments). These results were most easily interpreted as an enhanced turnover of the deacylation-reacylation cycle. To exclude the possibility, though unlikely, that the effects observed were due to a small contamination of macrophages, the experiments were repeated with the rat cell line G2. In these pure T-cells fatty acids were also released. As can be seen from fig.1b, there was also some

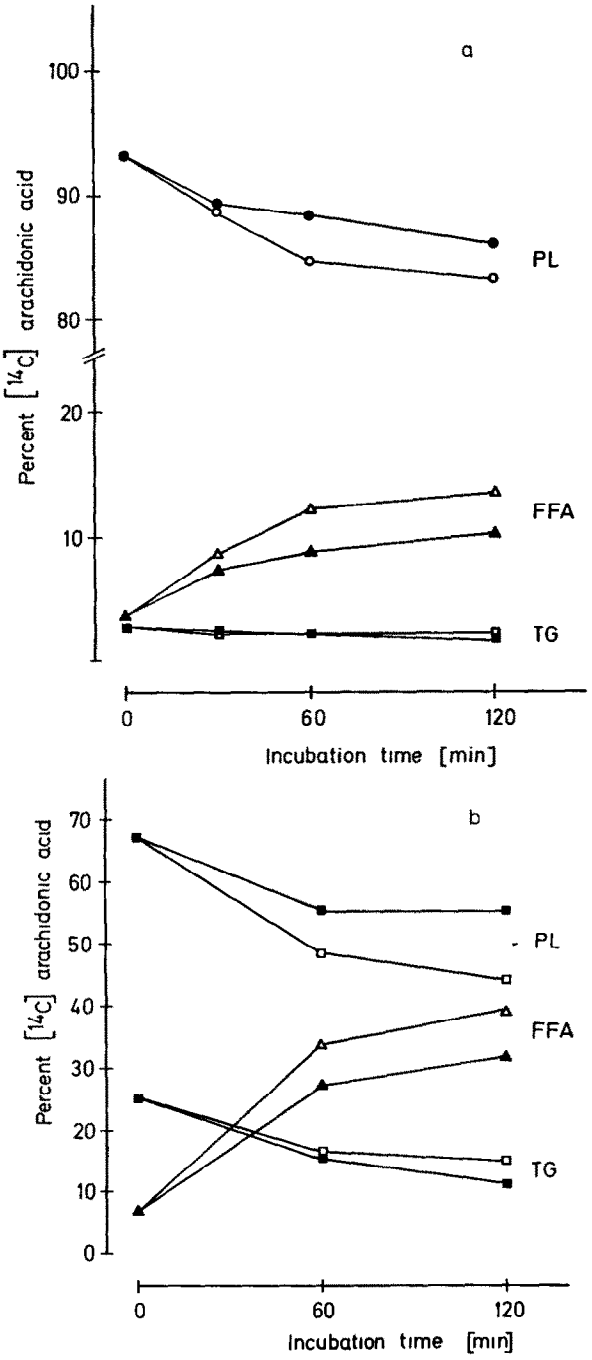

Fig.1. Liberation of arachidonic acid from prelabelled T-lymphocytes. Thymocytes from rats (a) or G2 T-cells (b) were prelabelled with [^{14}C]arachidonic acid. After washing they were further incubated in the presence (open symbols) or absence (closed symbols) of exogenous oleic acid. Data shown are from typical experiments with duplicate incubations; differences were less than the symbols.

release of fatty acids from the triacylglycerol pool, phospholipids, however, were the main source of arachidonic acid.

3.2. *Characterization of phospholipase A_2 activity in T-lymphocytes*

Most of the measurements were performed with labelled *E. coli* as substrate. Phospholipid vesicles were used to prove the specific cleavage of a fatty acid from position 2 of a defined phosphatidylcholine. Phospholipase A_2 activity was linear over several hours, the specific activity being 0.8 ± 0.1 nmol oleic acid/mg protein per h (two independent preparations with multiple determinations). The enzyme showed some similarities to the one described in the lymphocyte preparation from peripheral blood [9]: the pH optimum was at pH 7.5 (fig.2); the enzymatic activity was suppressed at Ca^{2+} concentrations higher than 5 mM and by detergents such as Triton X-100, cholate and deoxycholate. There was a marked increase in enzymatic activity when Hepes was used as buffer compared to Tris-HCl with the same molarity and pH. Addition of SDS to the cells prior to cell

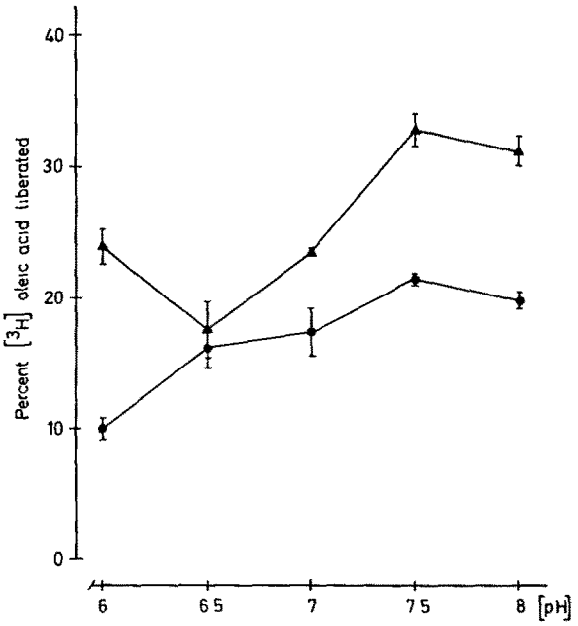

Fig.2. pH dependence of phospholipase A_2 activity. Phospholipase A_2 activity was determined with labelled *E. coli* as substrate. Assay conditions: 1 mM $CaCl_2$, 50 mM Hepes, 0.1 mg/ml protein, 60 min, 37°C. Data are means of two preparations of thymocytes (●) and G2 T-cells (▲).

disruption by sonication did not increase the phospholipase A_2 activity as described for blood lymphocytes.

Similar characteristics were found for the phospholipase A_2 activity of the G2 line. As an example the pH dependence is shown in fig.3.

4. DISCUSSION

We could show by direct and indirect methods that T-lymphocytes from rat thymus contain phospholipase A_2 activity at neutral, i.e. physiological pH. The results obtained with thymocytes could be confirmed by experiments with an interleukin 2-dependent T-cell line from rat, which also demonstrated phospholipase A_2 activity.

Using similar methods, we could not detect respective activities in other species, i.e. calf and mouse. In homogenates prepared from mouse thymus (DBA/2), calf thymus and an interleukin 2-dependent mouse T-cell helper clone no phospholipase activity was detectable at neutral pH under varying assay conditions (ionic strength, Ca^{2+} concentration, addition of SDS). In calf thymocytes we could detect phospholipase activity only at an acid pH. Several reasons may account for these differences among preparations from different species. (i) Optimal assay conditions vary widely for different phospholipases, especially with regard to detergent requirements. Eukaryotic phospholipases are often membrane bound and thus embedded in a lipid surrounding that ensures easy substrate access. In vitro, neither the enzyme nor the substrate is water-soluble and, if no activity is detectable, this may just be a problem of accessibility. Accessibility of the enzyme could be improved by SDS at low concentrations in the case of peripheral blood lymphocytes [9], but was without effect in our system. (ii) Endogenous inhibitors of phospholipase activity were recently described. Ballou and Cheung [16] demonstrated that unsaturated fatty acids were potent inhibitors of human platelet phospholipase A_2. Hydroxyeicosapentanoic acid, an arachidonic acid derivative, could be shown to inhibit phospholipase A_2 activity when added exogenously [17] and might be an endogenous inhibitor as well. These or other not yet detected inhibitors might be also active in lymphocytes.

In different species, different mechanisms of long-chain fatty acid metabolism may be predominantly operative. Our results with a T-cell line show unambiguously that T-lymphocytes, at least from rats, contain phospholipase A_2.

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