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Characterization of phospholipase A_2 in monocytic cell lines

Functional and biochemical aspects of membrane association

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Phospholipase A_2 activity was characterized in the human monocytic tumour-cell lines U937 and THP1. The enzyme showed an alkaline pH optimum and substrate specificity for arachidonoyl-phosphatidylcholine. The activation of phospholipase A_2 required bivalent cations ($Ca^{2+} > Mg^{2+} = Sr^{2+} > Ba^{2+}$). Investigation of the subcellular distribution of the enzyme revealed that the phospholipase A_2 activity was shifted to the cytosol in the presence of EDTA, indicating that the association of the enzyme with the cellular membranes is Ca^{2+} (bivalent-cation)-dependent. Stimulation of THP1 cells for 2–4 h with the phorbol ester phorbol 12-myristate 13-acetate (PMA) activated cytosolic and membrane-bound phospholipase A_2. At this time, no effect of PMA on phospholipase A_2 activity was observed in the less mature U937 cells. However, when both cell lines were induced to differentiate along the monocytic pathway by a 2–3-day treatment with PMA, the cells released significant amounts of arachidonic acid and prostanoids. Compared with undifferentiated control cells, these PMA-differentiated cells showed a decrease in cytosolic phospholipase A_2 activity and an increase in membrane-bound activity. Membrane-bound and cytosolic enzyme showed the same pH optimum, Ca^{2+}-dependency and substrate specificity. These data indicate that membrane-bound and cytosolic phospholipase A_2 activities represent one enzyme and that the membrane-bound form is the biologically active phospholipase A_2.

INTRODUCTION

Monocytes and macrophages are involved in many physiological and pathophysiological situations, owing to their capacity to interact with other cells and tissues by means of a large variety of products [1]. A major group of mediators involved in inflammatory and immunological reactions are arachidonic acid metabolites such as leukotrienes and prostanoids [2]. The synthesis of these compounds is regulated by the availability of arachidonic acid, as well as the enzymes of the cyclo-oxygenase and lipoxygenase pathways respectively. It is thus of major interest to characterize the enzyme responsible for the release of arachidonic acid from membrane phospholipids, phospholipase A_2, in these cells. The enzyme has been purified from different mouse macrophage cell lines, P388d1 [3] and RAW264.7 [4], and mouse peritoneal macrophages [5]. However, the results are contradictory with respect to the Ca^{2+}-dependency, pH optimum and molecular mass. Very recently, different molecular masses have also been reported for the enzyme purified from the monocytic cell line U937 [6,7]. The different molecular masses have not yet been confirmed by immunological methods, nor have the enzymes been cloned.

Very little is known about the phospholipase A_2 activity in human monocytes. Although the arachidonic acid metabolism in these cells has been characterized with respect to various stimuli (e.g. [8–10]), phospholipase A_2 activity has only been assessed by the release of labelled arachidonic acid from whole cells [11]. A more detailed characterization of the phospholipase A_2 from monocytes is complicated by donor variability and adhering platelets, which contain high phospholipase A_2 activity. Therefore, we used the monocytic cell lines U937 and THP1 as model systems [12,13]. Both cell lines represent monocytic precursor cells which can be induced to differentiate along the monocyte pathway by various physiological and non-physiological stimuli, including vitamin D_3 and the phorbol ester phorbol 12-myristate 13-acetate (PMA) respectively. In the present study PMA was used, since differentiation of the monocytic phenotype with the phorbol ester is well characterized ([14,15] and citations therein). Moreover, PMA is the only agent known so far to induce differentiation of the whole cell population, whereas other differentiation-inducing compounds only affect certain subpopulations.

Previous studies showed that PMA-induced differentiation of U937 cells is associated with increased phospholipase A_2 activity, as indirectly evaluated by arachidonic acid release [16] and also as membrane-bound activity with exogenous substrate [17]. Furthermore, phospholipase A_2 activity has been reported in the cytosol of these cells, although the relationship between the cytosolic and membrane-bound activity remained unclear. A Ca^{2+}-dependent shift between cytosol and membranes had been described in platelets [18] and mesangial cells [19] and in the mouse macrophage line RAW264.7 [20]. Since the present manuscript was submitted, Ca^{2+}-dependent translocation of phospholipase A_2 has been described by Diez & Mong in U937 cells [6].

It was thus the aim of the present study to characterize further the relationship of membrane-bound and cytosolic phospholipase A_2 activity in undifferentiated and PMA-differentiated U937 and THP1 cells. The present data demonstrate that functional activation of these cells during differentiation correlates with a translocation of the phospholipase A_2 activity from the cytosol to the membranes. *In vitro*, the enzyme may be shifted to the cytosol by EDTA, which facilitates the characterization of the enzyme.

EXPERIMENTAL

Cell culture

U937 and THP1 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The cells were

Abbreviation used: PMA, phorbol 12-myristate 13-acetate.
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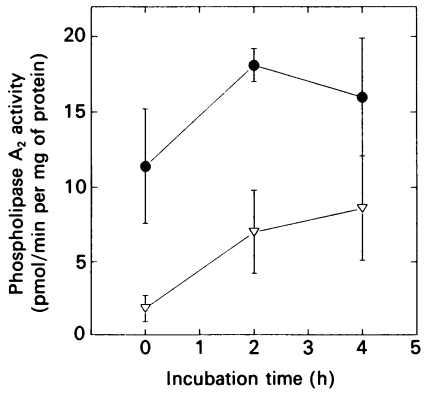

Fig. 1. Stimulation of phospholipase A_2 by PMA

THP1 cells were stimulated with PMA (5 nM) for 2 or 4 h. Membranes (▽) and cytosol (●) were separated by differential centrifugation. Phospholipase A_2 activity was determined with arachidonyl phosphatidylcholine as substrate as described in the Experimental section. Data are means ± S.D. of 3–5 preparations.

Table 1. Changes in phospholipase A_2 activity during differentiation

U937 and THP1 cells were incubated with 5 nM-PMA for the times indicated. Phospholipase A_2 activity was determined in the subfractions. For U937 cells, data are means ± S.D. of six preparations: * $P < 0.05$, ** $P < 0.005$ determined by the two-sided Student t test for independent measurements. For THP1 cells, data are means ± range of two preparations.

	Phospholipase A_2 activity (pmol/min per mg of protein)		
Incubation time...	0	48 h	72 h
U937 cells			
Cytosol	24.2±16.3	13.6±13.4**	9.9±8.2*
Membrane	2.0±2.2	4.7±2.2*	6.5±2.6**
THP1 cells			
Cytosol	7.5±2.0	0.5±0.2	0.1±0.1
Membrane	0.8±0.4	2.8±0.3	4.1±0.7

cultured and differentiated with PMA (5 nM) as described elsewhere [14].

Release of arachidonic acid

U937 and THP1 cells were differentiated with PMA in 96-well microtitre plates for 72 h. After washing, both undifferentiated and PMA-differentiated cells were incubated overnight with 0.04 μCi of [^{14}C]arachidonic acid (Amersham Buchler, Braunschweig, Germany; sp. radioactivity 50 mCi/mmol) per 10^5 cells in medium with 1 mg of BSA (essentially fatty acid-free; Sigma, München, Germany)/ml. The cells were washed extensively and further incubated in medium supplemented with BSA. At zero time the cells were stimulated with PMA or ionomycin (Sigma) as described below. After 30 min or 4 h, samples of the supernatant were removed and the radioactivity was determined by liquid-scintillation counting. The values obtained were corrected for the radioactivity found in the supernatants at zero time, the beginning of the stimulation period. In order to compare different experiments, the released radioactivity was calculated as a percentage of the total radioactivity incorporated.

Cell fractionation

At the times indicated the undifferentiated cells were washed once with phosphate-buffered saline (137 mM-NaCl/2.7 mM-KCl/8 mM-Na_2HPO_4/1.5 mM-KH_2PO_4) and collected by centrifugation. The adherent differentiated cells were washed and removed from the culture plates with a rubber policeman. Routinely the cells were resuspended in 10 mM-Tris/HCl (pH 7.6)/10% (v/v) glycerol/5 mM-2-mercaptoethanol/0.1 mM-phenylmethanesulphonyl fluoride and disrupted by sonication. Cellular organelles were removed by centrifugation at 6500 g for 20 min. Cytosol and membranes were separated by centrifugation at 100000 g for 60 min in a Beckman TL100 ultracentrifuge. Membranes were resuspended in the same buffer as described above. Protein was determined by a micro-titre Bradford assay with BSA as standard [21].

Partial purification of phospholipase A_2

The cytosolic fraction was prepared as described above with buffer supplemented with 1 mM-EDTA. It was loaded on a DEAE-cellulose column equilibrated with the same buffer. Elution was performed with a linear gradient from 0 to 0.5 M-NaCl. The enzyme was eluted as a single peak at about 0.3 M-NaCl.

Determination of phospholipase A_2 activity

This was done with α-1-palmitoyl-2-[1-^{14}C]arachidonyl phosphatidylcholine (NEN, Boston, MA, U.S.A.) as substrate as described previously [22]. If not indicated otherwise, the assay mixture contained 1 μM phospholipid, corresponding to about 30000 c.p.m., 5 mg of BSA/ml and 5 mM-$CaCl_2$ in 100 mM-Tris/HCl, pH 9.5. Reaction time was 30 min at 37 °C. The liberated fatty acid was separated from substrate that had not reacted by heptane extraction [22], and the radioactivity was determined by liquid-scintillation counting. In control experiments, the heptane phase was analysed by t.l.c. and contained exclusively free arachidonic acid.

Statistical analysis

Statistics were calculated with the two-sided Student t test for independent measurements.

RESULTS AND DISCUSSION

Short-term effect of PMA on phospholipase A_2 activity in U937 and THP1 cells

U937 cells were incubated with PMA (5 nM) for short periods of time (up to 4 h). After separation of membranes and cytosol by differential centrifugation, the phospholipase A_2 activity remained unchanged in these subcellular fractions compared with untreated U937 cells. In contrast, the phospholipase A_2 activity of the more mature THP1 cells was increased in PMA-stimulated cells (Fig. 1). The enhanced activity was detectable in both compartments, membranes and cytosol, but was more pronounced in the membrane fraction. It was also reflected by an increased release of labelled arachidonic acid from cellular lipids: when labelled THP1 cells were incubated for 4 h in the absence or presence of PMA (5 nM), the release was stimulated 3.5±1.3-fold (255±104 compared with 795±149 c.p.m./well; $P < 0.005$, $n = 4$ independent experiments). The stimulation of the arachidonic acid release was thus of the same order of magnitude as the activation of membrane-bound phospholipase A_2 after 4 h (4.0±0.7-fold; $n = 4$, $P < 0.05$). Similar PMA-induced activation of phospholipase A_2 has been shown previously in various types of macrophages, e.g. mouse [23] and rat [24] peritoneal

Table 2. Release of arachidonic acid from undifferentiated and PMA-differentiated cells

U937 and THP1 cells were labelled with radioactive arachidonic acid overnight and stimulated with ionomycin for 30 min as indicated. The release was calculated as percentage liberated of the total radioactivity incorporated into the cells. Data are means ± S.D. of n independent experiments. Stimulation with ionomycin was statistically significant at * $P < 0.05$ or ** $P < 0.02$ as indicated.

	Arachidonic acid release (%)	
Cells	U937 cells ($n = 5$)	THP1 cells ($n = 3$)
Control	0.5±0.3	1.1±0.7
+ionomycin (1 μg/ml)	1.1±0.7	1.0±0.7
+ionomycin (10 μg/ml)	2.5±1.3**	2.0±0.5
PMA-differentiated	2.4±1.6	0.9±0.8
+ionomycin (1 μg/ml)	6.7±3.7*	5.0±3.3
+ionomycin (10 μg/ml)	6.4±2.7**	6.0±2.7*

Table 3. Ratio of cytosolic to membrane-bound phospholipase A_2 activity is dependent on the conditions of the preparation *in vitro*

Equal numbers of undifferentiated THP1 cells were fractionated in Tris/HCl buffer, pH 7.6, containing EDTA or Ca^{2+} as indicated. Phospholipase A_2 activity was measured in the cytosol and the membrane fraction, and the ratio of cytosolic to membrane-bound activity calculated. Data are means ± S.D. of three preparations: * $P < 0.05$, ** $P < 0.005$ compared with control cells without addition of EDTA or bivalent ions.

	Cytosol/membrane ratio		
Buffer	Specific activity	Total activity	Protein
1.0 mM-EDTA	10.7±2.9*	17.8±1.5**	1.6±0.5
0.5 mM-EDTA	10.7±2.6*	16.0±3.2**	1.4±0.3
No addition	2.1±1.1	3.1±1.1	1.5±0.3
0.5 mM-Ca^{2+}	2.2±1.6	2.1±1.1	1.5±0.5
1.0 mM-Ca^{2+}	1.8±0.6	2.0±0.2	1.2±0.2
1.0 mM-Mg^{2+}	0.9±0.8	1.2±1.0	1.6±0.3

macrophages or bone-marrow-derived macrophages [25]. It is thus noteworthy that the less mature U937 cells do not respond to this short-term PMA treatment with an increase in phospholipase A_2 activity, although TPA activates and translocates its receptor, protein kinase C, within 2–5 min in these cells, followed by expression of differentiation-associated genes. Although the mechanism of PMA-induced increase in phospholipase A_2 activity is still unknown, and the relevant phosphorylation substrates for PMA-induced protein kinase C have not yet been identified [23], this model might be very useful to elucidate a possible pathway of protein kinase C-mediated phospholipase A_2 activation. Since in THP1 cells a significantly increased phospholipase A_2 activity was detectable in the membranes as well as in the cytosolic fraction, these data might indicate a rather stable modification of the enzyme or of a not yet identified regulatory protein. As 'activation' was determined by measuring enhanced enzyme activity in whole membranes or cytosols, it might also reflect enhanced biosynthesis *de novo*.

Phospholipase A_2 activity is translocated in U937 and THP1 cells during PMA-induced differentiation

U937 and THP1 cells were incubated with low doses of PMA

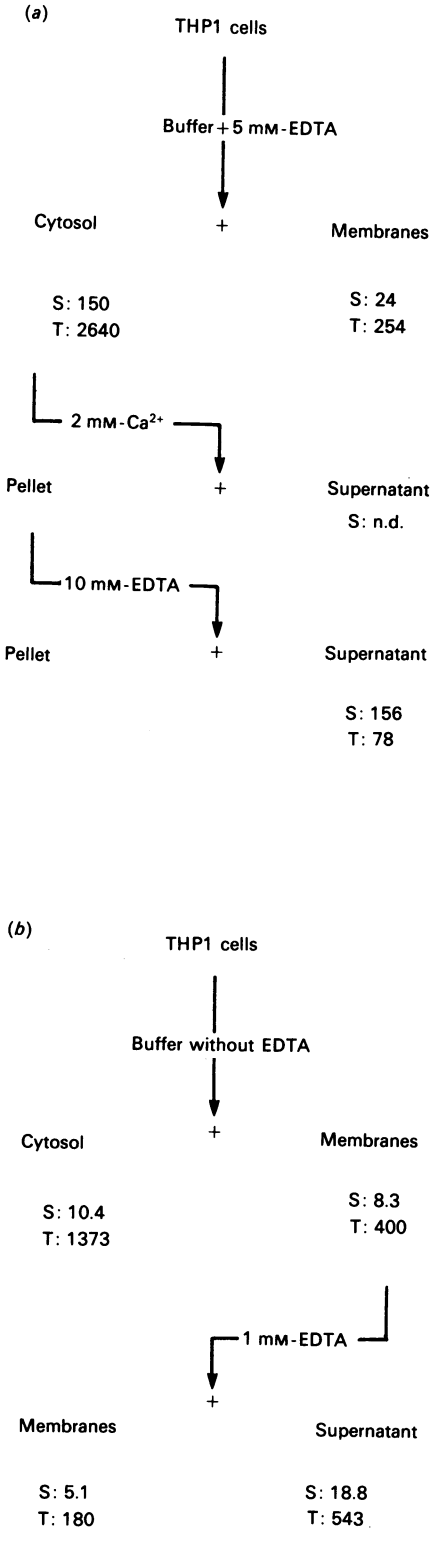

Fig. 2. Buffer-dependent distribution of phospholipase A_2 activity between cytosol and membranes

THP1 cells were disrupted in buffer with (*a*) or without (*b*) 5 mM-EDTA, and membranes and cytosol were prepared as described in the Experimental section. (*a*) The cytosolic protein was precipitated by an excess of 2 mM-Ca^{2+}. The resulting pellet was solubilized in buffer containing 10 mM-EDTA. Pellets 1 and 2 were insoluble and therefore not analysed for activity. (*b*) A portion of the membranes was sonicated in buffer containing 1 mM-EDTA and centrifuged at 100000 *g* for 60 min. Data are from typical experiments: n.d., not detectable; S, specific activity (pmol/min per mg of protein); T, total activity (pmol/min).

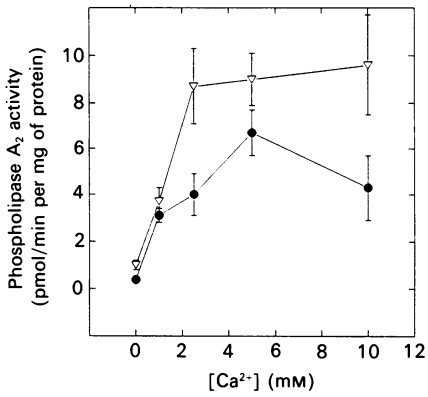

Fig. 3. Ca^{2+}-dependency of cytosolic and membrane-bound phospholipase A_2 activity

Phospholipase A_2 activity was determined in the cytosolic (▽) and membrane-bound (●) fraction of THP1 cells in the presence of the Ca^{2+} concentrations indicated. Zero Ca^{2+} was measured with no additional Ca^{2+} in the assay *in vitro*. Data are means ± S.D. of three preparations.

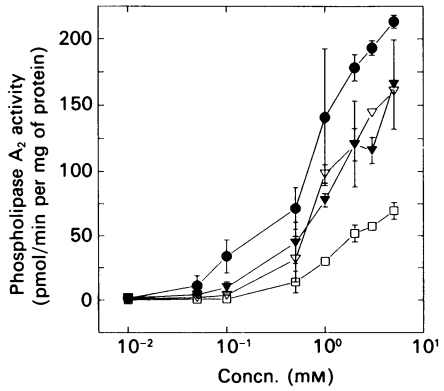

Fig. 4. Phospholipase A_2 activity is dependent on bivalent cations

The activity of the partially purified phospholipase A_2 from THP1 cells was determined in the presence of Ca^{2+} (●), Mg^{2+} (▽), Sr^{2+} (▼) or Ba^{2+} (□) in the concentrations indicated. Data are means ± range of two experiments.

(5 nM) for up to 72 h. During this time, the cells become adherent and develop functional characteristics associated with mature macrophages [14]. At various time points phospholipase A_2 activity was determined in the cytosol and membrane fraction. In undifferentiated cells, the major part of the activity was found in the cytosol. After 48 and 72 h the specific as well as the total cytosolic activity was decreased. During the same time period the membrane-bound activity increased. The shift of the enzyme activity after 48 and 72 h of incubation with PMA is shown in Table 1 by the ratio of cytosolic to membrane-bound activity. The total activity measured in cytosol and membrane fractions apparently decreased. The most likely explanation is the 'dilution' of the exogenous substrate by endogenous phospholipids when the membrane-bound enzyme activity is determined *in vitro* (see also below). The molecular mechanisms involved in the apparent translocation, which might also reflect tighter binding to the membrane, have not been investigated so far. It is very likely that Ca^{2+} ions play a substantial role in the binding, as described by Channon & Leslie [20] and discussed below.

Translocation of phospholipase A_2 is accompanied by functional activation

The shift of the enzyme to the membrane during differentiation is accompanied by an increase in functional activity. The availability of arachidonic acid, the precursor of prostanoids and leukotrienes, is enhanced in differentiated U937 cells compared with control cells ($P < 0.05$; Table 2). These data are in good agreement with those obtained by Wiederhold *et al.*, who measured an increased release of both labelled and non-labelled arachidonic acid from differentiated U937 cells [16]. The release from control U937 cells and from differentiated cells was stimulated by increasing concentrations of ionomycin, reaching maximal values of 5–8% release. Stimulation rates were comparable in these cells, indicating that the membrane-bound enzyme responded to an increase in intracellular Ca^{2+}. The basal release from THP1 cells was not significantly different between differentiated and control cells. In the presence of ionomycin, however, the release was strongly enhanced in the differentiated cells, whereas very high concentrations of ionomycin (10 μg/ml) were necessary to effect arachidonic acid release in control cells. Therefore the high capacity for prostanoid synthesis in differentiated U937 and THP1 cells seems to be associated with both the induction of cyclo-oxygenase [17] and the functionally enhanced phospholipase A_2 activity.

The cellular distribution of phospholipase A_2 activity is bivalent-cation-dependent

The distribution of the specific phospholipase A_2 activity between cytosol and membrane fraction was dependent on the buffer used for cell disruption and membrane preparation. In the presence of EDTA, the specific activity of the membrane-bound phospholipase A_2 was about one-tenth of the cytosolic activity. It increased when the preparation was performed in the presence of Ca^{2+}. Concomitantly, the cytosolic activity decreased, as shown by the decrease in the ratio of cytosolic and membrane-bound phospholipase A_2 activities in THP1 cells (Table 3). The shift was also evident in the total activities, but the protein distribution was not significantly affected by the different preparation protocols. Similarly, Mg^{2+} ions were effective in stabilizing the membrane–enzyme interaction. Corresponding data were obtained in U937 cells. In the presence of EDTA the total cellular phospholipase A_2 activity apparently increased. This may be explained by facilitated interaction between the phospholipid substrate and the solubilized enzyme, or by an underestimated specific activity of the membrane-bound enzyme, since the protein-dependency of the membrane preparations was not linear. Interactions of phospholipase A_2 with regulatory Ca^{2+}-dependent proteins or sensitivity to the product arachidonic acid, as described for the membrane-bound enzyme of the macrophage cell line p388D1 [26], may be involved in the inhibition. Additionally, the enzyme activity was determined with exogenous substrate, but the simultaneous cleavage of endogenous substrate was not detected.

As described for other Ca^{2+}- and phospholipid-binding proteins such as lipocortins (e.g. [27]), the EDTA-solubilized cytosolic enzyme can be precipitated by an excess of Ca^{2+}. The recovery of the enzyme activity from the precipitate was accompanied by a high loss of total activity (Fig. 2*a*). By following the protocol for the purification of lipocortins from monocytes [27], phospholipase A_2 could be recovered quantitatively from a Mono Q ion-exchange column, co-eluting with numerous other proteins detectable by polyacrylamide-gel electrophoresis. The column was not further analysed for lipocortin activity; however, no fractions which contained a pure protein were obtained. Therefore in our hands precipitation of phospholipase A_2

with Ca^{2+} was not a suitable step for purification of the enzyme.

After preparation in buffer without EDTA, the membrane-bound enzyme is easily released from the membranes by sonication of the membranes in the presence of EDTA (Fig. 2*b*). The membrane-bound and the released phospholipase A_2 showed the same pH- and Ca^{2+}-dependency. As discussed above, the total activity apparently increased in the presence of EDTA, when a higher proportion of the enzyme was soluble.

A buffer-dependent distribution of phospholipase A_2 between cytosol and membranes has also been described in other cell types, such as platelets [18], mesangial cells [19] and recently in a mouse macrophage cell line [20]. There is good evidence now that the activities determined in the membrane fraction and in the cytosolic represent the same phospholipase A_2, rather than two different enzymes. In THP1 and U937 cells, both enzyme activities have optima at pH 8–10 and show the same Ca^{2+} requirements under the assay conditions *in vitro* (Fig. 3). In both cell types, phosphatidylcholine with arachidonic acid in position 2 was the preferred substrate. Enzyme activity was decreased to about 10% with the corresponding phosphatidylethanolamine as substrate. Exogenous phosphatidylinositol was not a substrate for the enzyme; however, it has been demonstrated in macrophages that phosphatidylinositol may well become a substrate *in vitro* in certain combinations with other phospholipids [5]. No phospholipase A_2 activity was detectable towards autoclaved *Escherichia coli* labelled with oleic acid or towards the extracted lipids, which consist primarily of phosphatidylethanolamine (results not shown).

Thus the biochemical evidence supports the concept of one enzyme becoming biologically active in the membrane environment of U937 and THP1 cells. However, the conclusive evidence needs to be investigated by immunological and molecular-biology methods.

Characterization of the cellular phospholipase A_2 of THP1 cells

In this study we demonstrate that the phospholipase A_2 in U937 and THP1 cells belongs to the group of cellular phospholipases which is only weakly attached to the membrane, in contrast with tightly associated enzymes which need detergents for solubilization, e.g. the phospholipase A_2 from sheep erythrocytes [28]. In order to characterize further the phospholipase A_2 activity of these human monocytic cell lines, the enzyme from THP1 cells was shifted to the cytosol by EDTA-containing buffer and enriched by DEAE-cellulose chromatography as described in the Experimental section. The enzyme activity was eluted with about 0.3 M-NaCl as a single peak. The specific activity was increased about 5-fold. The enzyme showed a broad alkaline pH optimum with maximal activity at pH 8–10; protein-dependency was linear up to 10 μg per assay. The phospholipase A_2 had an absolute requirement for bivalent cations; in the presence of EDTA no enzyme activity was detectable. Optimal activity was measured at 5 mM-Ca^{2+}, with no decrease in activity up to 10 mM-Ca^{2+}. Similarly high concentrations of other bivalent cations were also able to support the enzyme activity; however, the optimal activity was decreased by about 20% in the presence of Mg^{2+} and Sr^{2+} and 70% in the presence of Ba^{2+} (Fig. 4). The cation requirement seems to vary widely between phospholipases A_2 from different sources, e.g. the activity of the platelet enzyme is sustained by Sr^{2+}, but not by Mg^{2+} [18], whereas the secretory enzyme from parotid glands was active half-maximally in the presence of Mg^{2+} and to a lesser extent in the presence of Ba^{2+}, Sr^{2+} or Cd^{2+} [29].

Interestingly, very low concentrations of Ca^{2+} were sufficient to activate the phospholipase A_2 further in the presence of high concentrations of Mg^{2+} (Table 4).

Table 4. Maximal Mg^{2+}-dependent phospholipase A_2 activity is enhanced by low concentrations of Ca^{2+}

Phospholipase A_2 activity was determined in the presence of 10 mM-$MgCl_2$ alone or plus the indicated concentrations of $CaCl_2$. To compare different preparations, the activity in the presence of 10 mM-$MgCl_2$ was taken as 1. Data are means ± S.D. of three experiments.

	Phospholipase A_2 activity
10 mM-Mg^{2+}	1
10 mM-Mg^{2+} + 0.01 mM-Ca^{2+}	1.7 ± 0.2
10 mM-Mg^{2+} + 0.1 mM-Ca^{2+}	1.8 ± 0.3
10 mM-Mg^{2+} + 0.5 mM-Ca^{2+}	1.9 ± 0.2
10 mM-Mg^{2+} + 1.0 mM-Ca^{2+}	1.8 ± 0.2

In conclusion, we have thus shown that in the two human monoblastoid cell lines U937 and THP1 the subcellular localization of phospholipase A_2 is dependent on bivalent cations. The enzyme requires Ca^{2+} for optimal activity and seems to be functionally active in its membrane-bound form.

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REFERENCES

1. Ziegler-Heitbrock, H.-W. L. (1989) Eur. J. Cell Biol. **49**, 1–12
2. Schade, U. F., Burmeister, I., Elekes, E., Engel, R. & Wolter, D. T. (1989) Blut **59**, 475–485
3. Ulevitch, R. J., Watanabe, Y., Sano, M., Lister, M. D., Deems, R. A. & Dennis, E. A. (1988) J. Biol. Chem. **263**, 3079–3085
4. Leslie, C. C., Voelker, D. R., Channon, J. Y., Wall, M. M. & Zelarney, P. T. (1988) Biochim. Biophys. Acta **963**, 476–492
5. Wijkander, J. & Sundler, R. (1989) FEBS Lett. **244**, 51–56
6. Diez, E. & Mong, S. (1990) J. Biol. Chem. **265**, 14654–14661
7. Clark, J. D., Milona, N. & Knopf, J. L. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 7708–7712
8. Pawlowski, N. A., Kaplan, G., Hamill, A. L., Cohn, Z. A. & Scott, W. A. (1983) J. Exp. Med. **158**, 393–412
9. Williams, J. D., Robin, J.-L., Lewis, R. A., Lee, T. H. & Austen, K. F. (1986) J. Immunol. **136**, 642–648
10. Browning, J. L. & Ribolini, A. (1987) J. Immunol. **138**, 2857–2863
11. Hoffman, T., Lizzio, E. F., Suissa, J., Rotrosen, D., Sullivan, J. A., Mandell, G. L. & Bonvini, E. (1988) J. Immunol. **140**, 3912–3918
12. Sundström, C. & Nilsson, K. (1976) Int. J. Cancer **17**, 565–577
13. Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T. & Tada, K. (1980) Int. J. Cancer **26**, 171–176
14. Hass, R., Bartels, H., Topley, N., Hadam, M., Koehler, L., Goppelt-Struebe, M. & Resch, K. (1989) Eur. J. Cell Biol. **48**, 282–293
15. Hass, R., Koehler, L., Rehfeldt, W., Lessmann, V., Mueller, W., Resch, K. & Goppelt-Struebe, M. (1990) Cancer Res. **50**, 323–327
16. Wiederhold, M. D., Anderson, K. M. & Harris, J. E. (1988) Biochim. Biophys. Acta **959**, 296–304
17. Koehler, L., Hass, R., Wessel, K., DeWitt, D. L., Kaever, V., Resch, K. & Goppelt-Struebe, M. (1990) Biochim. Biophys. Acta **1042**, 395–403
18. Kramer, R. M., Checani, G. C., Deykin, A., Pritzker, C. R. & Deykin, D. (1986) Biochim. Biophys. Acta **878**, 394–403
19. Gronich, J. H., Bonventre, J. V. & Nemenoff, R. A. (1988) J. Biol. Chem. **263**, 16645–16651
20. Channon, J. Y. & Leslie, C. C. (1990) J. Biol. Chem. **265**, 5409–5413
21. Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
22. Goppelt-Struebe, M., Wolter, D. & Resch, K. (1989) Br. J. Pharmacol. **98**, 1287–1295
23. Wijkander, J. & Sundler, R. (1989) Biochim. Biophys. Acta **1010**, 78–87

24. Ohuchi, K., Watanabe, M., Yoshizawa, K., Tsurufuji, S., Fujiki, H., Suganuma, M., Sugimura, T. & Levine, L. (1985) Biochim. Biophys. Acta **834**, 42–47
25. Goppelt-Struebe, M. & Moeller, A. (1990) Biol. Chem. Hoppe-Seyler **371**, 369–374
26. Lister, M. D., Deems, R. A., Watanabe, Y., Ulevitch, R. J. & Dennis, E. A. (1988) J. Biol. Chem. **263**, 7506–7513
27. Rothut, B., Comera, C., Prieur, B., Errasfa, M., Minassian, G. & Russo-Marie, F. (1987) FEBS Lett. **219**, 169–175
28. Kramer, R. M., Wüthrich, C., Bollier, C., Allegrini, P. R. & Zahler, P. (1978) Biochim. Biophys. Acta **507**, 381–394
29. Castle, A. M. & Castle, J. D. (1981) Biochim. Biophys. Acta **666**, 259–274

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