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Purification of a 110-kilodalton cytosolic phospholipase A_2 from the human monocytic cell line U937

(arachidonic acid/prostaglandins/leukotrienes/platelet-activating factor/signal transduction)

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ABSTRACT **The major dithiothreitol-resistant phospholipase A_2 activity present in the cytosol of U937 cells has been purified >200,000-fold by sequential chromatography on phenyl-5PW, heparin-Sepharose CL-6B, high-performance hydroxylapatite, TSK-gel G3000-SW, and Mono Q columns. This 110-kDa cytosolic phospholipase A_2 is distinct from the relatively small (14-kDa) dithiothreitol-sensitive phospholipases A_2 that are secreted from many cell types. This additional phospholipase A_2 selectively hydrolyzes fatty acid at the *sn*-2 position of the glycerol and favors phospholipids containing arachidonic acid, which is the rate-limiting precursor for prostaglandin and leukotriene production. Interestingly, a >5-fold increase in phospholipase A_2 activity is noted as the calcium concentration increases from the levels found in resting cells to those observed in activated macrophages. We suggest that this enzyme and not the previously described secretory phospholipase A_2 is activated by cytosolic effectors such as GTP-binding regulatory proteins and protein kinases to initiate the production of prostaglandins, leukotrienes, and platelet-activating factor. To distinguish this cytosolic enzyme from the previously described secretory ones, we suggest referring to it as cPLA$_2$ for cytosolic phospholipase A_2 and collectively referring to the secretory phospholipases A_2 as sPLA$_2$s.**

Arachidonic acid is released from the *sn*-2 position of the glycerol in membrane phospholipids by an activated form of phospholipase A_2 (PLA_2) to initiate the synthesis of two potent classes of inflammatory mediators, prostaglandins and leukotrienes (1–3). The other product of the PLA_2 reaction is a lysophospholipid, which can serve as a precursor to the inflammatory mediator platelet-activating factor (PAF) (4). It is widely held that the production of arachidonic acid and lysophospholipid represents the rate-limiting step in the production of prostaglandins, leukotrienes, and platelet-activating factor. Therefore, agents that stimulate the production of these inflammatory mediators are likely to do so by modulating the activity of PLA_2. However, the mechanism by which PLA_2 activity is regulated remains poorly understood. To date, most of the direct studies on PLA_2 have been structural or mechanistic and have focused on the extracellular enzymes isolated from snake venom or mammalian pancreas (5–9). Recently, the secretory granules of platelets and neutrophils have been shown to contain phospholipases A_2 (PLA_2s) that are structurally similar to the venom and pancreatic enzymes (10–12). There is accumulating evidence, however, that eicosanoid production is regulated by a PLA_2 located in the cytosol.

A number of different studies have shown that when cells are treated with phorbol 12-myristate 13-acetate (PMA), a potent activator of the cytosolic enzyme protein kinase C, an increase in prostaglandin, leukotriene, and platelet-activating factor production is observed (13–16). Interestingly, when the PLA_2 activity present in the cytosolic fraction of PMA-treated cells is measured, a several fold enhancement is detected (17). Similar results have been reported for cells treated with either platelet-derived growth factor or epidermal growth factor (17, 18). Bradykinin, serotonin, and histamine, whose signal-transducing GTP-binding regulatory proteins are exposed to the cytosol, also have been shown to regulate PLA_2 activity (15, 16, 19–21). Several of these effectors of PLA_2 also raise intracellular Ca^{2+} levels (15, 20). Interestingly, a cytosolic PLA_2 activity present in RAW 264.7 cells has been shown to translocate to the membrane fraction in response to Ca^{2+} concentrations normally found in stimulated cells (22). Although the relative importance of each of these regulatory mechanisms is not known, it is clear that there must be a Ca^{2+}-responsive cytosolic PLA_2 capable of interacting with G proteins and protein kinases. Cytosolic forms of the previously described secretory enzymes are unlikely candidates to serve this function because the seven common disulfide bonds required for activity (11) would be reduced by the glutathione present in the cytosol. Recently, the Ca^{2+}-dependent PLA_2 activity from RAW 264.7 cells was shown to be resistant to reducing agents (23). Following partial purification of this PLA_2, sodium dodecyl sulfate (SDS)/polyacrylamide gel analysis of this PLA_2 revealed a major polypeptide of ≈60 kDa (23). In this paper, we report the purification of a cytosolic 110-kDa PLA_2. Our 110-kDa PLA_2 is activated by physiological levels of Ca^{2+}, is resistant to reducing conditions, and preferentially hydrolyzes phospholipids containing arachidonoyl (Δ_4Ach) in the *sn*-2 position of the glycerol.

METHODS

Materials. Unlabeled phospholipids were purchased from Avanti Polar Lipids, and all radiolabeled phospholipids including 1-[9,10-^{3}H]stearoyl-2-arachidonoyl phosphatidylcholine (1-[^{3}H]Ste-2-Δ_4Ach-PtdCho) were purchased from New England Nuclear. The Ca^{2+} for the Ca^{2+}-dependent experiment was purchased from VHG Labs (Manchester, NH) as a 2.006% solution, and EGTA used in the same experiment was Fluka puriss. p.a. >99.0% grade.

Assays. PLA_2 enzymatic activity was detected by measuring the release of radiolabeled arachidonic acid from the *sn*-2 position of 1-palmitoyl-2-[1-^{14}C]arachidonoyl phosphatidylcholine (1-Pam-2-[^{14}C]Δ_4Ach-PtdCho) as follows. 1-Pam-2-[^{14}C]Δ_4Ach-PtdCho (100 μM; 1 × 10^5 cpm) was dried under

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Abbreviations: PLA_2, phospholipase A_2; PLA_2s, phospholipases A_2; PMA, phorbol 12-myristate 13-acetate; DTT, dithiothreitol; PtdCho, phosphatidylcholine; Ste, stearoyl; Δ_4Ach, arachidonoyl; Pam, palmitoyl; HPHT column, high-performance hydroxylapatite column.

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nitrogen and then resuspended in 0.5 ml of 80 mM glycine buffer (pH 9.0) containing 200 μM Triton X-100, 5 mM $CaCl_2$, 250 μg of fatty acid-free bovine serum albumin per ml, and 70% (vol/vol) glycerol. The suspension was then sonicated to form mixed micelles of phospholipid and Triton X-100. Aliquots of the solution to be assayed were added to the mixed micelle preparation and incubated at 37°C in a shaking water bath. After a defined time period, the reaction was quenched by mixing the incubation mixture with 2.5 ml of isopropyl alcohol/heptane/0.5 M H_2SO_4, 20:5:1 (vol/vol). Heptane (1.5 ml) and water (1.0 ml) were added, and the solution was vigorously mixed for 10 sec. One-half of the heptane phase was loaded on a silica column (200 mg), and the free fatty acid was eluted with 1 ml of diethyl ether. The eluant was mixed with scintillant, and the radioactivity was measured.

The enzymatic activity is stable for at least 1 hr at 37°C, and the kinetics of the assay are linear to >10% conversion. Addition of 70% glycerol to the incubation mixture increases the activity by >15-fold without affecting the stability of the enzyme.

Protein concentrations were determined with Bio-Rad protein assay solution and bovine serum albumin as a standard.

Purification Procedure. The human monocytic cell line U937 [ATCC CRL2093] was chosen as the source for the purification of the human cytosolic PLA_2. Cells were grown in Dulbecco's modified Eagle's medium/Ham's F-12, 50:50 (vol/vol), supplemented with 10% (vol/vol) fetal bovine serum. Cells were first grown in a humidified 7.5% CO_2/92.5% air incubator in Bellco 1- or 3-liter spinners and then transferred for large-scale production to 8-liter Bellco spinners in a 37°C warm room. The head space of the spinners was gassed continuously with 7.5% CO_2/92.5% air. Cell density at harvest was $\approx 1.5 \times 10^6$ cells per ml. Cells were harvested by tangential flow filtration with a Millipore Prostak mammalian cell system fitted with 30 ft^2 (2.9 m^2) of 0.65-μm-pore size filters. After concentrating 50 liters to ≈8 liters, cells were pelleted by centrifugation at $\approx 800 \times g$ for 10–20 min. The cell pellet was washed twice with ice-cold phosphate-buffered saline.

The washed cells were suspended in 0.5 liter 10 mM Hepes (pH 7.5) lysis buffer containing 0.34 M sucrose, 1 mM EDTA, 0.1 mM dithiothreitol (DTT), 1 mM ATP, 1 μg of leupeptin per ml, and freshly added 1 mM phenylmethylsulfonyl fluoride. The cells were lysed by N_2 cavitation at 600–700 psi (1 psi = 6.89 Pa), the lysate was centrifuged at $50,000 \times g$ for 60 min, and PLA_2 was purified from the supernatant.

The supernatant was adjusted to 0.5 M $(NH_4)_2SO_4$, centrifuged at $50,000 \times g$ for 60 min, filtered through a 0.22-μm Millipak 60 (Millipore) filter unit, and loaded onto a Toso Haas (Southborough, MA) phenyl-5PW column (2.15 × 15 cm) that had been equilibrated with 20 mM Tris·HCl, pH 7.5/5 mM DTT/0.5 M $(NH_4)_2SO_4$. The PLA_2 activity was eluted at 8 ml/min with a 250-ml reverse gradient from 0.5 to 0 M $(NH_4)_2SO_4$, followed by a 500-ml wash at 0 M salt. The activity eluted at 0 M (fractions 29–34) salt was concentrated to ≈10 ml by using an Amicon filtration apparatus with a PM10 membrane; 0.4 ml of 1 M Mes buffer (pH 6.8), 1 ml of glycerol, and 0.05 ml of 1 M DTT were added; and the solution was passed through a heparin-Sepharose CL-6B column (1 × 12 cm; Pharmacia LKB) equilibrated with 40 mM Mes, pH 6.8/10% glycerol/5 mM DTT. Potassium phosphate buffer (pH 6.8) and $CaCl_2$ were added to the heparin column eluant to yield final concentrations of 10 mM phosphate and 10 μM Ca^{2+}, and the sample was loaded onto a Bio-Gel high-performance hydroxylapatite (HPHT) column (0.78 × 10 cm) equilibrated with the same concentrations of phosphate buffer and $CaCl_2$. By using a 60-ml linear potassium phosphate gradient (10–500 mM) at a flow rate of 0.4 ml/min, the activity was eluted near 140 mM phosphate. The active fractions were concentrated with an Amicon Centricon-30 microconcentrator and injected onto a TSK-gel G3000-SW column (0.75 × 60 cm; Toso Haas), which was both equilibrated and run in 40 mM Mes, pH 6.5/300 mM KCl/5 mM DTT/3 mM octyl glucoside. The flow rate was 0.4 ml/min, and the fraction size was 0.5 ml. The activity was eluted at a molecular weight of ≈100 kDa. The fractions containing activity were diluted 1:3 with 20 mM Tris·HCl, pH 7.5/10% glycerol/5 mM DTT, loaded onto a Mono Q HR 5/5 column (Pharmacia LKB) equilibrated with the same buffer, and eluted with a 60-ml 0 to 1 M KCl linear gradient at a flow rate of 1 ml/min while collecting 1-ml fractions. The PLA_2 activity was eluted in fractions 38–40 at ≈400 mM salt. Half of fraction 39 was rechromatographed on the G3000-SW column, and 0.25-ml fractions were collected.

RESULTS

Isolation of a Cytosolic PLA_2 from the Human Monocytic Line U937. To select for the purification of a cytosolic PLA_2 activity, we inactivated the noncytosolic PLA_2s by adding DTT to all buffers (11). A survey of cell lines indicated the human monocytic cell line U937 contained relatively high levels of DTT-insensitive PLA_2 activity (50–300 pmol/min per mg) and therefore was chosen as the source for the purification.

For each purification, the cells from a 50-liter ($\approx 5 \times 10^{10}$ cells) U937 suspension culture were lysed by N_2 cavitation at 600 psi, and the lysate was centrifuged at $50,000 \times g$ for 60 min. The partitioning of the PLA_2 activity between supernatant and pellet was dependent on the Ca^{2+} concentration in the lysis buffer. When cells were lysed in the presence of 1 mM EDTA, the majority of the activity was found in the supernatant. The addition of 5 mM $CaCl_2$ to the lysis buffer

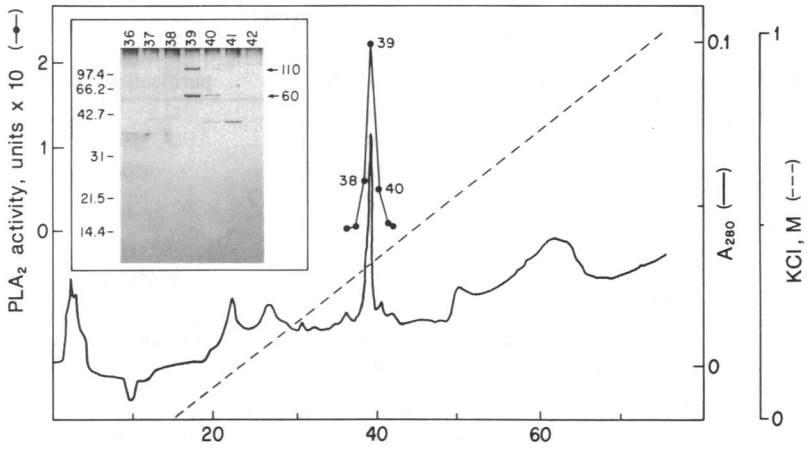

FIG. 1. Elution of PLA_2 from Mono Q. PLA_2 activity from the G3000-SW column was applied to a Mono Q HR5/5 column and eluted with a 60-ml 0–1 M KCl gradient. Fractions were 1 ml. For clarity, only the fractions containing activity are shown. (*Inset*) Fractions 36–42 (10-μl aliquots) were analyzed on a 12% polyacrylamide/SDS gel visualized with silver stain. The position of the molecular weight standards ($\times 10^{-3}$) are indicated to the left of the gel.

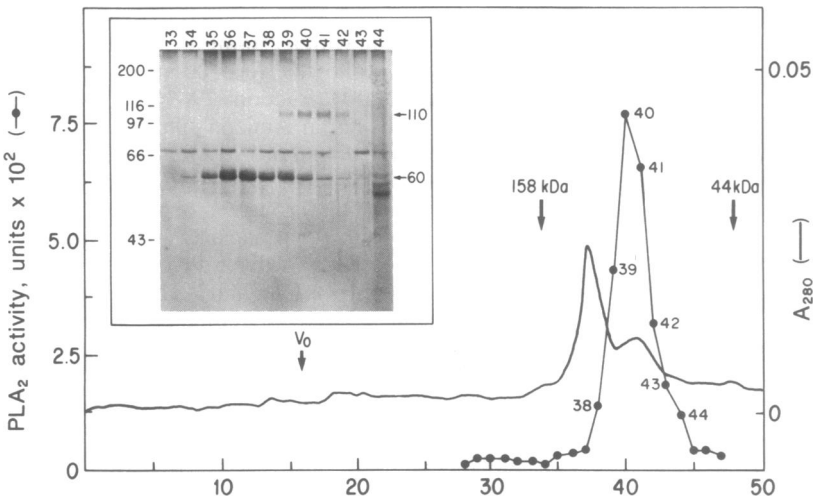

FIG. 2. Separation of the 60-kDa and 110-kDa proteins on a size-exclusion column; coelution of PLA_2 activity and the 110-kDa protein. Half of the peak activity fraction (fraction 39) from the Mono Q column was chromatographed again on the G3000-SW column. Fractions were 0.25 ml. The void volume (V_0) and the elution volume of the molecular weight markers bovine gamma globulin (158 kDa) and ovalbumin (44 kDa) are shown. (*Inset*) Fractions 33–44 (6-μl aliquots) were analyzed on a 9% acrylamide/SDS gel visualized with silver stain. The position of the molecular weight standards ($\times 10^{-3}$) are indicated to the left of the gel.

caused the activity to partition into the particulate fraction. However, both recovery and specific activity were maximized when EDTA was included in our lysis buffer.

Therefore, the PLA_2 activity was purified from the supernatant by sequential chromatography on phenyl-5PW, heparin-Sepharose Cl-6B, HPHT, size-exclusion, and anion-exchange columns as documented in Table 1. In three separate purifications, chromatography of the supernatant on a phenyl-5PW column reduced the protein by a factor of 40–50 with a 50–75% recovery of activity. The activity was tightly bound to the phenyl-5PW column and was not eluted until the 0 M salt wash after the reverse gradient. An additional peak of activity, equal to ≈10% of the activity recovered in the 0 M salt wash, could be eluted by 15 mM octyl glucoside after the low salt wash.

The peak fractions from the phenyl-5PW column were combined and concentrated 7-fold with an Amicon ultrafiltration apparatus with a PM-10 membrane. The total activity increased 11-fold upon concentration, suggesting that a low molecular weight (<10 kDa) inhibitor had been removed. The concentrated solution was passed through a heparin-Sepharose CL-6B column, which bound 80% of the total protein while allowing the PLA_2 activity to flow through the column; only a trace (≈2%) of activity was eluted in the 0–1 M KCl gradient. The activity that flowed through the column was chromatographed on a Bio-Gel HPHT column, which reduced the total protein by an additional factor of 6. A single peak of activity was eluted at ≈140 mM potassium phosphate. When the peak fractions from the HPHT column were concentrated and injected on the G3000-SW gel filtration column, a single peak of activity was detected at an elution volume consistent with a 100-kDa protein.

The penultimate purification step utilized a Mono Q HR5/5 column and a 0–1 M KCl gradient. The A_{280} elution profile of the Mono Q column (Fig. 1) showed one major protein peak eluted at ≈400 mM KCl, which was coincident with activity. Fractions through this region were analyzed on a 12% polyacrylamide/SDS gel and visualized by silver staining (Fig. 1 *Inset*). In the lane corresponding to fraction 39, which contained the most activity, two major polypeptides of 60 kDa and 110 kDa were observed. No protein bands were detected near 14 kDa. To determine if either protein was PLA_2, half of fraction 39 was rechromatographed over the gel filtration column resulting in the resolution of two A_{280} peaks (Fig. 2). Fractions 33–44, which contained both A_{280} peaks, were analyzed on a 9% polyacrylamide/SDS gel. As can be seen in the inset to Fig. 2, the 60-kDa protein was eluted as the first A_{280} peak, with the majority eluting before the PLA_2 activity. The 110-kDa polypeptide was eluted in the second A_{280} peak along with the PLA_2 activity. The intensity of the 110-kDa band was found to correlate with the amount of PLA_2 activity found in each of the fractions 39–42, indicating that the 110-kDa protein, not the 60-kDa protein is PLA_2. The anomalous behavior of the 60-kDa protein on the size-exclusion column indicates that it probably exists as a dimer and, as such, tailed into the PLA_2 peak during the first gel filtration step. A third band can be seen in every lane of the polyacrylamide gel and is likely to be a contaminant in either the column buffer or the SDS sample buffer. Our assignment of PLA_2 activity to the 110-kDa protein is consistent with results we have obtained recently with the purification of a 105-kDa PLA_2 from a murine macrophage-like cell line RAW 264.7 (unpublished data).

The specific activity of pure U937 PLA_2 is ≈20 units/mg (Table 1). Overall, the purification has resulted in a reduction

Table 1. Purification of a cytosolic PLA_2 from the human monocytic cell line U937

Step	Protein, mg	Activity, unit*	Specific activity, unit/mg	Fold purification
Supernatant	2100	0.15	0.000071	—
Phenyl-5PW	46	0.11	0.0024	34
PM-10 ultrafiltration	41	1.2	0.029	410
Heparin-Sepharose CL-6B	8.6	1.2	0.14	2,000
HPHT column†	1.4	—	—	—
G3000-SW	0.2‡	0.29	1.5	20,000
Mono Q	0.08‡	0.33	4.1	58,000
G3000-SW§	0.011‡	0.22	20¶	280,000

*A unit is defined as the amount of PLA_2 necessary to produce 1 μmol of arachidonic acid per minute.
†Phosphate inhibits the U937 PLA_2 activity and thus precludes an accurate determination of recovery.
‡Protein was estimated by the absorbance at 280 nm.
§A portion (0.11 unit) of the activity from the Mono Q column was applied to the G3000-SW column.
¶The final specific activity was estimated based on the measured activity and the absorbance at 280 nm.

by a factor of 200,000 in protein and a 280,000-fold increase in specific activity.

Positional Specificity of the U937 PLA_2. During the purification, the PLA_2 activity was measured with 1-Pam-2[^{14}C]Δ_4Ach-PtdCho as a substrate. The radiolabeled arachidonic acid released by enzymatic activity was separated from the radioactive substrate and measured as described. To determine the positional specificity of our 110-kDa enzyme, we incubated the enzyme with either 1-Ste-2[^{14}C]Δ_4Ach-PtdCho or 1-[^{3}H]Ste-2Δ_4Ach-PtdCho and measured the amount of radioactivity released as free fatty acid. To verify that release of radiolabel from either position would be detected in the assay, porcine pancreatic PLA_2 and lipase from *Rhizopus arrhizus*, which are specific for cleavage at the *sn*-2 and *sn*-1 positions, respectively (24), were incubated with the two radiolabeled substrates. Pancreatic PLA_2 caused the release of radioactivity from the *sn*-2 position (Fig. 3*B*) with the characteristic lag phase (25, 26), and the lipase from *R. arrhizus* released fatty acid from the *sn*-1 position (Fig. 3*C*), thus validating our methodology. The U937 PLA_2 hydrolyzes the *sn*-2 ester 25 times faster than the *sn*-1 ester during the linear phase of the reaction (Fig. 3*A*). These data demonstrate that the U937 enzyme is indeed a PLA_2 with little phospholipase A_1 or B activity.

Effect of pH and Ca^{2+} on the Activity of U937 PLA_2. The Ca^{2+} dependence of the U937 PLA_2 was determined at pH 7.4 (Fig. 4*A*) with Ca^{2+}/EGTA buffers to accurately maintain the

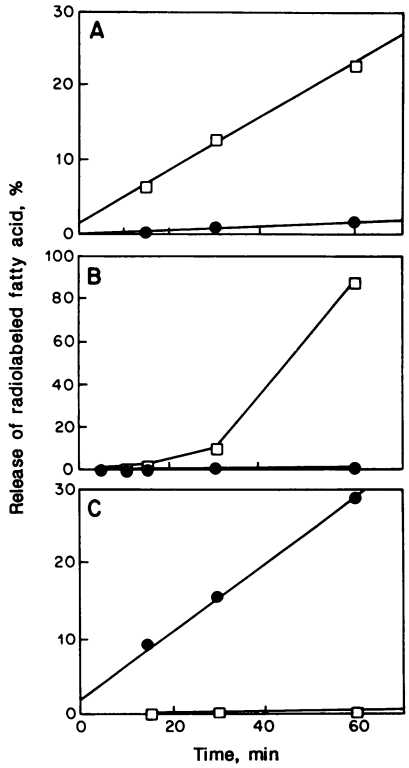

FIG. 3. Positional specificity of the U937 PLA_2. (*A*) Purified PLA_2 was added to either 1-Ste-2-[^{14}C]Δ_4Ach-PtdCho (□) or 1-[^{3}H]Ste-2-Δ_4Ach-PtdCho (●), and the radioactivity released as free fatty acid was measured. As a control, the substrates were also incubated with either porcine pancreatic PLA_2 (*B*) or lipase from *R. arrhizus* (*C*). Assay mixtures (1.2 ml total) containing 100 μM 1-Ste-2-Δ_4Ach-PtdCho (2×10^6 cpm as 1-Ste-2-[^{14}C]Δ_4Ach-PtdCho or 3×10^6 cpm as 1-[^{3}H]Ste-2-Δ_4Ach-PtdCho), 200 μM Triton X-100, 80 mM glycine (pH 9.0), 5 mM $CaCl_2$, and 70% glycerol were incubated with U937 PLA_2 (0.1 μg) (*A*), porcine pancreatic PLA_2 (1.2 μg) (*B*), or lipase from *R. arrhizus* (152 μg) (*C*). At defined times, two 100-μl aliquots were removed from each assay and processed as described. Each point is corrected for nonenzymatic hydrolysis.

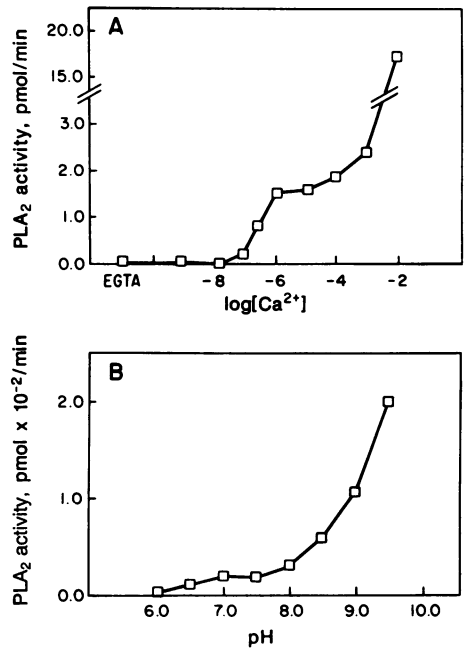

FIG. 4. Effect of Ca^{2+} and pH on the activity of U937 PLA_2. (*A*) The Ca^{2+} dependence of the purified PLA_2 toward mixed micelles was determined. Each assay (100-μl total) contained 100 μM 1-Pam-2-[^{14}C]Δ_4Ach-PtdCho (2×10^5 cpm), 200 μM Triton X-100, 70% glycerol, 250 μg of fatty acid-free bovine serum albumin per ml, 80 mM Hepes (pH 7.4), 1 mM EGTA, and 0.025–11 mM Ca^{2+}. Equal aliquots of PLA_2 (7.5 ng per assay) were added to each assay and incubated at 37°C. (*B*) The pH dependence of the purified PLA_2 toward Triton X-100 mixed micelles was determined. Each assay (100-μl total volume) contained 100 μM 1-Pam-2-[^{14}C]Δ_4Ach-PtdCho (2×10^5 cpm), 200 μM Triton X-100, 5 mM $CaCl_2$, 70% glycerol, 250 μg of fatty acid-free bovine serum albumin per ml, and 20 mM buffer. The buffers used to span pH 6.0–9.5 were Mes (pH 6.0 and 6.5), Hepes (pH 7.0 and 7.5), Tris (pH 8.0 and 8.5), and glycine (pH 9.0 and 9.5). Equal aliquots of PLA_2 (5 ng per assay) were added to each sample and incubated at 37°C. For both experiments, the incubation mixtures were quenched and processed as described. The incubation time was adjusted (2.5–60 min) to maintain <10% conversion. The points are corrected for nonenzymatic hydrolysis.

free Ca^{2+} levels (27). Interestingly, the PLA_2 activity was found to increase significantly in response to changes in Ca^{2+} concentrations that are normally observed in whole cells (28, 29). The response was biphasic, however, and an additional increase in activity was noted at Ca^{2+} concentrations between 1 and 10 mM.

The effect of pH on PLA_2 activity toward 1-Pam-2[^{14}C]Δ_4Ach-PtdCho mixed micelles was also investigated (Fig. 4*B*). Measurable PLA_2 activity was detected at a pH as low as 6.0, with the release of arachidonic acid >20-fold above nonenzymatic levels. Gradual increases were detected up to pH 8.0, after which the activity increased dramatically.

Substrate Specificity of U937 PLA_2. Arachidonic, linoleic, oleic, and palmitic acids are the major fatty acids esterified in the *sn*-2 position of PtdCho in macrophages (30). Using 1-Pam-2-[^{14}C]acyl-PtdCho/Triton X-100 mixed micelles, we determined the relative rates of hydrolysis of these fatty acid esters by the purified U937 PLA_2. The U937 enzyme prefers phospholipids with polyunsaturated fatty acids (Table 2). In fact, arachidonic acid is liberated almost 50-fold faster than palmitic acid. The enzyme's preference for Δ_4Ach-containing phospholipids is consistent with the involvement of the U937 PLA_2 in eicosanoid biosynthesis. It should be stressed, however, that the selectivity of the enzyme *in vivo* may be different from that observed using artificial mixed micelles.

Table 2. Substrate specificity of the U937 PLA_2

Substrate	Activity, pmol/min
1-Pam-2-[^{14}C]Δ_4Ach-PtdCho	12 ± 1.5
1-Pam-2-[^{14}C]Lin-PtdCho	4.3 ± 0.24
1-Pam-2-[^{14}C]Ole-PtdCho	2.1 ± 0.06
1-Pam-2-[^{14}C]Pam-PtdCho	0.26 ± 0.05

Each assay (100 μl total) contained 100 μM 1-Pam-2-[^{14}C]acyl-PtdCho, 200 μM Triton X-100, 80 mM glycine (pH 9.0), 5 mM $CaCl_2$, 70% glycerol, and 250 μg of fatty acid-free bovine serum albumin per ml. Equal aliquots of PLA_2 (5 ng per assay) were added to each assay and incubated at 37°C. Incubation times were adjusted to maintain <3% conversion. The values listed are corrected for nonenzymatic hydrolysis and are the average of three assays ± SD. Ole, oleoyl; Lin, linoleoyl.

DISCUSSION

A 110-kDa PLA_2 has been purified from the cytosol of the human monocytic cell line U937. The assignment of PLA_2 activity to the 110-kDa polypeptide is based on the coelution of this protein with PLA_2 activity. The majority of the PLA_2 activity following the Mono Q column was present in a single fraction. Analysis of this fraction by SDS/PAGE and silver staining revealed two major protein bands of 60 and 110 kDa. Upon further purification on a gel filtration column, the 60-kDa protein was eluted before the PLA_2 activity, indicating that the 60-kDa protein is not a PLA_2. In contrast, the intensity of the 110-kDa band in each lane of the polyacrylamide gel correlated with the amount of PLA_2 activity present in the corresponding fraction. The final specific activity of this PLA_2 preparation is 20 μmol/min per mg, yielding a turnover number for the 110-kDa PLA_2 of 40 sec^{-1} which is close to that of the secretory PLA_2s (50 sec^{-1}), thus supporting the claim that the 110-kDa polypeptide contains the PLA_2 activity (10–12).

Recently, a PLA_2 activity with similar chromatographic properties to our 110-kDa enzyme was purified from the cytosol of the RAW 264.7 macrophage cell line to a point where the specific activity was 2 μmol/min per mg, and the last major band on a Coomassie-stained SDS/polyacrylamide gel was a 60-kDa protein (23). However, we have used a similar purification protocol to that reported here to purify a 105-kDa PLA_2 to homogeneity from the RAW 264.7 cell line (unpublished data). In this purification, we observed a single major DTT-resistant PLA_2 activity, and again, a 60-kDa protein as one of the last contaminants. Therefore, the 60-kDa protein noted by Leslie *et al.* (23) is not likely to be a PLA_2.

We have found that the 110-kDa PLA_2 is the major DTT-insensitive activity present in U937 cells. Throughout the purification, all fractions were assayed for PLA_2 activity by using the PtdCho/Triton X-100 mixed micelle assay. Although additional peaks did elute from the phenyl-5PW column and the heparin column, the 110-kDa PLA_2 accounted for 90% and 98% of the activity, respectively.

The U937 phospholipase selectively hydrolyses fatty acid at the *sn*-2 position and favors phospholipids containing arachidonic acid in the *sn*-2 position, indicating it is likely to participate in prostaglandin and leukotriene biosynthesis. Interestingly, significant PLA_2 activity is observed at physiological concentrations of Ca^{2+}, and a >5-fold increase in PLA_2 activity is noted as the Ca^{2+} concentration increases from the levels seen in resting cells to those seen in activated macrophages (28, 29). The mechanism of this Ca^{2+} regulation of activity is currently unknown; however, the cytosolic PLA_2 activity in RAW 264.7 cell lysates has been observed to translocate to the cell membrane pellet when the Ca^{2+} concentrations were increased over this same range (22).

Upon close inspection, the 110-kDa PLA_2 is observed to migrate as a doublet (Fig. 1). These two species are partially resolved from one another on the Mono Q anion exchange column (Fig. 1, lanes 38–40). The apparent charge difference of these species could be due to differences in phosphorylation or could be indicative of multiple isoforms of the enzyme.

In conclusion, we have purified a PLA_2 that behaves as a cytosolic protein of 110 kDa and favors the hydrolysis of arachidonic acid-containing phospholipids. Furthermore, the activity of this 110-kDa PLA_2 is regulated by changes in Ca^{2+} concentration normally encountered in whole cells. We suggest that the 110-kDa protein be called cytosolic PLA_2 ($cPLA_2$) to distinguish it from the secretory isoforms ($sPLA_2$s) of the enzyme.

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