The Ca\(^2+\)-sensitive Cytosolic Phospholipase A\(_2\) Is a 100-kDa Protein in Human Monoblast U937 Cells*

(Received for publication, October 15, 1990)

Ruth M. Kramer†, Edda F. Roberts, Joseph Manetta, and James E. Putnam

From Lilly Research Laboratories, Indianapolis, Indiana 46285

Human monoblast U937 cells contain a soluble phospholipase A\(_2\) (PLA\(_2\)) that is activated over the range of 150–600 nm Ca\(^2+\) and is stable at only neutral pH. We have purified this PLA\(_2\) over 34,000-fold to near homogeneity using sequential ion exchange, hydrophobic interaction, and gel filtration chromatography steps. The protein has a \(M_r\) of \(\sim 100,000\) (by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and an isoelectric point of 5.1. Four lines of evidence indicate that this 100-kDa polypeptide represents the PLA\(_2\). (i) The intensity of staining of the 100-kDa protein was proportional to the degree of purification of PLA\(_2\) activity, (ii) the relative staining intensity of the 100-kDa protein precisely paralleled the elution profile of PLA\(_2\) activity during chromatography steps, (iii) the PLA\(_2\) activity recovered from a nondenaturing gel (>60% of the total activity applied) coincided exactly with the major high molecular weight protein detected by silver staining, and (iv) monoclonal and (ii) antibodies against the 100-kDa protein immunoprecipitated the PLA\(_2\). We conclude that the cytosolic PLA\(_2\) isolated from U937 cells represents a novel, high molecular weight PLA\(_2\) responding to physiological (intracellular) changes in Ca\(^2+\) concentration and therefore may play a critical role in cellular signal transduction processes and the biosynthesis of lipid mediators.

Phospholipases A\(_2\) (phosphatide 2-acylhydrolases, EC 3.1.1.4) are a diverse family of enzymes that hydrolyze the sn-2 fatty acyl ester bond of phosphoglycerides producing free fatty acids and lysophospholipids (1, 2). They play crucial roles in normal cellular functions involving metabolism of phospholipids and produce rate-limiting precursors for the biosynthesis of biologically active lipids, including eicosanoids and platelet-activating factor (3–5). The secretory PLA\(_2\),\(^1\) from human platelets possesses the structural and functional hallmarks of the well known PLA\(_2\)s from pancreas and the venoms of snake (6). It is an extremely stable 14-kDa protein that depends on millimolar amounts of Ca\(^2+\) for activity. In contrast, there is increasing evidence to indicate that the intra-

---

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Biochemistry Research, Lilly Corporate Center, Indianapolis, IN 46285. Tel.: 317-278-1264; Fax: 317-276-5431.

1 The abbreviations used are: PLA\(_2\), phospholipase A\(_2\); Hepes, 4-(2-hydroxyethyl)-1-piperazineneethanesulfonic acid; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; PAGE, polyacrylamide gel electrophoresis.

---

2 After completion of our manuscript Clark et al. (32) reported on the purification of a 110-kDa PLA\(_2\) from U937 cells. It is noteworthy that taking into account all differences in the respective PLA\(_2\) assay systems (e.g. our incorporation of dioleoyl glycerol into phosphatidylcholine liposomes resulting in a 4-fold enhancement of PLA\(_2\) activity versus their addition of 70% glycerol to incubations stimulating the PLA\(_2\) activity >15-fold) the cytosolic PLA\(_2\) prepared by Clark et al. and the one described here exhibit similar specific activities.
fluoride at 1 ml, cells (~10^7) were lysed by nitrogen cavitation using a Parr cell disruption bomb (600 p.s.i. for 20 min at 4 °C). The lysate was centrifuged (150,000 × g, 60 min, at 4 °C), and the supernatant media were collected and filtered through a Millipak (0.22 μm) filter (Millipore). The resulting clear filtrate was applied at a flow rate of 6 ml/min to a column of Q Sepharose (3.2 × 14 cm), pre-equilibrated with buffer A. The column was washed with 25 ml of buffer A containing 150 mM NaCl. After extensive washing, a 1500-ml linear salt gradient was developed from 150–600 mM NaCl in buffer A. Fractions were collected (12 ml), and those with PLA_2 activity were pooled (120 ml) and concentrated to 40 ml. The concentrate was applied to a phenyl-Superose HR 10/10 column, pre-equilibrated in buffer A containing 750 mM NaCl, at a flow rate of 1 ml/min. After extensive washing of the column at the same flow rate, a 120-ml linear gradient of ethylene glycol was generated by mixing buffer A containing 750 mM NaCl and buffer A containing 50% ethylene glycol. The flow rate was 0.75 ml/min, and 4-ml fractions were collected. The active fractions were pooled (60 ml), concentrated to <1 ml, resuspended in 15 volumes of buffer A containing 30% ethylene glycol and 150 mM NaCl, and reconstituted to 1 ml to give a final ethylene glycol concentration of 30%. The concentrate was chromatographed on tandem Superose 12 columns (each 1.6 × 50 cm) for 7.5 h at 200 ml/h, with buffer A containing 50% ethylene glycol and 150 mM NaCl. The flow rate was 0.15 ml/min, and 1.5-ml fractions were collected. The fractions containing PLA_2 activity were pooled (15 ml), concentrated to <0.65 ml, resuspended in 20 ml of buffer A containing 150 mM NaCl (to give a final ethylene glycol concentration of <1%), and reconstituted to <0.5 ml. The concentrate was chromatographed on a Mono Q HR 5/5 column pre-equilibrated with buffer A containing 150 mM NaCl. The column was washed with the same buffer collecting 0.5-ml fractions. The PLA_2 activity eluted after 2 column volumes in 7 ml. In some preparations combined active fractions from the phenyl-Superose column were collected directly to a Mono Q HR 5/5 column previously equilibrated with buffer A containing 150 mM NaCl. PLA_2 activity was eluted at 0.5 ml/min with a linear salt gradient (150–600 mM NaCl) collecting 0.5-ml fractions.

Polyacrylamide Gel Electrophoresis (PAGE)—SDS-PAGE was performed according to Laemmli (14). Reduction PAGE samples were mixed with 1 volume of 5% Tris acetate, pH 7.5, containing 20% glycerol, loaded onto a 6% polyacrylamide minigel prepared in 50 mM Tris acetate, pH 7.5, and electrophoresed at 30 mA for 2 h at 4 °C using 50 mM Tris acetate, pH 7.5, as electrophoresis buffer. For elution of PLA_2, gel slices (1 × 0.3 mm) were transferred into 150 ml of 150 mM NaCl, 3 mM 2-mercaptoethanol, 1 mM EDTA, 25 mM Tris/HCl, pH 8, containing 1% CHAPS (Sigma) and 2 mg/ml BSA and incubated overnight at 4 °C. Proteins were visualized on the gels by silver staining (15). Isoelectric focusing was performed with a Bio-Rad 111 mini isoelectric focusing cell following the instructions of the manufacturer.

Immunoblotting—Mono Q purified PLA_2 (80 ng/lane) was subjected to SDS-PAGE electrophoresis on 8–16% gels (Novex) and transferred to nitrocellulose (Novex) using the Sartoblot electrobolting system (Sartorius). The nitrocellulose sheets were treated with 0.1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 10 mM Tris/HCl, pH 7.5 (buffer B) containing 3% BSA and then incubated with the appropriate immune serum or hybridoma supernatant media in the presence of 0.1% Triton X-100. The sheets were washed with buffer B, incubated for 4 h at room temperature with [3H]labeled sheep anti-mouse IgG Fab′/f (Amersham Corp.), washed again, and exposed to x-ray film for 7.5 h at −70 °C.

Assay of PLA_2 Activity—PLA_2 activity was assayed using sonicated liposomes containing 1-palmitoyl-2-[[3]arachidonoyl-sn-glycerol-3-phosphocholine (52 mCi/mmol, from Du Pont-New England Nuclear) and sn-1,2-dioleoyl glycerol (Avanti Polar Lipids) at a molar ratio of 2:1 as previously described (8) and modified as follows. The assay mixture contained 1 mM CaCl_2, 2 mM 2-mercaptoethanol, 150 mM NaCl, 50 mM Hepes, pH 7.4, and 1 mg/ml BSA. The substrate consisted of 2 μM radiolabeled phosphatidylcholine liposomes (60,000 dpm per incubation) containing 1 μM dioleoyl glycerol. As reported earlier (16), dioleoyl glycerol incorporated into the phosphatidylcholine liposomes inhibited the activity of PLA_2. To determine PLA_2 activity accurately, 4-fold. The pH optimum for the cytosolic PLA_2 is 9, but in an attempt to assay under more physiological conditions, incubations were performed at pH 7.4. At this pH the PLA_2 activity is 2-fold less than at pH 9. To probe for secretory PLA_2 in acid-extracted U937 cells, the Escherichia coli assay system was used (17).

Preparation of Antibodies—Immunization was performed by injecting 8 μg of antigen (phenyl-Superose HR 5/5-purified PLA_2) in Freund's complete adjuvant intraperitoneally into BALB/c mice. Two additional injections with 8 μg of antigen and a third with 4 μg (all emulsified in Freund's incomplete adjuvant) were given at an interval of 3 weeks. A final injection of 9 μg (without adjuvant) was administered 3 days prior to the fusion. Fusion of spleen cells was performed using the LMT system modified by Goding (18). Splenocytes (2 × 10^7) were fused with 150 μl Flurote anti-(mouse-IgG) coated beads (Pandex MS-00-1) overnight at 4 °C with gentle rocking. The beads were washed with 1 ml of 150 mM NaCl, 10 mM Tris/HCl, pH 7.5, containing 1 mg/ml BSA and supplemented with 200 mM NaCl. 100 μl of 10/10 purified PLA_2, added in 100 μl of the same buffer for 4 h at room temperature. PLA_2 assays were performed on supernatants and pelleted beads to estimate mouse antibody-mediated binding of PLA_2 to anti-(mouse-IgG)-coated Pandex beads.

Other Methods—Protein measurements in fractions were made using the BCA protein assay (Pierce Chemical Co.). The protein content of highly purified PLA_2 preparations was estimated using staining intensity on SDS-polyacrylamide gels and/or absorbance at 280 nm. Free Ca^2+ was measured using the Ca^2+ fluorescence probe Fluo-3 (Molecular Probes) and a SLM model 48000 spectrophotometer.

RESULTS

Purification of PLA_2 from U937 Cells—U937 cells do not express detectable levels of the 14-kDa secretory PLA_2 as determined by extracting U937 lysates with acid and assaying PLA_2 activity with the E. coli substrate (6). However, as demonstrated below, U937 cells provide an excellent source for isolation of the cytosolic PLA_2. The purification protocol used to first isolate and identify the PLA_2 is summarized in Table I and Fig. 1. Upon ultracentrifugation of disrupted cells, the PLA_2 activity was recovered in the soluble fraction and the membrane fraction contained less than 10% of the lysate PLA_2 activity. The recovery of PLA_2 in the soluble fraction was critically dependent on the presence of Ca^2+-chelating agents and protease inhibitors. The apparent increase in total PLA_2 activity observed in the cytosolic compared with the lysate fraction could be due to the removal of an endogenous inhibitor of PLA_2 and/or membrane phospholipid competing with the radiolabeled substrate in the PLA_2 assay.

The cytosolic PLA_2 was purified by sequential anion exchange, hydrophobic interaction (high salt), gel filtration, and hydrophobic interaction (low salt) chromatography. First, the 150,000 × g supernatant (fraction I) was applied to an anion exchange column that was developed with a gradient of NaCl. The PLA_2 activity eluted as a single peak with 380–450 mM salt (fraction II) and was subsequently bound to a phenyl hydrophobic interaction column equilibrated with buffer containing 750 mM NaCl. Under these conditions the PLA_2 strongly bound and could only be eluted with ethylene glycol (15–35%) (fraction III). The concentrated active fractions were chromatographed on a Superose 12 column. The PLA_2 activity eluted from the gel filtration column as a single peak with an apparent molecular mass of 70 kDa (fraction IV). The active fractions were concentrated, resuspended, and rechromatographed to lower the ethylene glycol to <1% and applied to a phenyl hydrophobic interaction column equili-
Ca\textsuperscript{2+}-sensitive Cytosolic Phospholipase A\textsubscript{2}

| Fraction | Protein (mg) | Specific PLA\textsubscript{2} activity* (milliunits/mg) | Total PLA\textsubscript{2} activity (milliunits) | Yield (%) | Purification (fold) |
|----------|--------------|--------------------------------------------------------|---------------------------------|-----------|------------------|
| (0) Cell lysate | 15,621       | 0.05                                                  | 748                             | 100       | 1                |
| (I) Cytosol    | 6,066        | 0.31                                                  | 1,882                           | 252       | 6                |
| (II) Q-Sepharose| 293          | 3.74                                                  | 1,095                           | 146       | 74               |
| (III) Phenyl-Superose\textsuperscript{b} | 6.3          | 76.0                                                  | 529                             | 71        | 1,520            |
| (IV) Superose 12 | 0.51         | 517                                                   | 265                             | 35        | 10,340           |
| (V) Phenyl-Superose\textsuperscript{c} | <0.100       | >1,722                                                 | 172                             | 23        | >34,440          |

* PLA\textsubscript{2} activity was determined using the standard assay as detailed under “Experimental Procedures.” The phosphatidylcholine concentration used (2 \textmu M) was subsequently found to be nonsaturating (see “Results”), and the values indicated represent ~67% of the activity attainable with saturating substrate concentrations. PLA\textsubscript{2} activity is expressed as milliunits, where 1 milliunit is defined as the amount of enzyme required to hydrolyze 1 nmol of phospholipid/min.

\textsuperscript{b} Phenyl-Superose columns were equilibrated and PLA\textsubscript{2} activity loaded in buffer containing 750 mM NaCl (III) and 150 mM NaCl (V).

\textsuperscript{c} As described in the text and demonstrated in Fig. 3, further purification (~2-fold) was obtained, when fraction V was subjected to chromatography on a Mono Q HR 5/5 column.

![SDS-PAGE of the purification](image1)

**Fig. 1. SDS-PAGE of the purification.** The pooled fractions from each step were subjected to SDS-PAGE (10% gel) under reducing conditions and then silver-stained. Fractions 1-5 (Table I) are in lanes 1-5, respectively. St, standard proteins.

![Detection of PLA\textsubscript{2} activity after native PAGE of purified PLA\textsubscript{2}](image2)

**Fig. 2. Detection of PLA\textsubscript{2} activity after native PAGE of purified PLA\textsubscript{2}.** Aliquots of purified PLA\textsubscript{2} (50 ng, fraction V) and a standard protein (BSA) were applied in parallel lanes and subjected to electrophoresis under nondenaturing conditions. One lane was sliced into 20 pieces that were eluted with buffer containing 1% CHAPS, and 5-\mu l aliquots of these extracts were assayed for PLA\textsubscript{2} activity as detailed under “Experimental Procedures.” The remaining gel was silver-stained.

Electrophoresis under Native Conditions—In order to investigate whether the 100-kDa protein represented the PLA\textsubscript{2}, the final enzyme preparation (fraction V) was subjected to electrophoresis under native conditions as described under “Experimental Procedures” applying 50 ng of protein in duplicate lanes. The two lanes were cut from the gel. One was stained with silver and the other sliced into small pieces that were eluted with buffer containing 1% CHAPS and assayed for PLA\textsubscript{2} activity. We found that greater than 60% of the applied PLA\textsubscript{2} activity was recovered after elution and that the PLA\textsubscript{2} activity profile coincided with the major, darkly stained high molecular weight band indicating that this band represents the PLA\textsubscript{2} protein (Fig. 2). Isoelectric focusing of the purified PLA\textsubscript{2} revealed that the 100-kDa protein has an isoelectric point of 5.1.

Correlation of PLA\textsubscript{2} Activity and 100-kDa Protein upon Mono Q Chromatography—In some PLA\textsubscript{2} preparations the active fractions from the phenyl-Superose HR 5/5 column were directly loaded onto a Mono Q HR 5/5 column that was subsequently eluted with a NaCl gradient. Fractions from this column containing protein and PLA\textsubscript{2} activity were analyzed by SDS-PAGE. As demonstrated in Fig. 3, only a single major intense band at 100 kDa was observed in the fractions containing PLA\textsubscript{2} activity. Furthermore, the relative intensity of the 100-kDa band precisely paralleled the elution profile of PLA\textsubscript{2} activity from the Mono Q column. It should be noted that this chromatography not only provided an excellent means for concentration of the PLA\textsubscript{2} but also resulted in a further 2-fold increase in the specific activity.

Immunoadsorption of PLA\textsubscript{2}—In a complementary approach to verify that the 100-kDa protein was indeed the PLA\textsubscript{2}, antibodies were raised against the 100-kDa protein and tested for immunoreactivity with the PLA\textsubscript{2} (monitored via its enzymatic activity). Antibodies against the 100-kDa protein were identified in an enzyme-linked immunosorbent assay using Mono Q-purified 100-kDa protein for selection as detailed under “Experimental Procedures.” As demonstrated in Fig. 4A all antibodies were able to immunoprecipitate PLA\textsubscript{2}, albeit with distinct affinities. The antibody-mediated removal of PLA\textsubscript{2} activity from the solution was accompanied by a concomitant appearance of PLA\textsubscript{2} activity in the immunoprecipitate. In addition, the polyclonal antibody P 132 and the monoclonal antibody M 3–1 also reacted strongly with the 100-kDa band in a Western blot indicating that they were able to recognize the protein in its denatured form (Fig. 4B).
To examine the Ca\(^{2+}\) sensitivity of the cytosolic PLA\(_2\) from U937 cells more closely, purified enzyme was assayed in EGTA/CaCl\(_2\) buffers with nanomolar free Ca\(^{2+}\) concentrations. As shown in Fig. 5 there was a low basal PLA\(_2\) activity at 100 nM Ca\(^{2+}\), followed by a sharp 4-fold increase in PLA\(_2\) activity achieving maximal activity at 600 nM Ca\(^{2+}\).

Determination of kinetic parameters of the cytosolic PLA\(_2\) using <2 ng of Mono Q-purified enzyme in assays with increasing amounts (0.8–10 \(\mu\)M) of substrate revealed linear kinetics in a Lineweaver-Burk plot and gave an apparent \(K_m\) of 0.9 \(\mu\)M and a \(V_{max}\) of 12.4 pmol/min corresponding to >6.2 \(\mu\)mol/min/mg.

**DISCUSSION**

We report here the purification to near homogeneity of a cytosolic PLA\(_2\) from U937 cells that responds to Ca\(^{2+}\) concentrations similar to those associated with cytoplasmic free Ca\(^{2+}\) transients observed during cell activation. The conclusion that the PLA\(_2\) is a 100-kDa protein is based on several lines of evidence. (i) The intensity of staining of the 100-kDa protein was proportional to the degree of purification of PLA\(_2\) activity (Fig. 1 and Table I). (ii) When resolved from minor protein contaminants by native gel electrophoresis, the isolated 100-kDa protein band contained all the recovered PLA\(_2\) activity. (iii) The 100-kDa band co-fractionated precisely with the PLA\(_2\) activity on the final purification steps, as shown for the Mono Q column (Fig. 3). (iv) Monoclonal antibodies raised against the 100-kDa protein recognized and were able to immunoprecipitate the PLA\(_2\).

Convincing evidence has been presented for the existence of Ca\(^{2+}\)-independent PLA\(_{2b}\) in intestinal brush-border membranes (\(M\), 98,000) (20) and myocardial cytosol (\(M\), 45,000) (21). Other studies reported the presence of a Ca\(^{2+}\)-dependent PLA\(_2\) in the cytosolic fraction of various cells elucidating some unique functional properties of this enzyme (8–13). Although purification schemes and tentative molecular weights have been reported, the complete characterization of this PLA\(_2\) has been hampered by the low abundance and apparent lability upon purification. Recently, Diez and Mong (13) reported the purification to homogeneity of a Ca\(^{2+}\)-dependent cytosolic PLA\(_2\) from U937 cells. Our results differ dramatically from the results of these authors. They purified the PLA\(_2\) activity only 1900-fold over the cell lysate and concluded, based solely

Functional and Kinetic Properties—The cytosolic PLA\(_2\) was active in the absence of added Ca\(^{2+}\) and was apparently Ca\(^{2+}\)-independent. However, it was inhibited by 5 mM EGTA suggesting a Ca\(^{2+}\) requirement in the submicromolar range.
on the SDS-PAGE analysis of the purification process, that the cytosolic PLA₂ has a molecular mass of 56 kDa. However, the intensity of this 56-kDa band was not proportional to the degree of purification in the final purification steps, and the specific activity of the purified PLA₂ was substantially less than determined in the present study. Therefore, in the final enzyme preparation obtained by Diez and Mong the cytosolic PLA₂ still comprised only a small fraction of the total protein, and the 56-kDa band represented a major contaminating protein and not the PLA₂.

An important physiological role of the Ca²⁺-sensitive cytosolic PLA₂ may be the release of arachidonic acid and generation of lysophospholipids for the biosynthesis of eicosanoids and platelet-activating factor in response to hormones, neurotransmitters, growth factors, autacoids, and other receptor ligands (10, 22, 23). The mechanism(s) responsible for receptor-mediated activation of intracellular PLA₂ have not yet been elucidated, but there is indirect evidence to indicate that various factors may be involved, including elevated cytoplasmic free Ca²⁺ (24), influx of extracellular Ca²⁺ (25, 26), diacylglycerol (16, 27), and protein kinase C (28-30). More directly couple receptors to PLA₂ (23, 31), although the specific GTP-binding proteins involved have not yet been identified. The mechanism(s) responsible for receptor-mediated activation of intracellular PLA₂ have not been elucidated, but there is indirect evidence to indicate that various factors may be involved, including elevated cytoplasmic free Ca²⁺ (24), influx of extracellular Ca²⁺ (25, 26), diacylglycerol (16, 27), and protein kinase C (28-30). More recently, it was suggested that GTP-binding proteins may directly couple receptors to PLA₂ (23, 31), although the specific GTP-binding proteins involved have not yet been identified. The availability of reasonable quantities of cytosolic human PLA₂, as well as PLA₂ antibodies that may serve as immunoprobes, will allow for the molecular studies that are needed to further elucidate the structure and function of this novel intracellular PLA₂. Such knowledge will facilitate delineation of the molecular events that control the activity of the cytosolic PLA₂ and provide a new insight into the involvement of PLA₂ in cellular signal transduction processes and the generation of lipid mediators.

Acknowledgments—We thank Jacques Chiller, Jerome Fleisch, Richard DiMarchi, and Gary Kaiser for advice and continuing support; Paul Hyslop for his help with the measurements of free Ca²⁺; Theresa Gygi and Tony Sheppard for their contributions to this work; Ed Mihelich, Richard Sportsman, LeRoy Baker, Victor Chen, and Bill Heath for valuable discussions; and Jerry Becker and Joe Jakubowski for critical reading of the manuscript.

REFERENCES
1. Dennis, E. A. (1983) in The Enzymes (Boyer, P. D., ed) Vol. 16, pp. 307-353, Academic Press, New York
2. Waite, M. (1987) in Phospholipases (Hanahan, D. J., ed) pp. 111-133, Plenum Publishing Corp., New York
3. Irvine, R. F. (1982) Biochem. J. 204, 3-16
4. Dennis, E. A. (1987) Bio/Technology 5, 1294-1300
5. Snyder, P. (1989) Proc. Soc. Exp. Biol. Med. 190, 125-135
6. Kramer, R. M., Hess, C., Johansen, B., Hayes, G., McGrey, P., Chow, E. P., Tizard, R., and Pepinsky, R. B. (1989) J. Biol. Chem. 264, 5768-5775
7. Kramer, R. M., Johansen, B., Hess, C., and Pepinsky, R. B. (1990) Adv. Exp. Med. Biol. 275, 35-55
8. Kramer, R. M., Jakubowski, J. A., and Deykin, D. (1988) Biochim. Biophys. Acta 959, 269-279
9. Loeb, L. A., and Gross, R. W. (1986) J. Biol. Chem. 261, 10467-10470
10. Gronich, J. H., Bonventre, J. V., and Nemenoff, R. A. (1988) J. Biol. Chem. 263, 16645-16651
11. Wijkander, J., and Sundler, R. (1989) FEBS Lett. 244, 51-56
12. Leslie, C. C., Voelker, D. R., Channon, J. Y., Wall, M. M., and Zelarney, P. T. (1988) Biochim. Biophys. Acta 963, 476-492
13. Diez, E., and Mong, S. (1990) J. Biol. Chem. 265, 14654-14661
14. Lasemil, U. K. (1970) Nature 227, 680-685
15. Wray, W., Boulikas, T., Wray, V. P., and Hancock, R. (1981) Anal. Biochem. 118, 197-203
16. Kramer, R. M., Checani, G. C., and Deykin, D. (1987) Biochem. J. 248, 779-783
17. Kramer, R. M., and Pepinsky, R. B. (1990) Methods Enzymol. 197, 373-381
18. Goding, J. W. (1986) in Monoclonal Antibodies: Principles and Practice, Academic Press, London
19. Ausubel, F. M. (1987) in Current Protocols in Molecular Biology, Wiley-Interscience, New York
20. Gassama-Diagne, A., Fauvel, J., and Chap, H. (1990) J. Biol. Chem. 264, 9470-9475
21. Hazen, S. L., Stuppy, R. J., and Gross, R. W. (1990) J. Biol. Chem. 265, 10457-10460
22. Suga, K., Kawasaki, T., Blank, M. L., and Snyder, F. (1990) J. Biol. Chem. 265, 12363-12371
23. Axelrod, J. (1990) Biochem. Soc. Trans. 18, 503-507
24. van den Bosch, H. (1980) Biochim. Biophys. Acta 604, 191-246
25. Brooks, R. C., McCarthy, K. D., Lapetina, E. G., and Morell, P. (1990) J. Biol. Chem. 265, 20157-20163
26. Balsinde, J., Fernandez, B., and Diez, E. (1990) J. Immunol. 144, 4298-4304
27. Burch, R. M. (1988) FEBS Lett. 234, 283-286
28. Parker, J., Daniel, L. W., and Waite, M. (1987) J. Biol. Chem. 262, 5386-5393
29. Bonventre, J. V., and Swidler, M. (1988) J. Clin. Invest. 82, 165-176
30. Halenda, S., Bangs, J. H., Zavoico, A., Lai, L., and Feinstein, M. B. (1989) Biochemistry 28, 7356-7363
31. Burch, R. M. (1989) Mol. Neurobiol. 3, 155-171
32. Clark, J. D., Milona, N., and Knopf, J. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7708-7712