We have recently identified a cytosolic calcium-independent phospholipase A₂ (PLA₂) that represents the major measurable PLA₂ activity in rabbit proximal tubules (Portilla, D., Shah, S. V., Lehman, P. A., and Creer, M. H. (1994) J. Clin. Invest. 93, 1609–1615). We now report the 3200-fold purification of this PLA₂ to homogeneity from rabbit kidney cortex through sequential column chromatography including anion exchange, hydrophobic interaction, Mono Q, hydroxylapatite, phenyl-Sepharose, and chromatofocusing fast protein liquid chromatography from rabbit kidney cortex. The purified enzyme had a molecular mass of 28 kDa, possessed a specific activity of 1.2 μmol/mg min and a neutral pH optimum, and exhibited a preferential hydrolysis toward sn-2 fatty acid from diradylglycerophospholipids. The purified polypeptide hydrolyzed plasmenylcholine > phosphatidylcholine glycerophospholipids and selectively cleaved phospholipids containing arachidonic acid at the sn-2 position in comparison to oleic acid. Antibodies against the purified protein precipitated all of the soluble calcium-independent PLA₂ activity from rabbit kidney cortex. These data altogether suggest that the 28-kDa protein in the kidney represents a novel class of calcium-independent PLA₂.

Phospholipase A₂ (PLA₂) enzymes (EC 3.1.1.4) are a group of lipolytic enzymes that catalyze the hydrolysis of the acyl ester bond at the sn-2 position of glycerophospholipids resulting in the release of arachidonic acid from membrane phospholipids and the production of lysophospholipids (1, 2). Mammalian PLA₂s are structurally a diverse group of enzymes that include the calcium-dependent secreted PLA₂ forms, characterized by their low molecular weight, high disulfide bond content, and an absolute requirement of calcium during hydrolysis (3, 4). A second recently identified group of intracellular cytosolic PLA₂s comprise the low calcium-requiring, 85-kDa cytosolic PLA₂ (5, 6) and two new calcium-independent PLA₂ enzymes, a 40-kDa plasmalogen-selective, calcium-independent PLA₂ purified from canine myocardium (7–9) and a 80-kDa cytosolic calcium-independent PLA₂ recently purified from the macrophase-like cell line P388D1 (10, 11).

In kidney and heart tissue (12–14), early studies have demonstrated that activation of intracellular PLA₂ represents an important mechanism leading to the development of metabolic alterations that precede cell death during ischemia. Recent observations from our laboratory demonstrated that the majority of measurable PLA₂ activity in freshly isolated rabbit proximal tubules was present in the cytosolic compartment and was calcium-independent and arachidonate-selective (14). These studies also suggest that activation of this calcium-independent PLA₂ enzyme during hypoxia precedes cell death and is accompanied by hydrolysis of endogenous proximal tubule plasmalogen substrates, leading to the generation of arachidonic acid and accompanying phospholipid catabolism (15). Since the release of arachidonic acid and the concomitant accumulation of amphiphilic products derived from PLA₂ activation, such as lysoplasmalogens, have been shown to be important modulators of renal Na⁺-ATPase (16), the purification of the peptide(s) that account for this calcium-independent PLA₂ activity are of obvious importance.

In the present study, we report the purification and characterization of a novel calcium-independent PLA₂ from rabbit kidney cortex. The present data report a 28-kDa protein with calcium-independent PLA₂ activity selective against arachidonoylated plasmalogen phospholipid substrates. This enzyme differs from the previously reported calcium-independent PLA₂ enzymes from other tissues (7–11, 17, 18) in that it is not modulated by ATP and it does not appear to require association with a high molecular weight complex to express catalytic activity. Immunoprecipitation and Western blot analysis confirm the association of a 28-kDa protein with calcium-independent, plasmalogen-selective PLA₂ activity.

**MATERIALS AND METHODS**

Radioactive lipids were purchased from DuPont NEN. Two plasmalogen substrates were used in our studies: 16:0, 18:1 plasmalogen and 16:0, 20:4 plasmalycholine. Synthesis of sn-2 radiolabeled plasmalycholine substrates was performed by dichlohexylcarbodimide-mediated synthesis of radiolabeled fatty acid anhydride, followed by its condensation to the sn-2 hydroxyl of 1-O-hexadec-1'-enyl glycerophospholipid using N,N-dimethyl-4-aminopyridine as a catalyst. Each radiolabeled plasmalycholine glycerophospholipid was purified by high-performance liquid chromatography as described previously (7, 14). To compare activity against arachidonic acid-labeled plasmalycholine substrate, we used commercially available 1-stearyloyl-2-(15α)-docosahexaenoyl-sn-glyco-3-phosphocholine. The phenyl-Sepharose CL-4B, Mono Q HR 10/10 Mono P Chromatofocusing, and FPLC system were from Pharmacia Biotech, Inc. Hydroxylapatite (BioGel HTP) and CM Affi-Gel Blue gel were purchased from Bio-Rad. PLA₂, assay—PLA₂ activity in column chromatographic fractions was assessed by incubating enzyme with 1 μM 1-O-hexadecanoyl-2,3-diacyl-sn-1,2-dioleoyl-sn-glyco-3-phosphocholine (introduced by ethanolic injection (10 μl)) in assay buffer containing 50 mM Tris-HCl, 4 mM EGTA (pH 7.3) at 37°C for 15 min. All measurements of PLA₂ activity were made under initial reaction conditions where the observed rates of fatty acid release were linear with respect to time and protein content.

**Received for publication, January 5, 1996, and in revised form, April 3, 1996.**
Reactions were quenched by the addition of butanol (100 μl) and vortexed; then the organic phase was separated by centrifugation. Released radioabeled fatty acid was separated by silica columns or thin layer chromatography, followed by subsequent quantification by scintillation spectrometry as described previously (14). Lysophospholipase activity was assayed in the phenyl-Sepharose fraction containing PLA2 activity. A 100-μl sample containing 100 μg protein was incubated with 25 mM imidazole, 25 mM NaCl, 1 mM EDTA, 0.5 mM Triton X-100, 10% glycerol, 0.1 mM PMSF, 1 μg/ml pepstatin A, and 1.5 μg/ml leupeptin. The detergent was separated from the medulla and homogenized in KEI buffer using a blender and LSC homogenizer (LH21 Yamamoto) at 900 rpm. The homogenate was centrifuged at 100,000 g for 40 min and solubilized with 10 mM imidazole, 1 mM potassium phosphate, 1 mM EDTA-Tris, 0.5 mM Triton X-100, 10% glycerol, 0.1 mM PMSF, 1 μg/ml pepstatin A, and 1.5 μg/ml leupeptin, pH 6.8.

Phenyl-Sepharose Column—The sample containing the pellet from the ammonium sulfate precipitation step was directly loaded onto a phenyl-Sepharose 4B previously equilibrated with a buffer consisting of 10 mM imidazole, 1 mM potassium phosphate, 1 mM EDTA-Tris, 0.5 mM Triton X-100, 10% glycerol, 0.1 mM PMSF, 1 μg/ml pepstatin A, and 1.5 μg/ml leupeptin, pH 6.8, and 0.15 M ammonium sulfate (HIC-A buffer). After extensively washing with this buffer, the column was developed with 10 mM imidazole, 1 mM potassium phosphate, 1 mM EDTA-Tris, 0.5 mM Triton X-100, 10% glycerol, 0.1 mM PMSF, 1 μg/ml pepstatin A, and 1.5 μg/ml leupeptin, pH 6.8 (HIC-B buffer). The eluent was collected in 10-ml fractions. The active fractions from the phenyl-Sepharose column were pooled and concentrated prior to the next step.

CM-Blue Sepharose Column—A sample obtained from the hydrophobic interaction column was directly loaded onto a CM-Blue column that was previously equilibrated with HIC-B buffer. The column was eluted with buffer C (0.5 mM potassium phosphate, pH 6.8), and PLA2 activity was obtained in the washed fraction.

Mono Q FPLC Column—The active fractions from the CM-Blue Sepharose column were pooled and adjusted to pH 8.0 with 1 M Tris base and passed over a Mono Q HR 10/10 FPLC column (flow, 1 ml/min); that had been pre-equilibrated with a buffer consisting of 25 mM Tris, 10 mM NaCl, 1 mM potassium phosphate, 0.5 mM Triton X-100, 10% dithiotreitol, and 10% glycerol, pH 8.0. The majority of the enzyme activity (peak I) was eluted with the same buffer; however, a second peak of activity (peak II) was eluted in a linear gradient from 0 to 1 M NaCl. Only the active fractions corresponding to peak I were pooled and used for further purification of the enzyme.

Hydroyxylapatite FPLC—The pooled fractions from peak I of the Mono Q column were loaded onto a hydroxylapatite FPLC column that was previously equilibrated with a buffer consisting of 25 mM Tris, 10 mM KCl, 1 mM potassium phosphate, 0.5 mM Triton X-100, 10% dithiotreitol, and 10% glycerol, pH 8.0. Most of the enzyme activity was eluted with a similar buffer (peak A), but a small peak of activity (peak B) was eluted at the beginning of a linear gradient from 0 to 0.5 M potassium phosphate. The fraction corresponding to peak A containing the majority of PLA2 activity was further characterized.

Phenyl-Sepharose FPLC Column—The pooled fractions corresponding to the majority of PLA2 activity (peak A) from the hydroxylapatite column were concentrated and brought up to 0.25 M ammonium sulfate by the addition of solid ammonium sulfate and the pH was adjusted to 6.8 and then loaded onto a phenyl-Sepharose HR 5/5 FPLC column. After loading the sample, the column was washed with a buffer consisting of 10 mM imidazole, 1 mM potassium phosphate, 1 mM EDTA-Tris, 0.5 mM Triton X-100, 10% glycerol, 0.1 mM PMSF, 1 μg/ml pepstatin A, and 1.5 μg/ml leupeptin, pH 6.8, and 0.1 M ammonium sulfate, pH 6.8, until the absorbance at 280 nm dropped to 0.2. The column was then developed with a buffer containing 5 mM EDTA, 50 mM NaCl, pH 7.3, at 30 mA for 30 min. A 6-mm-wide strip was cut at the beginning of the gradient and eluted in a linear gradient from 0.1 to 0 M ammonium sulfate. This active fraction was used to study the pH dependence of PLA2 activity. The assay buffers to test pH preference contained 5 mM EDTA, 50 mM NaCl, 50 mM Bis-Tris, and a pH gradient from 4 to 10 adjusted with HCl and Tris.

Native gel electrophoresis was carried out following the method of Kramer et al. (6). Briefly, 6% separating gel, pH 7.3, and 4% stacking gel, pH 6.8, of polyacrylamide in 40 mM Tris acetate were casted in a Bio-Rad Mini Gel II with 1.5-mm-thick spacer and prep-comb. The purified fraction containing PLA2 activity obtained from the phenyl-Sepharose FPLC column was concentrated with Centri-Prep (Amicon), and an aliquot of the preparation corresponding approximately to 20 μg of protein was mixed with 0.2 g of SM-2 (Bio-Rad) and kept on ice for 2 h to remove the Triton X-100 in the sample. The detergent-removed sample was directly loaded onto the gel and run in 50 mM Tris acetate, pH 7.3, at 30 mA for 100 min at 4 °C. A 6-mm-wide strip was cut at the position of the 28-kDa band and transferred to a mini test tube. After rinsing with running buffer, the slices were incubated overnight on a rotating plate in 150 μl of a buffer containing 4 mM EGTA, 0.5 mM Triton X-100, 0.2 mM ATP, and 1 mM dithiotreitol, pH 7.5. PLA2 activities were measured using a 100-μl volume of each sample.

**RESULTS**

Purification of Rabbit Kidney Cytosolic Calcium-independent PLA2—Since approximately 90% of calcium-independent PLA2...
activity was present in the cytosol of rabbit kidney cortex when compared to the membrane-associated fraction, the purification of this enzyme activity was performed using the soluble fraction of rabbit kidney cortex.

Similar to Ackermann et al. (10), the inclusion of Triton X-100 (0.5 mM) in the buffers used for the purification helped to stabilize the rabbit kidney calcium-independent PLA₂ activity. Cytosolic rabbit kidney calcium-independent PLA₂ was purified to homogeneity by sequential hydrophobic interaction, CM-Blue Sepharose affinity chromatography, Mono Q FPLC, hydroxylapatite FPLC, phenyl-Sepharose FPLC, and chromatofocusing. Typical column profiles are shown in Fig. 1, and data from the purification of a single enzyme preparation are shown in Table I.

The first chromatographic step used a phenyl-Sepharose CL-4B hydrophobic interaction column, and the majority (80–90%) of the PLA₂ activity retained by the column was quantitatively eluted by application of a low salt buffer without ammonium sulfate. A typical chromatographic profile is shown in Fig. 1A. Since previous studies have identified the potential association of ATP with calcium-independent PLA₂, we explored the interaction of calcium-independent PLA₂ with various immobilized nucleotide affinity matrices. Kidney calcium-independent PLA₂ activity did not bind to ATP-agarose or Blue Sepharose affinity matrices, and more than 90% of the activity was recovered in the void volume. Because of the significant amount of protein without PLA₂ activity that was retained by the Blue Sepharose column, we used this step to further purify calcium-independent PLA₂ activity after the hydrophobic interaction column.

PLA₂ activity was further purified by application of the Blue Sepharose eluate onto a FPLC-Mono Q anion exchange column that was subsequently eluted using a linear NaCl gradient. A major peak of activity was obtained in the washed fraction (Fig. 1B). This step achieved a 5–6-fold purification with recovery of at least 60–70% of the loaded PLA₂ activity. The Mono Q active fraction was directly loaded onto an FPLC-hydroxylapatite column, and PLA₂ activity was eluted with a linear K[PO₄]₃ gradient, as described under “Materials and Methods” (Fig. 1C). Two peaks of PLA₂ activity consistently eluted from this column. Peak A eluted in the wash fraction and represented 60–90% of the loaded activity. Peak B eluted between 0 and 200 mM phosphate, along with other proteins (Fig. 1C), and comprised only 10–15% of the loaded activity. Therefore, the PLA₂ activity in peak B was discarded, and the purified enzyme activity in peak A was characterized further. PLA₂ activity eluted from the hydroxylapatite column was further purified by phenyl-Sepharose hydrophobic interaction. PLA₂ activity was eluted off this column in a single peak at low salt buffer (Fig. 1D). Typical recoveries from this column ranged from 50–80% (Fig. 1D). This calcium-independent PLA₂ activity was purified over 3000-fold with a final specific activity roughly of about 1 μmol/min/mg protein. Assessment of purity was carried out using SDS-PAGE under reducing conditions. In every purification, the final fraction containing calcium-independent phospholipase A₂ activity obtained from the phenyl-Sepharose column displayed a single major band with an apparent molecular mass of 28 kDa (Fig. 2).

The 28-kDa Protein Purified to Near Homogeneity Has Calcium-independent PLA₂ Activity—To investigate whether the 28-kDa protein contained PLA₂ activity, the enzyme preparation obtained from the phenyl-Sepharose column was subjected to electrophoresis under native conditions as described under “Materials and Methods.” Approximately 20 μg of protein were applied to one preparative gel. The two lanes were cut from the gel. One was stained with Coomassie Blue, and the other was sliced into small pieces, incubated overnight with buffer containing Triton X-100, and assayed for PLA₂ activity. Greater than 60–70% of the applied PLA₂ activity was recovered, and the profile for calcium-independent PLA₂ activity coincided with the major, darkly stained 28-kDa protein, indicating that this band represents the PLA₂ protein (Fig. 3). The corresponding band stained by Coomassie Blue was cut out, electroeluted, and run on SDS-PAGE. This fraction eluted as a single band at 28 kDa (Fig. 4, lane 9).

Chromatofocusing of Phenyl-Superose Fraction Containing Calcium-independent PLA₂ Activity—To further characterize the pl of the calcium-independent PLA₂ activity obtained from the phenyl-Superose column and examine its purity, chromatofocusing of this fraction was performed. PLA₂ activity was eluted by the generation of a shallow pH gradient, which resulted in a single sharply focused peak of activity with an apparent isoelectric point of 5.6 (Fig. 1E). When we examined the proteins at various stages of purification on SDS-PAGE, we found that the intensity of the 28-kDa band increased as the preparation was enriched in activity (Fig. 2).

Further characterization of the molecular weight of the fraction containing calcium-independent PLA₂ activity was accomplished by gel filtration using a Superose 12 column. Gel filtration molecular weight markers were used to calibrate the column. The active fraction containing PLA₂ activity corresponded to M₉, 19,000. However, subsequent separation of this protein by SDS-PAGE demonstrated again that the calcium-independent PLA₂ activity purified corresponded to a 28-kDa protein. Therefore, it is likely that interaction of this protein with the gel filtration column may account for the estimated smaller size in this column. Similar observations have been described for low molecular weight PLA₂ when separated by gel filtration columns. Fig. 4 shows that the fraction containing PLA₂ activity obtained from the phenyl-Superose column, gel filtration column, chromatofocusing, and electroelution from native gel (Fig. 4, lanes 3, 4, 7, and 9, respectively) all contain the 28-kDa protein.

Characterization of the Substrate Specificity of Purified Calcium-independent PLA₂ Activity—Characterization of the phospholipid substrate specificity of purified kidney cytosolic

---

**Table I**

Typical purification of the rabbit kidney calcium-independent PLA₂

| Protein            | Total activity | Specific activity | Purification | Yield |
|--------------------|----------------|------------------|--------------|-------|
| Homogenate         | 4,537          | 1,642            | 0.362        | 1     | 100  |
| Ammonium sulfate   | 2,014          | 793              | 0.394        | 1.1   | 50   |
| Phenyl-Sepharose   | 628            | 606              | 0.966        | 2.7   | 36   |
| Blue Sepharose     | 483            | 938              | 1.942        | 5.4   | 57   |
| Mono Q FPLC        | 241            | 575              | 2.386        | 6.6   | 35   |
| Hydroxylapatite FPLC | 23            | 261              | 11.367       | 31.4  | 16   |
| Phenyl-Superose FPLC | 0.18          | 208              | 1,160        | 3,204 | 12   |

The data are taken from a single enzyme preparation starting with 20 rabbit kidneys and carried through each purification step.
phospholipase A₂ was performed by kinetic analysis of the phenyl-Superose hydrophobic interaction eluent, which yielded more than 3000-fold purification and a specific activity of 1.2 μmol/min/mg protein.

Examination of the choline glycerophospholipid subclass specificity of the purified enzyme revealed that hydrolysis of ³H-labeled plasmenylcholine substrates was more rapid than the hydrolysis of ³H-labeled phosphatidylcholine substrates. Comparison of the PLA₂ activity using phosphatidylcholine molecular species containing palmitate at the sn-1 position and

Fig. 1. Typical column chromatography profiles of the calcium-independent PLA₂ from rabbit kidney cortex. A, phenyl-Sepharose CL-4B; B, Mono Q FPLC; C, hydroxylapatite FPLC; D, phenyl-Superose FPLC; E, chromatofocusing FPLC.
either oleic or arachidonic acid at the sn-2 position as substrates demonstrated a 4–5-fold preference for cleavage of arachidonate over oleate, and this occurred predominantly at a neutral pH (Fig. 5). To determine if the purified enzyme was specific for the hydrolysis of the sn-2 position of phospholipids, we tested also for lysophospholipase activity using 1-[1-14C]-palmitoyl-lyso-PC. The enzyme demonstrated very little lysophospholipase activity when compared to sn-2-labeled substrates. Thus, it appears as if the hydrolysis of fatty acid detected in our assay was specific for the sn-2 rather than the sn-1 position.

Characterization of the 28-kDa Protein in the Purified Preparation—To verify that the 28-kDa protein was indeed responsible for the PLA2 activity, mouse polyclonal antibodies were raised against the rabbit kidney 28-kDa protein and tested for immunoreactivity with PLA2. As shown in Fig. 6A, antibodies against the 28-kDa protein were able to immunoprecipitate soluble rabbit kidney cortex PLA2 obtained from the phenyl-Sepharose column. In addition, the mouse polyclonal antibody reacted strongly with the 28-kDa band in a Western blot, indicating that they were able to recognize the protein in its denatured form (Fig. 6B).

The 28-kDa Protein Binds to the sn-2 Position of Labeled Phospholipid Substrate—To determine whether the isolated 28-kDa protein could cleave fatty acid esterified to the sn-2 position of phosphatidylcholine, the 28-kDa protein isolated from the hydrophobic interaction column was incubated with arachidonic acid, 1-palmitoyl-2-[1-3H]arachidonoyl-sn-glycero-3-phosphocholine and [3H]oleate-labeled plasmenylcholine. Subsequent SDS-PAGE and autoradiogram demonstrated a predominant 28-kDa band that was labeled with both arachidonic acid and arachidonate containing phosphatidylcholine substrate. Calcium did not affect the formation of the arachidonoyl-enzyme intermediate (Fig. 7).

**DISCUSSION**

The results presented here represent the first purification of a novel calcium-independent PLA2 activity from rabbit kidney, which has specificity for cleavage of the sn-2 acyl linkage in diradyl glycerophospholipids. Rabbit kidney cortex calcium-independent PLA2 activity.
independent PLA2 activity was the major measurable enzyme activity in the kidney, and it was very low in abundance, requiring more than 3200-fold purification to reach apparent homogeneity. The degree of purification of kidney calcium-independent PLA2 was facilitated by the use of small amounts of Triton X-100. This property represents a major difference in the ATP-affinity column matrices in the presence or absence of Triton X-100. This property represents a major difference in the scheme of purification from the previously purified calcium-independent PLA2 enzymes. The major steps of purification in our experiments were obtained using hydrophobic interaction columns as well as hydroxyapatite columns, suggesting that we had an enzyme that readily interacts on a lipid-water interface. Substrate specificity of the purified protein demonstrated that rabbit kidney PLA2 selectively hydrolyzed plasmalogen substrates and arachidonylated glycerophospholipids.

Our final step of purification demonstrated the enrichment of a single 28-kDa protein on SDS-PAGE visualized by Coo massie Blue staining. To further confirm that the molecular mass of calcium-independent PLA2 corresponded to this protein, we subjected this fraction to native gel electrophoresis analysis and excised the band that corresponded to this protein. This observation suggests that the major protein isolated from the hydrophobic interaction column, which has an estimated size of 28 kDa, had indeed intrinsic calcium-independent PLA2 activity. Chromatofocusing analysis of this fraction as well as isoelectrical focusing gel again confirmed the presence of a 28-kDa protein with a pI of 5.6 with calcium-independent PLA2 activity. In addition, the immunoprecipitation of PLA2 activity by a mouse polyclonal antibody against the 28-kDa protein generated in our laboratory further suggests that this protein is associated with calcium-independent PLA2 activity in the kidney.

Our studies also show that the incorporation of radiolabeled sn-2 fatty acid into the isolated 28-kDa peptide was highly selective for arachidonic acid. Furthermore, calcium was not an obligatory cofactor in the formation of the arachidonoyl-enzyme intermediate. This observation suggests that rabbit kidney PLA2 may catalyze the cleavage of the ester linkage through the formation of an acyl intermediate, as described previously for other mammalian lipases (19, 21-22).

The PLA2 activity purified in the present study is unique when compared to other previously described calcium-independent PLA2s. The two best characterized calcium-independent PLA2 activities correspond to the plasmalogen-specific canine myocardial enzyme (7-9) and the recently described P388D1 macrophage cell line cytosolic calcium-independent PLA2 (10, 11). Both of these enzymes differ from our purified enzyme by the following features: 1) they both bind ATP-affinity matrices constituting this one, the major step of purification; 2) both appear to be associated with high molecular weight complexes, the myocardial enzyme associated with a Mr 400,000 protein likely to be a phosphofructokinase, and the macrophage enzyme associated to a Mr 330,000 protein by radiation-inactivation studies; 3) both have different molecular sizes, the myocardial one of 40 kDa, and the P388D1 of approximately 80 kDa. Nevertheless, the final purified form of rabbit kidney calcium-independent PLA2 shares some similarities with the myocardial enzyme. For example, the rabbit kidney calcium-independent PLA2 has a substrate preference for plasmalogen > diacyl PC and also displayed a fatty acid preference for arachidonyl > palmitoyl. Furthermore, internal amino acid sequence analysis of three peptides obtained from trypsin digestion of the 28-kDa protein (results not shown) do not match any protein sequence found in either GenBank or Protein Data Bank. This observation further excludes the possibility of the 28-kDa rabbit kidney protein being related to either the platelet activating factor-acetyl hydrolase (23, 24) or 14-3-3 proteins (19).

We have recently demonstrated that rabbit proximal tubule plasmalogens highly enriched in arachidonic acid represent the primary target for accelerated phospholipid hydrolysis during
hypoxia (15). Since the rabbit kidney cortex PLA₂ activity purified here could potentially have direct physical access to the proximal tubule membranes and selectively hydrolyzes plasmalogen substrate and arachidonylated glycerophospholipids, this phospholipase has the catalytic potential to hydrolyze the predominant proximal tubule phospholipid components enriched in arachidonic acid during ischemia. Accordingly, the activation of this polypeptide is expected to result in the selective release of arachidonic acid and catabolism of proximal tubule phospholipids similar to that seen during ischemia-hypoxic cell injury to the kidney. Although the mechanisms of activation and regulation of this new group of calcium-independent PLA₂ remain unknown, future efforts to identify the molecular mechanism responsible for activation of calcium-independent PLA₂ should provide insight into the mechanisms leading to cell death during renal ischemia.

Acknowledgments—We thank Dr. Michael Creer for providing the ³H-labeled plasmenylcholine substrates, Dr. Richard W. Gross for critical review of these studies, and Ellen Satter for secretarial assistance.

REFERENCES
1. Dennis, E. A. (1983) in The Enzymes (Boyer, P. D., ed) pp. 307–353, Academic Press, New York
2. Verheij, H. M., Slotboom, A. J., and de Haas, G. H. (1981) Rev. Physiol. Biochem. Pharmacol. 91, 91–203
3. Dennis, E. A. (1994) J. Biol. Chem. 269, 13057–13060
4. Walte, M. (1987) Handbook of Lipid Research, Vol. 5, Plenum Publishing Corp., New York
5. Clark, J. D., Milona, N., and Knoff, J. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7708–7712
6. Kramer, R. M., Roberts, E. F., Manetta, J., and Putnam, J. E. (1991) J. Biol. Chem. 266, 5265–5272
7. Wolf, R. A., and Gross, R. W. (1985) J. Biol. Chem. 260, 7295–7303
8. Hazen, S. L., Stuppy, R. J., and Gross, R. W. (1990) J. Biol. Chem. 265, 10622–10630
9. Hazen, S. L., and Gross, R. W. (1991) J. Biol. Chem. 266, 14526–14534
10. Ackermann, E. J., Kemper, E. S., and Dennis, E. A. (1994) J. Biol. Chem. 269, 9227–9233
11. Ackermann, E. J., Conde-Frieboes, K., and Dennis, E. A. (1995) J. Biol. Chem. 270, 445–450
12. Hazen, S. L., Ford, D. A., and Gross, R. W. (1991) J. Biol. Chem. 266, 5629–5633
13. Hazen, S. L., and Gross, R. W. (1993) J. Biol. Chem. 268, 9892–9900
14. Portilla, D., Shah, S. V., Lehman, P. A., and Creer, M. H. (1994) J. Clin. Invest. 93, 1609–1615
15. Portilla, D., and Creer, M. H. (1995) Kidney Int. 47, 1087–1094
16. Schonfeld, M., Mandel, L. J., Creer, M., and Portilla, D. (1994) J. Am. Soc. Nephrol. 5, 908
17. Fukushima, T., and Serrero, G. (1994) Lipids 29, 163–169
18. Nassama-Diagne, A., Fauvé, J., and Chap, H. (1989) J. Biol. Chem. 264, 4910–4915
19. Zupan, L. A., Steffens, D. L., Berry, C. A., Landt, M., and Gross, R. W. (1992) J. Biol. Chem. 267, 8707–8710
20. Portilla, D., Mandel, L. J., Bar Sagi, D., and Millington, D. S. (1992) Am. J. Physiol. 263, F354–F360
21. Gross, R. W., Drisdell, R. C., and Sobel, B. E. (1983) J. Biol. Chem. 258, 15165–15172
22. Jauhiainen, M., and Dolphin, P. J. (1986) J. Biol. Chem. 261, 7032–7043
23. Hattori, M., Hideki, A., Tsujimoto, M., Arai, H., and Inoue, K. (1994) Nature 370, 216–218
24. Tjoelker, L. W., Wilder, C., Eberhardt, C., Stafforini, D. M., Dietsch, G., Schimpf, B., Hooper, S., Le Trong, H., Coussens, L. S., Zimmerman, G. A., Yamada, Y., Kintyra, T. M., Prescott, S. M., and Gray, P. W. (1995) Nature 374, 549–553
