The C Terminus of Mammalian Phospholipase D Is Required for Catalytic Activity*

Mu-Ya Liu, Stephen Gutowski, and Paul C. Sternweis‡

From the Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9041

The activity of phospholipase D (PLD) is regulated by a variety of hormonal stimuli and provides a mechanistic pathway for response of cells to extracellular stimuli. The two identified mammalian PLD enzymes possess highly homologous C termini, which are required for catalytic activity. Mutational analysis of PLD1 and PLD2 reveals that modification of as little as the C-terminal threonine or the addition of a single alanine attenuates activity of the enzyme. Protein folding appears to be intact because mutant enzymes express to similar levels in Sf9 cells and addition of peptides representing the C-terminal amino acids, including the simple hexamer PMEVWT, restores partial activity to several of the mutants. Analysis of several mutants suggests a requirement for the hydrophobic residue at the −2 position but not an absolute requirement for the hydroxyl side chain of threonine at the C terminus. The inability of peptides amidated at their C termini to effect restoration of activity indicates the involvement of the C-terminal α carboxyl group in functional activity of these enzymes. The ability of peptides to restore activity to PLD enzymes mutated at the C terminus suggests a flexible interaction of this portion of the molecule with a catalytic core constructed on conserved HKD motifs. Participation of these C termini residues in either stabilization of the catalytic site or the enzymatic reaction itself remains to be determined. This requirement for the C terminus provides an excellent potential site for interaction with regulatory proteins that may either enhance or down-regulate the activity of these enzymes in vitro.

Phosphatidic acid plays a prominent role in phospholipid metabolism (1) and signal transduction. As a signaling molecule, phosphatidic acid is produced through the action of phospholipase D (PLD) in response to a variety of cellular stimuli and acts directly as a second messenger or as a precursor for the formation of another second messenger, diacylglycerol (see Refs. 2–5 for reviews).

Understanding of mechanisms by which mammalian PLD enzymes are regulated has been greatly accelerated by the development of a sensitive in vitro assay for the activity (6, 7) and identification of two mammalian genes (8–10). The regulation of PLD activity in vitro allowed the identification of four distinct mechanisms for stimulation of the enzymes. Phosphatidylinositol 4,5-bisphosphate (PIP₂) was the first activator and a key element in establishing assays for the enzymes (6, 11–13). This was followed by the elucidation of activation by members of both the Arf (6, 14) and Rho (15–19) families of small monomeric GTPases and by classical isoforms of protein kinase C (PKC) via a phosphorylation independent pathway (20, 21). Subsequent studies with recombinant proteins in vitro have shown that PLD1 responds to all of these activators (22), while PLD2 is only stimulated by PIP₂ and Arf (9, 23, 24). Elucidation of these regulatory mechanisms and investigations to determine which of these pathways are utilized in the cellular milieu in response to extracellular stimuli have been reviewed (see Refs. 25–27 for examples).

The availability of the cloned enzymes has also resulted in multiple attempts to define interaction sites of PLD isozymes with different activators. Constructs of PLD1 missing the N-terminal third of the protein yield selective attenuation of regulation by PKC (28–30), whereas stimulation by other activators remains intact. A site for interaction with Rho is located in the C-terminal third of the protein based on interaction of C-terminal regions of PLD1 with RhoA (31, 32) and selective attenuation of RhoA regulation by inclusion of PLD1 C-terminal constructs in assays (31). In contrast, regions of PLD1 involved in regulation by Arf have not been identified.

The identification of catalytic portions of PLD1 benefited from homology of the enzyme with other lipid transferases (33). The predicted participation of both conserved HKD (HXXKXXDXXXXXGXXN) motifs in catalytic function was verified by expression of mutagenized proteins (32). Extensive mutagenesis in other regions of the protein has led to a variety of catalytically defective enzymes (30), but the role of altered regions in catalysis, stability of the enzyme, or regulation remains to be defined.

Although modification of the N terminus of PLD1 allows apparent normal activity, modification of the C terminus of the mammalian PLD enzymes results in proteins that lack catalytic activity (29). In this study, we demonstrate that PLD1 and PLD2 with alterations in the C terminus are inactive but functionally intact. Partial restoration of the activity of these mutant enzymes by addition of various peptides strongly suggests that the C-terminal threonine of these mammalian enzymes is a required component of the active site or functionally helps stabilize the active site for hydrolytic activity.

**EXPERIMENTAL PROCEDURES**

*Materials—* Dipalmitoyl phosphatidylcholine (DPPC) and bovine brain phosphatidylethanolamine were purchased from Avanti. PIP₂ was purchased from Roche Molecular Biochemicals. 1-α-D-choline-meth-
yl-[3H]Dipalmityl PC was purchased from DuPont. Peptides and modified peptides were synthesized by Gensys Biotechnologies or the bio-synthesis facility of the Howard Hughes Medical Institute (University of Texas Southwestern Medical Center, Dallas, TX).

Assay of Synthetic peptides were cross-linked to tuberculin-purified protein derivate (Statens Seruminstitut) with glutaraldehyde (34). The conjugates were used to immunize rabbits (35). Sera were collected and screened by immunoblot analysis.

Preparation of the Protein Activators of PLD—Recombinant Arf proteins were expressed in bacteria and purified through two steps: batch elution from DEAE-Sephasel and a gel-filtration column as described (36). A plasmid encoding hexahistidine-tagged protein kinase Cα (6H-PKCα) was constructed using the polymerase chain reaction. Briefly, synthetic oligonucleotides encoding the amino acid sequence, NASMAGHHHHHHHGGALDR were inserted into the hinge region of the leptin PKCα gene in place of amino acids 307–327. The 6H-PKCα was expressed in Sf9 cells following infection with recombinant baculovirus and purified through consecutive steps of nickel-nitrilotriacetic acid affinity and Hi-Trap heparin chromatography. The final purity of proteins was evaluated by separation on SDS-polyacrylamide gel electrophoresis (37) and detection by staining with silver. Protein concentration was determined by staining with Amido Black (38).

Preparation of Light Membrane Fractions—Wild-type and mutant PLD enzymes were expressed in Sf9 cells grown in monolayer. About 1 × 10^6 Sf9 cells were infected with 0.5 ml of high titer recombinant virus (~1 × 10^9/ml) for 48 h. Cells were washed and harvested in buffer containing 20 mM NaHepes, pH 7.5, 1 mM EDTA, 1 mM diithiothreitol, and protease inhibitors (21 μg/ml N-succinyl-L-tosylamide-2-phenylethyl chloromethyl ketone, 21 μg/ml tosylphenylalanyl chloromethyl ketone, 21 μg/ml phenylmethylsulfonyl fluoride, 12.5 μg/ml pepstatin A, and 21 μg/ml N-succinyl-L-arginine methyl ester). The particulate materials of cells were sedimented by centrifugation at 40,000 × g for 30 min at 4 °C. Membranes that migrated to the interface between 20% and 40% sucrose were collected as the light membrane fraction enriched in PLD activity.

Preparation of Gel-filtered Substrate Vesicles—Substrate vesicles made by gel filtration have proven to be more stable and provide lower background for measurement of PLD activities. The procedure given here will be presented in more detail elsewhere.

A mixture of phosphatidylethanolamine (600 μM), PIPE (300 μM), DPPC (60 μM), and [3H]DPPC (about 500 cpm/pmol) was prepared and dried under a stream of nitrogen. The lipids were resuspended in PLD reaction buffer (50 mM NaHepes, pH 7.5, 80 mM KCl, 3 mM EGTA, and 1 mM DTT) containing 1% n-octyl-α-D-glucopyranoside and sonicated for 2 min at room temperature to form mixed micelles. Vesicles were formed by gel-filtration of the micelles through a column (0.7 cm × 30 cm) of AcA43 (BioGel), which had been equilibrated with PLD reaction buffer before loading. Fractions (~300 μl each) were collected and the peak of radioactive vesicles pooled and used as substrate for the assay of PLD activity. The recovery of [3H]DPPC and unlabelled lipids was uniform and ranged from 30% to 40%.

Assay of PLD Activity—Except for the preparation of substrate vesicles, the assay of PLD activity was measured by the release of [3H]choline as described previously (6, 7). Briefly, aliquots of light membrane fractions were mixed in 30 μl of a buffer containing 50 mM NaHepes, pH 7.5, 80 mM KCl, 3 mM EGTA, 1 mM DTT, and 10 μM GTPγS. When indicated, Arf and PKCα were added to final concentrations of 5 μM and 50 nM, respectively. Reactions were started by the addition of substrate vesicles and incubated at 30 °C for 60 min. Reactions were stopped by the addition of 200 μl of 10% trichloroacetic acid and 100 μl of 10 mg/ml bovine serum albumin. Samples were centrifuged to remove precipitated lipids and proteins and the supernatants analyzed for released [3H]choline by liquid scintillation spectroscopy. Unless indicated otherwise, all assays represent the average of duplicate samples.

Comparison of Mutant—The plasmid encoding human phospholipases D1 and D2 were kindly provided by Michael Frohman and Andrew Morris (8, 9). DNA encoding PLD1 was subcloned into pFast-BacTHb vector (Life Technologies, Inc.), and the fragment from SphI to the C terminus of the coding region was deleted and replaced with mutated products generated by polymerase chain reactions. The 3′ primers used for PCR reactions contained mutations and new stop codons to give the altered amino acid sequences indicated. All mutations were confirmed by DNA sequencing. Recombinant viruses were obtained by transformation of DH10Bac cells and selection by blue-white screening as described by the manufacturer. Viral DNA was isolated and used to transfect Sf9 cells for creation and amplification of baculoviruses encoding the mutated PLDs.

RESULTS

PLD1 with a Modified C Terminus Is Catalytically Compromised—Recombinant PLD1 that has been modified with 6 histidines at its C terminus was inactive when expressed in Sf9 cells (Fig. 1). Inactive enzyme was also obtained if the four C-terminal amino acids were deleted from the enzyme (PLD1-C4). This inactivity is due to an apparent catalytic deficiency, as activity could be partially restored to the expressed enzymes by addition of peptides representing the C terminus of PLD1 (Fig. 1). Although the addition of peptides at millimolar concentrations had little effect on activity of the wild-type enzyme, a peptide encoding ST, which represents the last 15 amino acids of PLD1, restored about 15% of wild-type activity to both enzymes that had been modified at the C terminus. At higher concentrations, all of the peptides had inhibitory effects on the assay of PLD activity. A shorter peptide (PT) containing only the last 6 amino acids of the C terminus could also restore activity to the inactive enzymes but was less potent than the longer ST peptide.

Fig. 1. Comparison of different peptides affecting wild type PLD1 and two PLD1 mutants. Assays were carried out with 3 μg of membrane protein, increasing concentrations of the peptides, PLDpepST, PLDpepSA, or PLDpepPT, as indicated, and in the presence of 50 mM PKCα, 5 μM Arf, and 10 μM GTPγS as described under “Experimental Procedures.”

2 W. D. Singer and P. C. Sternweis, manuscript in preparation.

3 X. Jiang, S. Gutowski, W. D. Singer and P. C. Sternweis, submitted for publication.
tide. In contrast, a peptide (SA) representing amino acids that were N-terminal to peptide PT was ineffective. This indicates that the C-terminal residues of PLD1 play a crucial role in PLD activity.

The concentrations of peptide that restore activity to mutationally compromised PLD are substantial, albeit specific. A more potent effect can be obtained if a myristoylated peptide is utilized. This is shown in Fig. 2. The acylated peptide shows an increase in potency of over 1000-fold but not a real increase in efficacy at the concentrations that can be utilized. The increase in potency can probably be attributed to localization of the peptide to the surface of substrate vesicles, thus allowing for more efficient competition of the peptide with the endogenous C terminus of PLD1.

Immunological Characterization of Expressed PLD1 Mutants—Further mutational analysis of PLD1 and PLD2 was done to investigate the role of C-terminal residues more fully. The mutant enzymes used in this study are described in Fig. 3A. All of the mutant enzymes could be expressed in Sf9 cells and were enriched in light membrane fractions. Antibodies used to detect the PLD1 proteins are described in Fig. 3B, and the level of expression of each mutant is shown in Fig. 4 (A and B). Antisera raised to peptides representing the N terminus (Q054) or two internal sequences of PLD1 (R654, R653) detected all of the expressed PLD1 enzymes (Fig. 4A) but not PLD2. In contrast, antisera to a peptide representing the C terminus of PLD1 recognized both PLD1 and PLD2, but discriminated strongly among the constructs with mutations near the C terminus. The recognition of both PLD1 and PLD2 by the C-terminal sera is indicative of their highly homologous C termini, whereas the N-terminal and internal peptides represent more divergent sequences between the two enzymes.

The C-terminal directed antisera recognize the very C terminus of the molecule. This is demonstrated by competition of this interaction with a peptide (PLDpepPT) representing the last 6 amino acids of PLD1 but not with a peptide (PLDpepSA) that represents the N-terminal half of the peptide used for production of the antisera (Fig. 4B). Further definition of this recognition is demonstrated by the mutant proteins. The C-terminal antisera did not detect proteins that had either deletions at the C terminus or the addition of either a 6-histidine tag (PLD1) or a single alanine residue (PLD2). In addition, mutation of the C-terminal tryptophan to alanine or the C-terminal threonine to lysine, glutamic acid, or alanine resulted in failure of recognition by the antisera. Only two conservative mutations in these residues, W1073F and T1074S, resulted in retention of detection. In total, these results suggest that the recognition site for the C-terminal antigenic region includes a hydrophobic pocket for the tryptophan and recognition of the threonine’s hydroxyl group and C-terminal carboxyl moiety. The latter is suggested by the failure of the antisera to recognize the enzymes with residues added to the C terminus.

Effect of C-terminal Mutations on the Activity of PLD1 and PLD2—The activities of various recombinant PLD1 enzymes...
are shown in Fig. 5A. Preparations derived from either expression of PLD1 containing the K466A mutation, which prevents catalytic activity, or expression of G protein bg subunits, provide controls for measurement of endogenous Sf9 PLD activity in these preparations of membranes. Truncation of the last two or four amino acids yielded inactive enzyme. Of six point mutations in the two C-terminal amino acids, three resulted in inactive proteins while three retained partial activity. Modification of tryptophan to phenylalanine was tolerated very well, but substitution of alanine in this position resulted in total loss of activity. This indicates that the aromatic residue is important for C-terminal function. Replacement of Thr-1074 with either serine or alanine allowed expression of about 20% of wild type activity. In contrast, substitution of this threonine with the charged residues, lysine or glutamic acid, completely attenuated activity of the enzyme.

Restoration by peptide of 20% of wild type activity to the inactive truncated proteins, PLD1-C_D2 and PLD1-C_4, PLD1-W1073A, and PLD1-CHis 6 indicates the these enzymes are expressed in a functionally intact form but are unable to maintain an active catalytic site. The inability of the peptide to restore activity to the PLD1-T1074K and PLD1-T1074E mutants may reflect the production of incorrectly folded protein but more likely reflects an inability of the peptide to compete with a more stable association of the endogenous C terminus (see “Discussion”). The addition of a C-terminal peptide to expressed enzymes with endogenous activity resulted in inhibitions of about 15–30%; this is due to use of the peptide at concentrations optimal for measuring restoration of activity to inactive proteins but at which inhibitory effects are beginning to be observed with wild type enzyme. Use of even higher concentrations of peptide will cause nonspecific inhibition of activities observed with both wild-type and restored enzymes.

Two mutations in the C terminus of PLD2 also attenuated activity of the enzyme. In both cases, addition of the C-terminal peptide could restore up to 30% of wild type activity. In the case of PLD2, activity is restored to enzyme assayed in the presence of PIP2. Wild type PLD1 has a lower activity in the presence of PIP2 and can be strongly activated by the small G protein, Arf, or PKCa. Fig. 6A shows that peptide could effectively restore stimulation by either regulatory molecule to catalytically compromised enzymes. These results indicate that the role of the C terminus in catalysis by the PLD isozymes is fundamental to activity and not selectively employed by these differential regulators of the enzymes.

Involvement of the C terminus with Rho appears more complex. Restoration of stimulation by RhoA can also be observed (Fig. 6B), but the lower efficacy of Rho makes these observa-
tions difficult and the extent of restoration relative to Arf and PKCa cannot be adequately assessed. However, some deficiency in the ability of RhoA to activate PLDs with C-terminal disruptions is indicated by the failure of RhoA to synergize with PKCa when the deficient enzymes are restored with peptide (Fig. 6C). Although this may indicate that Rho directly uses the intact C terminus to partially effect stimulation of activity, it is also consistent with an indirect role in which anchoring of the C-terminal residues is important to help stabilize other proximal elements of the protein which mediate regulation by Rho.

The C-terminal Carboxyl Group Is Required for Catalysis—A modest tolerance for change in the C-terminal threonine but the complete attenuation of activity by the attachment of a hexahistidine tag or alanine to this residue suggested that the α-carboxyl group of the threonine was functionally important. Therefore, amidated peptides were tested for their ability to restore activity to inactive enzymes (Fig. 7). Amidation of the C terminus of either the shorter or longer C-terminal peptides inhibited their ability to restore activity to inactive mutants of PLD1.

DISCUSSION

The results with mutations in the C termini of mammalian PLD1 and PLD2 indicate the fundamental role of this portion of the molecule in the catalytic action of these enzymes. We hypothesize that C-terminal residues stabilize a functional conformation of the active site. This may occur through interaction with residues directly involved in catalysis or with residues more remotely involved in formation of the site.

The catalytic core of PLD is predicted to form around the conserved HKD motifs (33, 39). The structure of Nuc, a bacterial endonuclease belonging to the superfamily of enzymes with these motifs, consists of a dimer in which the individual HKD motifs of the monomeric polypeptides interact in the dimeric molecule to form the active site (40). The recent structure of the PLD from Streptomyces sp. strain PMF confirms the coordinated interaction of two HKD motifs within a single polypeptide to form the catalytic site (41). Such coordination of the two motifs in the mammalian PLD enzymes is supported by mutagenesis experiments (32) and the evolution of PLD activity when the N-terminal and C-terminal halves of PLD1 were coexpressed but not when either half (single HKD domain) was expressed alone (42). One potential role for the C-terminal residues would be to stabilize the interaction between these domains. If so, partial restoration of activity by the free peptide indicates that the mechanism for such stabilization would not be a simple tethering of one domain to the other.

The discovery that peptides representing the C terminus can restore partial activity to inactive enzymes suggests a more localized function for the C terminus. It is likely that the mechanism for this restoration is binding of the peptide to a site normally occupied by the endogenous C terminus of the enzyme. To achieve this, peptides would have to compete with the endogenous C terminus, which will have a competitive edge.
by virtue of its attachment to the rest of the enzyme. Such a mechanism also indicates that positioning of the C terminus in the enzyme must be flexible. The inability of the peptides to fully restore activity may reflect an inability to use peptides at sufficiently high concentrations and transient interactions of the peptides with functional residues. Alternately, correct positioning of the endogenous C terminus may also have more global conformational effects on the active site that cannot be mimicked by the independent peptides.

One possibility is that the C terminus of one PLD molecule interacts with the active site of a second PLD molecule. Thus, the active enzyme would be a dimer and two enzymes with dissimilar mutations might be complementary. Although this mechanism could exist, complementation by coexpression of PLD1-K466A with PLD1-CHI8 was not observed (data not shown).

The importance of the last two residues of the PLD1 C terminus has been shown by mutagenesis and reconstitution; specific roles for other upstream residues have not been explored. Elimination of the hydrophobic side chain of tryptophan 1073 (PLD1-W1073A) results in complete loss of activity. It is possible that the aromatic group helps position residues in the catalytic site or that its interaction with a specific site in the enzyme helps stabilize positioning of the C-terminal threonine for effective interaction. A role in stable positioning of the C terminus is attractive in light of the ability of peptides to restore partial function of the W1073A mutant.

The inability of amidated peptides to restore activity to inactive enzymes indicates a requirement for an intact C-terminal α-carboxyl group. It is possible that this carboxyl group participates directly in catalytic action of the enzyme. However, conservation of the HKD motifs (HXXXXX-DXXXXXOXXX) and structural data from Nuc (40) suggest that all of the amino acid residues directly involved in catalysis are in place. It is more likely that the C-terminal carboxyl group may be stabilizing or activating catalytic residues through salt bridges or hydrogen bonds. In the structure of Nuc (40), both serines of the HKD motifs (GSXN) contribute symmetrically to stabilization of the active site. In the mammalian PLD isozymes, the serine position in the first HKD motif is a glycine, suggesting a significant departure in structure from this homologous core domain. It is tempting to speculate that the C-terminal threonine could supply this role in PLD1. However, the partial activity of the enzyme when alanine occupies this position rules out this role for the hydroxyl group and such a functional role would depend on interactions through the α-carboxyl moiety. A second invariant residue of interest in the HKD motif is the aspartyl residue. In Nuc (40), the aspartyl residue participates directly in catalytic action of the enzyme. How- ever, the C-terminal acronym proposed for the mammalian tryptophan and a free α-carboxyl group from the asparagine or aspartic acid could fulfill the requirement of the last amino acid. However, compensatory changes in the surfaces of the enzymes with which these C termini interact would presumably be required to accommodate the different side chains of these C-terminal residues. The C termini of identified PLDs in bacteria and plants are much more variable. This and overall differences in structural components and regulatory properties of the latter enzymes probably indicate that the functional requirement of the C terminus defined for the mammalian enzymes will not be a conserved feature in the broader family of PLD enzymes.

A requirement for interaction for C-terminal residues with the rest of the protein for expression of enzymatic activity offers great opportunity for regulation. Stabilization of this interaction would yield increased activity of the enzyme, whereas disruption would inhibit activity. Enhancement of interaction of the C terminus with the active site would be a means for one or more activators of PLD1 to exert their stimulatory effects, especially synergism observed by combinations of activators (16, 19, 20, 22, 43, 44). The apparent deficient recovery of stimulation with Rho, which is thought to interact with C-terminal regions of PLD (31, 32), is consistent with such a mechanism. However, the absence of activity in C-terminal mutants, the uniform recovery of stimulation by other activators in the presence of peptide, and similar behavior by PLD2 suggest this is not a major regulatory paradigm. Alternatively, a potential mechanism for inhibition or down-regulation of PLD could involve binding of an inhibitory molecule to or modification of the C terminus such that interaction of the C-terminal residues with the active site was disrupted. The similarity of C-terminal regulation in both PLD1 and PLD2 makes this a potential common site to attenuate both PLD pathways in a cell.

Acknowledgment—We thank Dr. William Singer for help with the manuscript.

REFERENCES

1. Athenstaedt, K., and Daum, G. (1999) Eur. J. Biochem. 266, 1–16
2. English, D. (1996) Cell Signal. 8, 341–347
3. Hodgkin, M. N., Pettit, T. R., Martin, A., Michell, R. H., Pemberton, A. J., and Wakeham, M. J (1998) Trends Biochem. Sci. 23, 200–204
4. Houle, M. G., and Bourgein, S. (1999) Biochim. Biophys. Acta 1439, 135–149
5. McPhail, L. C., Waite, K. A., Regier, D. S., Nixon, J. B., Quattrocchi-Dann, M., Zhang, W. X., Wallin, R., and Sergeant, S. (1999) Biochim. Biophys. Acta 1439, 277–290
6. Brown, H. A., Gutowski, S., Moonaw, C. R., Slaughter, C., and Sternweis, P. C (1995) Cell 75, 1137–1144
7. Brown, H. A., and Sternweis, P. C (1995) Methods Enzymol. 257, 313–324
8. Hammond, S. M., Alshullter, Y. M., Sung, T. C., Rudge, S. A., Rose, K., Engebrecht, J., Morris, A. J., and Frohman, M. A (1995) J. Biol. Chem. 270, 29640–29643
9. Colley, C. W., Sung, T. C., Roll, R., Jenco, J., Hammond, S. M., Alshullter, Y., Bar-Sagi, D., Morris, A. J., and Frohman, M. A (1997) Curr. Biol. 7, 191–201
10. Kodski, K., and Yamashita, S. (1997) J. Biol. Chem. 272, 11408–11413
11. Lisoviczich, M., Chalifa, V., Pertile, P., Chen, C. S., and Cantley, L. C (1994) J. Biol. Chem. 269, 21403–21406
12. Brown, H. A., Gutowski, S., Kahn, R. A., and Sternweis, P. C (1995) J. Biol. Chem. 270, 14935–14943
13. Rose, K., Rudge, S. A., Frohman, M. A., Morris, A. J., and Engebrecht, J. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 12151–12155
14. Cockcroft, S., Thomas, G. M., Fensome, A., Murray, B., Cunningham, E., Gout, I., Hiles, I., Totty, N. F., Truong, O., and Hsuan, J. J (1994) Science 263, 523–526
15. McCormick, R. A., Ross, A. H., Qi, R. G., Simons, M., and Engebrecht, J. H (1994) J. Biol. Chem. 269, 25561–25564
16. Brown, H. A., Bokoch, G. M., and Sternweis, P. C (1995) J. Biol. Chem. 270, 14944–14950
17. Kwak, J. Y., Lopez, I., Uhlinger, D. J., Ruy, S. H., and Lambeth, J. D (1995) J. Biol. Chem. 270, 27093–27098
18. Balboa, M. A., and Insel, P. A (1995) J. Biol. Chem. 270, 27093–27098
19. Balboa, M. A., and Insel, P. A (1995) J. Biol. Chem. 270, 28943–28947
20. Nakamura, H., Tago, K., Yonekawa, T., Sasaki, T., Takai, Y., Morii, N., Narumiya, S., Katada, T., and Kanaho, Y. (1995) J. Biol. Chem. 270, 25667–25671
21. Singer, W. D., Brown, H. A., Jiang, X., and Sternweis, P. C (1996) J. Biol. Chem. 271, 4504–4510
22. Conricode, K. M., Brewer, K. A., and Exton, J. H (1992) J. Biol. Chem. 267, 7199–7202
23. Hammond, S. M., Jenco, J. M., Nakashima, S., Cadwallader, K., Gu, Q., Cook, S., Nuzawa, Y., Prestwich, G. D., Frohman, M. A., and Morris, A. J (1997) J. Biol. Chem. 272, 3860–3865
24. Lopez, L., Arnold, R. S., and Lambeth, J. D (1998) J. Biol. Chem. 273, 12846–12852
25. Sung, T. C., Alshullter, Y. M., Morris, A. J., and Frohman, M. A (1999) J. Biol. Chem. 274, 494–502
The C Terminus of PLD Is Required for Catalytic Activity
