Kinetic Analysis of Arabidopsis Phospholipase D₀

SUBSTRATE PREFERENCE AND MECHANISM OF ACTIVATION BY Ca²⁺ AND PHOSPHATIDYLSERINE

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Phospholipase D (PLD) is a major plant phospholipase family involved in many cellular processes such as signal transduction, membrane remodeling, and lipid degradation. Five classes of PLDs have been identified in Arabidopsis thaliana, and Ca²⁺ and polyphosphoinositides have been suggested as key regulators for these enzymes. To investigate the catalysis and regulation mechanisms were carried out on the newly identified PLD from Arabidopsis. PLD₀ activity was dependent on both bulk concentration and surface concentration of substrate phospholipids in the Triton X-100/phospholipid mixed micelles. V_max, K_m, and K_n values for PLD₀ toward phosphatidylcholine or phosphatidylinositol 4,5-bisphosphate were determined; phosphatidylinositol 4,5-bisphosphate was the preferred substrate. PLD₀ activity was stimulated greatly by phosphatidylinositol 4,5-bisphosphate (PIP₂). Maximal activation was observed at a PIP₂ molar ratio around 0.01. Kinetic analysis indicates that PIP₂ activates PLD₀ by promoting substrate binding to the enzyme, without altering the bulk binding of the enzyme to the micelle surface. Ca²⁺ is required for PLD₀ activity, and it significantly decreased the interfacial Michaelis constant K_m. This indicates that Ca²⁺ activates PLD₀ by promoting the binding of phospholipid substrate to the catalytic site of the enzyme.

PLDs form a major family of phospholipases in plants (4). In Arabidopsis, 12 PLD genes have been identified. These are tentatively grouped into five classes, PLDα (1, 2, 3, and 4), -β (1 and 2), -γ (I, 2, and 3), -δ, and -ζ (1 and 2), based on gene architecture, sequence similarity, domain structure, biochemical properties, and the order of cDNA cloning (5). These PLDs display different dependences on two common cellular regulators, Ca²⁺ and phosphatidylinositol 4,5-bisphosphate (PIP₂). The activity of PLD₀ is independent of PIP₂ when assayed at millimolar concentrations of Ca²⁺, whereas PLDβ and -γ are PIP₂-dependent and most active at micromolar levels of Ca²⁺ (6). On the other hand, the most recently identified PLD₀ does not require Ca²⁺ or any divalent cations for activity (5). Some of the PLDs also differ in substrate specificity. PLD₀ selectively hydrolyzes phosphatidylcholine (PC) (5), whereas other PLDs are able to hydrolyze the common membrane lipids, PC, phosphatidylinositol 4,5-bisphosphate (PE), and phosphatidyglycerol (7). Recent analyses on stress-induced lipid hydrolysis show that PLD₀ primarily uses PC as a substrate (8, 9), although it can hydrolyze other phospholipids in vitro (7). These observations indicate that different PLDs are subjected to unique control, and their activation may lead to selective hydrolysis of different membrane lipids in the cell.

Knowing the kinetic properties of individual PLDs will greatly help understand the catalysis, substrate selectivity, and mechanism of regulation by cellular effectors, such as Ca²⁺ and PIP₂. Because PLDs act on substrates that form a lipid-water interface, both two-dimensional surface interactions and three-dimensional bulk interactions need to be considered in order to describe the action of these enzymes. Surface dilution kinetics, originally developed by Dennis and colleagues (10–13) to explain the action of cobra venom phospholipase A, is a kinetic model that takes into account both types of interactions. This analysis has been successfully used to define the catalytic mechanism, kinetics, activation, and inhibition of various lipid-metabolizing enzymes, including phospholipase C (14, 15), phospholipase A₂ (10–13), phosphatidate phosphatase (16), and diacylglycerol kinases (17, 18), but detailed kinetic analysis has not yet been applied to any purified PLD.

We report here the kinetic analysis of a recently identified PLD, PLD₀, from Arabidopsis (19, 20). Like most plant PLDs, the activity of PLD₀ requires Ca²⁺ and is stimulated by PIP₂ (19). On the other hand, the PLD₀ activity is distinctively activated through different pathways. Arabidopsis PLD₀ is a lipid substrate PLD that is activated through the involvement of PIP₂, phosphatidate phosphatase, and diacylglycerol kinases.

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**EXPERIMENTAL PROCEDURES**

**Materials**—All the phospholipids were purchased from Avanti Polar lipids. Dipalmitoylglycero-3-phospho-[methyl-3H]choline and dioleoylglycero-3-P-[14C]ethanolamine were products of PerkinElmer Life Sciences.

**Expression and Purification of the PLDs**—Full-length cDNAs of Arabidopsis PLDδ and PLDβ were cloned into pGEX-4T-1 and pGEX-2T-1 vectors (Amersham Biosciences) (19, 22), respectively, which produced a glutathione S-transferase (GST) fusion at the N terminus. The recombinant plasmids were transformed into *Escherichia coli* JM109 and then into BL21 (Promega). All cell cultures were grown in Luria-Bertani medium with 50 mg/liter ampicillin. The transformed cells were grown at 37°C to an absorbance of ~1.0 at 600 nm. Five milliliters of the cells was transferred into a 1-liter flask with 200 ml of LB medium containing 0.1 mM isopropyl-1-thio-β-galactopyranoside and incubated overnight at room temperature. Then the cells were harvested and lysed by sonication in phosphate-buffered saline buffer containing 140 mM NaCl, 2.7 mM KCl, 10 mM Na3HPO4, and 1.8 mM KH2PO4 (pH 7.3), with addition of 5 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride.

The GST fusion proteins were purified with glutathione-Sepharose 4B beads (Amersham Biosciences), following the manufacturer’s instructions. The GST tag was removed by thrombin digestion at room temperature overnight with gentle shaking. The protein purity was checked on SDS-PAGE gel stained with Coomassie Blue, and protein concentration was estimated by using a dye-binding protein assay kit (Bio-Rad) with bovine serum albumin as a standard. Purified protein (0.1–0.5 μg) was used per reaction, depending on different enzymes and substrates. Less than 5% of the substrate was hydrolyzed.

**Preparation of Triton X-100/Phospholipid Mixed Micelles**—Two μCi of [3H]PC or 0.5 μCi of [14C]PE was added to 1 μmol of cold PC or 1 μmol of cold PE in chloroform, respectively. The chloroform solutions were dried under a stream of nitrogen, and de-ionized water was added to give a final phospholipid concentration of 1 mM. The phospholipid suspension was probe-sonicated on ice until clear. To obtain the desired substrate concentration at the desired mole fraction, the phospholipid suspension was diluted with Triton X-100 stock solution (40 mM) using the following formula: mole fraction$_{\text{phospholipid}}$ = [phospholipid]/([phospholipid] + [Triton X-100 (free)]); [Triton X-100 (free)] = [Triton X-100 (total)] − critical micelle concentration of Triton X-100 (0.24 mM) (23). When the effect of PIP$_2$ mole concentration was to be tested, PIP$_2$ was added at this point, using the following formula: mole fraction$_{\text{PIP}_2}$ = [PIP$_2$]/([total phospholipid] + [Triton X-100 (free)]). The Triton X-100/phospholipid mixture was vortexed briefly and let stand at room temperature for half an hour. The total phospholipid concentration in Triton X-100/phospholipid mixed micelles did not exceed 25 mol % to ensure that the structure of the mixed micelles was similar to the structure of pure Triton X-100.

**PLD Assays**—PLD activity was measured by following the release of [3H]choline from dipalmitoylglycero-3-phospho-[methyl-3H]choline or [14C]ethanolamine from dioleoylglycero-3-P-[14C]ethanolamine. The basic assay mixture contained 100 mM Tris-HCl (pH 7.0), 80 mM KCl, 200 μM CaCl$_2$, 0.5 mM MgCl$_2$, the indicated concentrations of Triton X-100/phospholipid vesicles, and purified PLD protein, in a total volume of 100 μl. The reactions were initiated by adding enzyme proteins and were stopped by adding 1 ml of chloroform/methanol (2:1) and then 100 μl of 2 M KCl. The release of the radiolabeled head groups into the aqueous phase was quantitated by scintillation counting. All assays were conducted for 15 min at 30°C. The enzyme reactions were linear with time and protein concentration.

**RESULTS**

**Kinetic Analysis of PLDδ toward PC in mixed micelles with Triton X-100**—A. PLDδ activity measured as a function of the sum of the molar concentrations of Triton X-100 (TX) plus PC at a series of set mole fractions (MF) of PC. Data represent the average of three independent experiments. B, reciprocal plot of the data in A. C, replot of 1/V intercepts obtained in B versus the reciprocal of the mole fraction of PC. D, replot of slopes obtained in B versus the reciprocal of the mole fraction of PC.

**Kinetic Analysis of PLDδ toward PC in Triton X-100/PC Micelles**—To study the kinetic behavior of PLDδ, we used a detergent/phospholipid mixed micelle assay system, following the surface dilution model of enzyme kinetics (10–13, 24). This model takes into account both two-dimensional surface interaction and three-dimensional bulk interaction between an enzyme and lipid substrate. Such consideration is critical in determining the kinetic parameters of these enzymes because lipid-dependent enzymes, such as PLD, catalyze reactions at a water-lipid interface (10). The principle of the surface dilution model is presented in Equation 1 (24).

\[
E + A \xrightarrow{k_1} EA \xrightarrow{k_2} EAB \xrightarrow{k_3} EA + Q \quad (\text{Eq. 1})
\]

According to this model, the action of enzyme (E) consists of two consecutive steps. First, the enzyme interacts non-catalyt-
ically with the surface of detergent/lipid mixed micelles (A).
Subsequently, the enzyme-mixed micelle complex (EA) binds to an individual lipid substrate (B) at its catalytic site, which leads to the hydrolysis of substrate to product (Q). The first association depends on the bulk concentrations of both E and A, whereas the second step depends on the surface concentrations of EA and B. Here A represents the sum of the molar concentrations of detergent and lipid substrate, and B is the mole fraction of lipid substrate in the mixed micelle.

Equation 2 is the rate expression for surface dilution kinetic model (10, 12, 24).

\[
V = \frac{V_{\text{max}}(A)(B)}{K_s^A + K_m^B(A) + (A)(B)}
\]  

(Eq. 2)

Three kinetic parameters, \(V_{\text{max}}, K_s^A, \) and \(K_m^B\) can be determined from this equation. \(V_{\text{max}}\) is the true \(V_{\text{max}}\) when both the bulk concentration and the surface concentration of the lipid substrate approach infinity. \(K_s^A\), which is equal to \(k_{-1}/k_1\) and expressed in bulk concentration terms, is the dissociation constant describing the interaction of the enzyme with the mixed micelles.

**TABLE I**

|                  | PC | PE |
|------------------|----|----|
| \(V_{\text{max}}\) (\(\mu\text{mol/min/mg}\)) | 1.0 | 1.5 |
| \(K_s^A\) (mM)  | 1.5 | 3.5 |
| \(K_m^B\) (mole fraction) | 0.13 | 0.03 |
| \(V_{\text{max}}/K_m^B\) | 7.1 | 50 |

**FIG. 3.** Effect of PIP2 on the activities of PLD\(\Delta\) and PLD\(\beta\) toward PC in the mixed micelles with Triton X-100 (TX). A, PLD activity measured in the absence of PIP2, as a function of the mole fraction of PC at a set PC molar concentration of 0.2 mM. B, PLD activity measured as a function of the PIP2 mole fraction in the PC/Triton X-100/PIP2 mixed micelles at a set PC molar concentration of 0.2 mM and a set PC mole fraction of 0.2.

**FIG. 4.** Effect of PIP2 on the kinetic behavior of PLD\(\Delta\). A, PLD activity measured as a function of PC molar concentration at set mole fractions of PIP2. The mole fraction (MF) of PC was 0.2. B, PLD activity measured as a function of PC mole fraction at set mole fractions of PIP2. The PC molar concentration was 0.1 mM.

**Kinetic Analysis of Arabidopsis PLD\(\Delta\)**

These parameters were calculated from panel C and panel D of Fig. 2 (PC) and Fig. 6 (PE), according to Equation 2 of the surface dilution kinetic model. \(K_s^A\) is the dissociation constant describing the interaction of the enzyme with the mixed micelles, expressed in bulk concentration terms. \(V_{\text{max}}\) is the true \(V_{\text{max}}\) at an infinite mole fraction and an infinite bulk concentration of lipid substrate. \(K_m^B\) is the interfacial Michaelis constant, expressed in surface concentration units, mole fraction. Mole fraction = \([\text{PC or PE}]/([\text{PC or PE}] + \text{[Triton X-100 (free)]})\). \([\text{Triton X-100 (free)]} = \text{[Triton X-100 (total)]} - \text{critical micelle concentration of Triton X-100 (0.24 mM)}\) (21).

**FIG. 5.** Effect of PIP2 on the activities of PLD\(\Delta\) and PLD\(\beta\) toward PC in the mixed micelles with Triton X-100 (TX). A, PLD activity measured in the absence of PIP2, as a function of the mole fraction of PC at a set PC molar concentration of 0.2 mM. B, PLD activity measured as a function of the PIP2 mole fraction in the PC/Triton X-100/PIP2 mixed micelles at a set PC molar concentration of 0.2 mM and a set PC mole fraction of 0.2.
(Fig. 1B). The enzyme activity decreased when the surface concentration of the lipid substrate in the mixed micelle was diluted by the addition of detergent as the bulk concentration of the substrate was kept the same.

PLDδ activity was determined as a function of the sum of the molar concentrations of Triton X-100 and PC (A of Equation 2) at a series of mole fractions of PC (B of Equation 2) (Fig. 2A). The activity was dependent on the sum of molar concentrations of Triton X-100 and PC at each surface concentration. As the surface concentration of PC in the mixed micelles decreased, the apparent \( V_{\text{max}} \) decreased. A double-reciprocal plot of the results in Fig. 2A indicates that PLDδ exhibits saturation kinetics when the bulk concentration of Triton X-100 and PC was varied at each fixed mole fraction of PC (Fig. 2B). According to Equation 2, the replot of the \( 1/V \) intercepts versus \( 1/B \) from Fig. 2B should be linear; the intercept of the \( 1/V \) intercept axis is equal to \( 1/V_{\text{max}} \) and the intercept of the \( 1/B \) axis is equal to \(-1/K_m^B\). A replot of the data is linear (Fig. 2C), and the \( V_{\text{max}} \) and \( K_m^B \) are 1.0 \( \mu \)mol/min/mg and 0.13 mole fraction, respectively (Table I). Equation 2 also predicts that a replot of the slopes versus \( 1/B \) from Fig. 2B should be linear and cross the origin, and the slope of the replot is equal to \( K_s^A/K_m^B/V_{\text{max}} \). Fig. 2D shows that such a replot was linear and passed the origin. By using the slope of the line in Fig. 2D and the values for \( V_{\text{max}} \) and \( K_m^B \) obtained in Fig. 2C, the dissociation constant \( K_s^A \) for the Triton X-100/PC mixed micelle was calculated to be 1.5 mM (Table I).

**Fig. 5.** Effect of Ca\(^{2+}\) on the kinetic behavior of PLDδ. A, PLDδ activity toward PC/Triton X-100 mixed micelles measured as a function of the calcium concentration in the absence or presence of PIP\(_2\). The PC molar concentration was 0.1 mm, and the PC mole fraction was 0.1. B, PLDδ activity measured as a function of the PC mole fraction at two concentrations of Ca\(^{2+}\). The PC molar concentration was 0.1 mm.

**Fig. 6.** Activity of PLDδ toward PE in mixed micelles with Triton X-100. A, PLDδ activity measured as a function of the sum of the molar concentrations of Triton X-100 (TX) plus PE at a series of set mole fractions (MF) of PE. Data represent the average of three independent experiments. B, reciprocal plot of the data in A. C, replot of \( 1/V \) intercepts obtained in B versus the reciprocal of the mole fraction of PE. D, replot of slopes obtained in B versus the reciprocal of the mole fraction of PE.
of PC was set at 0.2 mM, and the PC mole fraction was set at 0.2. Dramatic increases of both PLDβ and PLDδ activities were observed when the PIP₂ mole fraction increased, with the maximal activation achieved at PIP₂ mole fraction around 0.01. The $K_{\text{act}}$ for PLDδ and -δ were similar, around 0.002 to 0.003 mole fraction of PIP₂ in the mixed micelles.

To study the possible mechanism by which PIP₂ stimulates the PLDδ activity, we compared the two constants, $K_{m}^{A}$ and $K_{m}^{R}$, in the absence or presence of PIP₂. For determining the effect of PIP₂ on $K_{m}^{A}$, PLDδ activity was measured as a function of the sum of the molar concentrations of Triton X-100 and PC at three set mole fractions of PIP₂ (0, 0.002, and 0.02) (Fig. 4A) with the PC mole fraction fixed at 0.1. Although increasing molar ratio of PIP₂ increased the apparent $V_{\text{max}}$, it did not significantly change the apparent $K_{m}^{A}$ (Fig. 4A). This suggests that PIP₂ does not affect the bulk binding of PLDδ to the mixed micelles. For examining the effect of PIP₂ on $K_{m}^{R}$, PLDδ activity was measured as a function of the mole fraction of PC at the same three set PIP₂ mole fractions (Fig. 4B). The apparent interfacial Michaelis constant, $K_{m}^{R}$, decreased about 2–3-fold in the presence of PIP₂, and the apparent $V_{\text{max}}$ increased with the increase of PIP₂ mole fraction (Fig. 4B). Overall, the specificity constant (apparent $V_{\text{max}}/K_{m}^{R}$) increased up to 10-fold in the presence of 0.02 mole fraction of PIP₂. These results suggest that PIP₂ may activate PLDδ by increasing the affinity of its catalytic site to the phospholipid substrate but may not affect its binding to the Triton X-100/phospholipid mixed micelle surface.

$Ca^{2+}$ Activates PLDδ by Decreasing the Interfacial Michaelis Constant $K_{m}^{R}-Ca^{2+}$ is another important regulator for plant PLD activity. The optimal $Ca^{2+}$ concentration for PLDδ activity in the Triton X-100/PC mixed micelle system was around 1 mM, and this optimum was not affected by the presence of PIP₂ in the mixed micelles (Fig. 5A). To determine the effect of $Ca^{2+}$ on $K_{m}^{R}$, PLDδ activity was measured as a function of the PC mole fraction in the mixed micelles at two $Ca^{2+}$ concentrations, 0.2 and 1 mM (Fig. 5B). Apparent $K_{m}^{R}$ at 1 mM of $Ca^{2+}$ was nearly 3-fold lower than the apparent $K_{m}^{R}$ at 0.2 mM of $Ca^{2+}$ (Fig. 5B). These results indicate that $Ca^{2+}$ may activate PLDδ by promoting the binding of the phospholipid substrate to the catalytic site of the enzyme.

PE Serves as a Better Substrate for PLDδ than PC—The substrate preference of PLDδ was studied by substituting PC with PE in the Triton X-100 mixed micelles and measuring the activity of PLDδ as a function of the sum of the molar concentrations of Triton X-100 plus PE (A) at a series of set mole fractions of PE (B). The PLDδ activity was dependent both on the sum of the bulk concentration of Triton X-100 and PE and on the surface concentration of PE in the mixed micelles (Fig. 6A). As predicted by Equation 2, both the replot of $1/V$ intercepts (from Fig. 6B) versus 1/PE and the replot of the slopes versus 1/PE were linear, with the latter going through the origin (Fig. 6, C and D). By using the two intercepts obtained from Fig. 6C and the line slope from Fig. 6D, we calculated the $V_{\text{max}}$, $K_{m}^{A}$, and $K_{m}^{R}$ for PE to be 1.5 μmol/min/mg, 3.5 mM, and 0.03 mole fraction, respectively (Table I).

The above results indicate that PLDδ follows the surface dilution kinetic model using two common phospholipid substrates, PC and PE, but the catalytic constants of these two phospholipids are quite different (Table I). The $V_{\text{max}}$ of PE is about 1.5-fold higher than that of PC, whereas the interfacial Michaelis constant, $K_{m}^{A}$ of PE is about 4–5-fold lower. Therefore, the specific constant ($V_{\text{max}}/K_{m}^{A}$) of PE is about 7-fold higher than that of PC, suggesting that PE has a higher affinity to the catalytic site of the enzyme. On the other hand, the dissociation constant, $K_{m}^{A}$ of PE is similar to or slightly higher than that of PC. This suggests that PLDδ has similar affinities for binding sites to Triton X-100/PC and Triton X-100/PE mixed micelles.

To confirm that PE indeed serves as a better substrate for PLDδ, we carried out side-by-side comparison toward the two substrates. In this analysis, PLDδ activity was measured as a function of the sum of the molar concentrations of Triton X-100 plus phospholipid, at a set phospholipid mole fraction of 0.1. B PLDδ activity measured as a function of the phospholipid mole fraction at a set phospholipid molar concentration of 0.1 mM.

![Comparison of the kinetic behavior of PLDδ toward PC and PE in the mixed micelles with Triton X-100 (TX).](image)

Fig. 7. Comparison of the kinetic behavior of PLDδ toward PC and PE in the mixed micelles with Triton X-100 (TX). A, PLDδ activity measured as a function of the phospholipid molar concentration, at a set phospholipid mole fraction of 0.1. B PLDδ activity measured as a function of the phospholipid mole fraction at a set phospholipid molar concentration of 0.1 mM.
The present data show that the oleic acid requirement by PLD partially purified rat brain PLD fraction showed maximal PIP2 vs. 

active phox homology/pleckstrin homology domains (5). Instead, basic residues are conserved in PLD many basic side chains (Lys, Arg, and His), and these critical residues cooperate with the active site of the enzyme, without affecting the bulk binding of PLD to the micelle surface. These results are consistent with the above-mentioned work showing that PLD binding enhancements PC binding to the catalytic site of PLD by 22,31. These studies show that PLD activity is also responsible to the PIP2 stimulation effect observed for PLD. The different PLD requirements suggest that other unique factor(s) may help activate PLD. Oleic acid has been shown to be a specific stimulator for PLD but not for other Arabidopsis PLDs. The present data show that the oleic acid requirement by PLD can be substituted by Triton X-100, consistent with the hypothesis that oleic acid may affect substrate presentation and modulating PLD-substrate interaction.

PLD, like most other plant PLDs, requires Ca2+ for activity. The only exception for the cation requirement to date is Arabidopsis PLD1 that is Ca2+-independent and contains putative phosphohomology/pleckstrin homology domains (5). Instead, most of the plant PLDs contains the Ca2+/phospholipid-binding C2 domain at their C terminus (5). The ability of the PLD C2 domain to bind Ca2+ has been demonstrated in the binding and structural studies on Arabidopsis PLD C2 and PLD C2 (31). However, how Ca2+ affects PLD catalysis is unknown. The present data show that Ca2+ decreases the apparent interfacial Michaelis constant, K_M (Fig. 5B), suggesting that it promotes lipid substrate binding to the active site of the enzyme.

Another significant finding of this analysis is that PLD prefers PE to PC as substrate and the kinetic description of this preference. This PE selectivity of PLD is in contrast to the preference of many other PLDs for PC as substrate. For example, the cloned mammalian PLD1 and -2 and Arabidopsis PLD1 use only PC as substrate (3). The most prevalent Arabidopsis PLD, PLDa, selectively hydrolyzes PE, as shown by lipid analysis in freezing-stressed Arabidopsis (9). Only Arabidopsis PLDy1 was previously indicated to have a higher activity toward PE than PC, but the kinetics and the extent of preference are not known (7). The present results show that PLD has similar dissociation constants, K_M, for the first binding step of PE and PC, but its specificity constant (V_max/K_M) for PE is about 7-9-fold higher than that of PC. Thus, it is conceivable that when PLD is activated in the cell, its higher affinity to PE may result in selective hydrolysis of PE and PE-enriched membranes. As two major lipids in eukaryotic membranes, PC and PE differ greatly in their membrane-forming properties and possibly cellular functions. Unlike PC, PE is a non-laminar lipid and prefers hexagonal phase formation. PE concentrations in vesicles and membranes have been shown to affect the activities of various proteins, including G-protein (32), phospholipase C (33), mammalian PLD (34), and Arabidopsis PLD and -γ (7). Thus, the cellular function and the mode of action of PLD could be distinctly different from the PC-selective PLDs.

REFERENCES
1. Wang, X. (2002) Curr. Opin. Plant Biol. 5, 408–414
2. Lisievska, M., Czarny, M., Fiuczi, G., and Tang, X. (2000) Biochem. J. 345, 461–475
3. Frohman, M. A., Sung, T. C., and Morris, A. J. (1999) Biochem. Biophys. Acta 1439, 175–186
4. Wang, X. (2001) Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 211–231
5. Qin, C., and Wang, X. (2002) Plant Physiol. 128, 1057–1068
6. Qin, W., Pappan, K., and Wang, X. (1997) J. Biol. Chem. 272, 28267–28273
7. Fappan, K., Austin-Brown, S., Chapman, K. D., and Wang, X. (1998) Arch. Biochem. Biophys. 353, 131–140
8. Zien, C. A., Wang, C., Wang, X., and Welti, R. (2001) Biochem. Biophys. Acta 1530, 236–248
9. Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H. E., Rajashekar, C. B., Williams, T. D., and Wang, X. (2002) J. Biol. Chem. 277, 31994–32002
10. Deems, R. A., Eaton, B. R., and Dennis, E. A. (1975) J. Biol. Chem. 250, 9013–9020
11. Roberts, M. F., Deems, R. A., and Dennis, E. A. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 1950–1954
12. Hendrickson, H. S., and Dennis, E. A. (1984) J. Biol. Chem. 259, 5734–5739
13. Hendrickson, H. S., and Dennis, E. A. (1984) J. Biol. Chem. 259, 5740–5744
14. James, S. R., Paterson, A., Harden, T. K., and Downes, C. P. (1995) J. Biol. Chem. 270, 11872–11881
15. Jones, G. A., and Carpenter, G. (1993) J. Biol. Chem. 268, 20845–20850
16. Lin, Y.-P., and Carman, G. M. (1990) J. Biol. Chem. 265, 166–170
17. McDonald, M. L., Mack, K. F., Williams, B. W., King, W. C., and Glomset, J. A. (1990) J. Biol. Chem. 265, 1112–1118
18. Walsh, J. P., Fahrenholz, L., and Bell, R. M. (1990) J. Biol. Chem. 265, 4374–4381
19. Wang, C., and Wang, X. (2001) Plant Physiol. 127, 1102–1112
20. Katagiri, T., Takahashi, S., and Shinozaki, K. (2001) Plant J. 26, 505–508
21. Gardner, J. C., Harper J. D. I., Weerakoon, N. D., Collings, D. A., Ritchie, S., Gilrey, S., Cyr, R. J., and Marc, J. (2001) Plant Cell 13, 2143–2158
22. Zheng, L., Shaw, J., Krishnamoorthy, R., and Wang, X. (2002) Biochemistry 41, 4546–4553
23. Lichtenberg, D., Robson, R. J., and Dennis, E. A. (1983) Biochem. Biophys. Acta 737, 285–304
24. Carman, G. M., Deems, R. A., and Dennis, E. A. (1985) J. Biol. Chem. 260, 18717–18714
25. Robson, R. J., and Dennis, E. A. (1985) Acc. Chem. Res. 18, 251–258
26. Robson, R. J., and Dennis, E. A. (1978) Biochim. Biophys. Acta 508, 513–524
27. Buxeda, R. J., Nickels, J. T., Jr., Belusin C. J., and Carman, G. M. (1991) J. Biol. Chem. 266, 13859–13865
28. Warner, T. G., and Dennis, E. A. (1975) J. Biol. Chem. 250, 8004–8009
29. Scislera, V. A., Rudge, S. A., Prestwich, G. D., Frohman, M. A., Kagebrecht, J., and Morris, A. J. (1999) EMBO J. 18, 5911–5921
30. Chalifa-Caspi, V., Eli, Y., and Lisievska, M. (1998) Neurochem. Res. 23, 589–598
31. Zheng, L., Krishnamoorthy, R., Zelikiewski, M., and Wang, X. (2000) J. Biol. Chem. 275, 19700–19706
32. Frohman, M. A., Ozasa, A., Rihats, C., Miralles, A., Fedor, K., Farkas, T., and Garcia-Sevilla, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11375–11380
33. Irvine, R. F., Letcher, A. J., and Dawson, R. M. C. (1984) Biochem. J. 218, 177–188
34. Nakamura, S., Kiyohara, Y., Jinnai, H., Hitomi, T., Ogino, C., Yoshida, K., and Nishizuka, Y. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4300–4304