The Regulation of Phospholipase C-γ1 by Phosphatidic Acid

ASSESSMENT OF KINETIC PARAMETERS*

(Received for publication, February 19, 1993, and in revised form, April 23, 1993)

Gwenith A. Jones§ and Graham Carpenter¶¶
From the Departments of Biochemistry and Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0416

A survey of lipids revealed that the anionic phospholipid phosphatidic acid activates both control and tyrosine-phosphorylated PLC-γ1. The mechanism by which phosphatidic acid activates both forms of PLC-γ1 was investigated using kinetic analysis. In the presence of phosphatidic acid, the substrate concentration response for control PLC-γ1 changes from sigmoidal to hyperbolic, while the cooperativity index decreases from 2.5 for control to 1.0 for tyrosine-phosphorylated PLC-γ1. The primary influence of phosphatidic acid on the control enzyme is on the cooperativity index and not the association of PLC-γ1 with substrate micelles, as phosphatidic acid had little effect on the micellar association constant, Kₐ. Phosphatidic acid also increases the activity of the tyrosine phosphorylated form of the enzyme. This increase is reflected in a decrease in the Kₐ from 0.3- to 0.03-m mol fraction phosphatidylinositol 4,5-bisphosphate. Phosphatidic acid has no effect on the Kₐ of the tyrosine-phosphorylated enzyme. From this data it is concluded that phosphatidic acid appears to activate PLC-γ1 by acting as an allosteric modifier.

One of the earliest responses that occurs in response to the activation of several growth factor receptors is the production of diacylglycerol and inositol 1,4,5-triphosphate (1). The enzyme that catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P₂) to diacylglycerol and inositol 1,4,5-triphosphate is phospholipase C (PLC). There are at least four known classes of phosphatidylinositol-specific PLC (-β, -γ, -δ, and -ε), and each class has a number of isoforms (2-5). Though there are structural similarities between each class of PLC, evidence suggests that they are regulated differently (2-6). Only PLC-γ has been shown to be tyrosine-phosphorylated by a number of receptor tyrosine kinases (2, 3, 5). We have shown previously that PLC-γ1 activity in vitro is increased by tyrosine phosphorylation in intact cells or in vitro (7) and that the mechanism by which tyrosine phosphorylation enhances PLC-γ1 activity is by acting as an allosteric modifier (8).

Tyrosine phosphorylation, however, may not be the only mechanism by which PLC-γ1 is regulated. Treatment of cells with phosphatidic acid or lysophosphatidic acid increases PtdIns-4,5-P₂ hydrolysis (9-13), and phosphatidic acid has been observed to increase the activity of PLC in vitro (14-16). Since phosphatidic acid is produced in cells in response to a number of agonists (17-25), including EGF (26, 27), the possibility that PLC-γ1 could be physiologically regulated by this phospholipid exists.

Using kinetic analysis, we demonstrated that PLC-γ1 activity in vitro can be increased by phosphatidic acid and that the mechanism of activation is by apparent allosteric modification of the enzyme.

EXPERIMENTAL PROCEDURES

Materials—Ptd[¹³C]Ins-4,5-P₂ was purchased from Du Pont-New England Nuclear. PtdIns-4,5-P₂ was purchased from Boehringer-Mannheim, and all other lipids were purchased from Sigma. Triton X-100 was purchased from Pierce Chemical Co., and Panobinostat. Staphylococcus aureus was purchased from Calbiochem. PLC-γ1 antibody used was generated against the peptide SFEEYQQPFD(E)C in rabbits. EGF was isolated from mouse submaxillary glands as described previously (28).

Cell Culture and Preparation of Cell Extracts—A-431 cells were maintained in Dulbecco's modified Eagle's medium with 10% calf serum at 37°C in a 5% CO₂ incubator. Cell lysates for enzyme assays were prepared as described previously (8). For assays performed on EGF-treated cytosol, the cytosol was incubated with IgG anti-epidermal growth factor-Sepharose beads (29) at 4°C for 4 h and washed with homogenization buffer, and the phosphotyrosyl-containing proteins were eluted from the beads with 20 mM phenylphosphate.

Preparation of Substrate—2 μl of Ptd[¹³C]Ins-4,5-P₂ was added to 1 mg of cold PtdIns-4,5-P₂ and the volume reduced to < 10 μl in a Savant Speedvac. Sufficient buffer (50 mM phosphate buffer, pH 6.8, 100 mM KCl) was added to give a final PtdIns-4,5-P₂ concentration of 10 mM. The phospholipid suspension was probe-sonicated (Branson cell disruptor 600) on ice until clear. To obtain the desired substrate concentration at the desired mole fraction, the 10 mM Ptd[¹³C]Ins-4,5-P₂ solution was diluted with Triton X-100 using the following formula: mole fraction PtdIns-4,5-P₂ = [PtdIns-4,5-P₂]/[PtdIns-4,5-P₂] + [Triton X-100]. If PLC-γ1 was to be assayed in the presence of phosphatidic acid, it was at this stage that phosphatidic acid was added, using the following formula: mole fraction phosphatidic acid = [phosphatidic acid]/[total phospholipid] + [Triton X-100].

PLC-γ Assay—For each assay tube, 100 μg of cytosolic protein was immunoprecipitated with 1 μl of PLC-γ1 antiserum and 10 μl of Panobinostat, and the assay was performed as described previously (8) using the desired concentration of PtdIns-4,5-P₂ at the desired mole fraction in an assay volume of 50 μl. The incubation time of the assay was varied so that less than 10% of the substrate was hydrolyzed. The amount of [¹³C]inositol 1,4,5-triphosphate produced was calculated based on the specific activity of Ptd[¹³C]Ins-4,5-P₂ (1.7-2.0 cpm/pmol). Enzyme activity values are based on the relative PLC-γ1 content in the immunoprecipitates as compared with cell lysate. To determine the relative PLC-γ1 content, aliquots of the immunoprecipitate and cell lysates were separated by SDS-polyacrylamide gel
electrophoresis and transferred to nitrocellulose. The nitrocellulose was incubated with PLC-γ1 antibodies and PLC-γ1 was visualized with 125I-protein A. The relative amounts of radioactivity were determined by quantitation with a Molecular Dynamics PhosphorImager.

Enzyme Kinetic Analysis—The kinetic parameters for PLC-γ1 were determined using Enzfitter kinetic program (BioSoft).

Protein Determination—Protein concentration was determined using BCA protein reagent (Pierce Chemical Co.) with bovine serum albumin as a standard.

RESULTS

Surface Dilution Model of Enzyme Kinetics—To study the influence of phosphatidic acid on PLC-γ1 activity, we used a detergent-phospholipid mixed micelle assay system. We determined previously (8) that with this assay, PLC-γ1 activity follows the surface dilution model of enzyme kinetics as described by Dennis and co-workers (30-32) to understand the kinetic behavior of phospholipase A2. The surface dilution model of enzyme kinetics takes into account that the reaction catalyzed by phospholipases occurs at a water-lipid interface. Interaction of the enzyme with the water-lipid interface of the mixed micelle is described by the kinetic parameter, $K_s$. This parameter is dependent on both the enzyme concentration and the molar substrate concentration. Catalysis of substrate at the micelle surface is described by the kinetic parameter, $K_m$. This interfacial kinetic constant is dependent on the mole fraction of substrate in the micelle. The absolute rate, $V_{max}$, occurs at infinite molar substrate concentration and infinite mole fraction. The surface dilution model has been successfully used to describe the behavior of a number of enzymes that, similar to phospholipase A2 and PLC-γ1, catalyze reactions at a micellar surface (33-39).

Anionic Phospholipids Increase PLC-γ1 Activity—Literature suggests that both phosphatidic acid and lysophosphatidic acid can increase PLC activity in intact cells (9-13) or in vitro (14-16). Various lipids were surveyed to determine which lipids could activate PLC-γ1 and to what degree. The ability of lipids to stimulate PLC-γ1 activity was determined for both tyrosine-phosphorylated and control PLC-γ1 (Fig. 1). The data show that phosphatidic acid, more than any other phospholipid, enhanced both control and tyrosine-phosphorylated PLC-γ1 activity. In the absence of lipid other than PtdIns-4,5-P₂, tyrosine-phosphorylated PLC-γ1 activity was 4-fold higher than the control non-tyrosine-phosphorylated PLC-γ1 activity. The addition of phosphatidic acid increased tyrosine-phosphorylated and control PLC-γ1 activity 6- and 40-fold, respectively. In the presence of phosphatidic acid, the specific activities of the control and the phosphotyrosine-containing enzyme were similar. The activating effect of phosphatidic acid is not due to contaminating amounts of lysophosphatidic acid, as lysophosphatidic acid only results in a small enhancement of PLC-γ1 activity (3- and 7-fold for tyrosine phosphorylated and control PLC-γ1, respectively) compared with phosphatidic acid. Other anionic phospholipids, phosphatidylglycerol and phosphatidylserine, were also able to activate PLC-γ1, though only to a very slight degree (<2-fold). Interestingly, the second messenger, 1,2-diacylglycerol, that is known to activate a number of proteins (1), had no effect on PLC-γ1 activity. Phosphatidylcholine, phosphatidylethanolamine, and free fatty acids (palmitate and oleic acid) also had no effect on PLC-γ1 enzymatic activity (Fig. 1 and data not shown).

PLC-γ1 requires calcium for activity. What role calcium plays in the hydrolysis of PtdIns-4,5-P₂ is not known. We determined whether the stimulation of PLC-γ1 activity by phosphatidic acid required calcium. No stimulation of PLC-γ1 activity by phosphatidic acid in the absence of calcium was observed (Fig. 2).

To determine the relative phosphatidic acid concentration that maximally activates PLC-γ1, PLC-γ1 activity was assayed at increasing mole fractions of phosphatidic acid (Fig. 3). Mole fractions of phosphatidic acid below 0.1 had little effect on control PLC-γ1 activity, while maximal enzymatic activity occurred at approximately 0.3-mol fraction of phosphatidic acid, with an $S_m$ of 0.2-mol fraction of phosphatidic acid. Phosphatidic acid also resulted in an increase in the activity of tyrosine-phosphorylated PLC-γ1, even at low (0.05) phosphatidic acid mole fractions, and maximal stimulation occurred at 0.2-mol fraction of phosphatidic acid, with an $S_m$ of 0.1-mol fraction of phosphatidic acid.

Phosphatidic Acid and Kinetic Parameters of Control and Tyrosine-phosphorylated PLC-γ1—To determine the influence of phosphatidic acid on PLC-γ1 kinetic parameters, the enzyme was assayed in the presence of 0.2-mol fraction of phosphatidic acid at increasing mole fractions and molar concentrations of PtdIns-4,5-P₂. Though 0.2-mol fraction of

---

**FIG. 1.** Effect of lipids on control and tyrosine-phosphorylated PLC-γ1 activity. PLC-γ1 was isolated from control and EGF-treated A-431 cells as described under "Experimental Procedures." PLC-γ1 activity was measured in duplicate at 200 μM micellar concentration, 0.1-mol fraction of PtdIns-4,5-P₂, in the presence of 0.3-mol fraction of the indicated lipids. NONE, no addition of exogenous lipid; PA, phosphatidic acid; LPA, lysophosphatidic acid; PG, phosphatidylserine; PC, phosphatidylcholine; DAG, sn-1,2-diacylglycerol. Data are from a representative experiment. The experiment was repeated three times with similar results. Open bars, PLC-γ1 isolated from control cells; closed bars, PLC-γ1 isolated from EGF-treated cells.

**FIG. 2.** The effect of calcium on PLC-γ1 activity. PLC-γ1 was isolated from control and EGF-treated A-431 cells as described under "Experimental Procedures." PLC-γ1 activity was measured in duplicate at 200 μM micellar concentration, 0.1-mol fraction of PtdIns-4,5-P₂, in the presence of 0 or 1 μM calcium. The experiment was repeated twice with similar results. Open bars, no addition of calcium; closed bars, 1 μM calcium.
PLC-γ1 and Phosphatidic Acid

The effect of phosphatidic acid on PLC-γ1 kinetic parameters

The presence of 0.2-mol fraction of phosphatidic acid, not only was the relative velocity rate increased at any given molar PtdIns-4,5-P_2 concentration, but the enzyme also no longer displayed cooperativity. When the Hill equation was used to calculate kinetic parameters, the control enzyme had a cooperativity index of 2.5, a V_max of 12 nmol/min/mg and a half-maximal substrate concentration of 0.28-mol fraction of PtdIns-4,5-P_2 in the absence of phosphatidic acid (Table I). Phosphatidic acid decreased the cooperativity index from 2.5 to 1.0 and produced an 8-fold reduction in the half-maximal substrate concentration (0.28- to 0.08-mol fraction of PtdIns-4,5-P_2) and increased the V_max 4-fold (Table I).

Tyrosine phosphorylated PLC-γ1 exhibited no cooperative effect in the absence or presence of phosphatidic acid. Rather, tyrosine-phosphorylated PLC-γ1 always displays apparent Michaelis-Menten kinetics (Fig. 5, A and B) (8). Phosphatidic acid produced a 10-fold decrease in the K_m without any change in the V_max for tyrosine-phosphorylated PLC-γ1 (Table I).

**Table 1**

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>-EGF</th>
<th>+EGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_max (nmol/min/mg)</td>
<td>2.8</td>
<td>11.2</td>
</tr>
<tr>
<td>S_0.5 (mole fraction)</td>
<td>0.28</td>
<td>0.08</td>
</tr>
<tr>
<td>n</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>K_m</td>
<td>1.0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Phosphatidic acid does not result in 100% activation of control PLC-γ1 activity, this mole fraction was selected in order to keep the total phospholipid mole fraction (PtdIns-4,5-P_2 and phosphatidic acid) below 0.4-mol fraction. At phospholipid mole fractions above 0.3, there is the possibility that not all of the phospholipid will be incorporated into the detergent micelle (40).

As reported previously (8), the control enzyme displays cooperativity toward the substrate, and this cooperative effect can not be overcome by increasing the molar concentration of PtdIns-4,5-P_2 (Fig. 4A). The data in Fig. 4B show that in the presence of 0.2-mol fraction of phosphatidic acid, not only was the relative velocity rate increased at any given molar PtdIns-4,5-P_2 concentration, but the enzyme also no longer displayed cooperativity. When the Hill equation was used to calculate kinetic parameters, the control enzyme had a cooperativity index of 2.5, a V_max of 12 nmol/min/mg and a half-maximal substrate concentration of 0.28-mol fraction of PtdIns-4,5-P_2 in the absence of phosphatidic acid (Table I). Phosphatidic acid decreased the cooperativity index from 2.5 to 1.0 and produced an 8-fold reduction in the half-maximal substrate concentration (0.28- to 0.08-mol fraction of PtdIns-4,5-P_2) and increased the V_max 4-fold (Table I).

Tyrosine phosphorylated PLC-γ1 exhibited no cooperative effect in the absence or presence of phosphatidic acid. Rather, tyrosine-phosphorylated PLC-γ1 always displays apparent Michaelis-Menten kinetics (Fig. 5, A and B) (8). Phosphatidic acid produced a 10-fold decrease in the K_m without any change in the V_max for tyrosine-phosphorylated PLC-γ1 (Table I).
It was possible that the observed phosphatidic acid increase in the $V_{\text{max}}$ of the control enzyme was partially due to micellar phosphatidic acid that promoted more efficient binding of PLC-γ1 to the micelles. If this were the case, it would be reflected in a change in the $K_c$. $K_c$ can be determined from the inverse reciprocal plot of reaction rate versus molar PtdIns-4,5-P$_2$ concentration at increasing mole fraction of PtdIns-4,5-P$_2$. The point at which the lines intersect in Fig. 6, $A$ and $B$ is the $K_c$ (41). These data, summarized in Table I, showed that phosphatidic acid did not significantly change the $K_c$ for either control (Fig. 6A) or tyrosine-phosphorylated PLC-γ1 (Fig. 6B).

The advantage of using immunoprecipitated enzyme is that we are able to use PLC-γ1 that is tyrosine-phosphorylated under physiological conditions, but the use of immunoprecipitated enzyme also raises the concern that the immunocomplex could affect the kinetic behavior of the enzyme. To partially address this concern, we compared the kinetic parameters of immunoprecipitated tyrosine-phosphorylated enzyme with soluble enzyme that was isolated and eluted from an anti-phosphotyrosine matrix. Though immunoprecipitation resulted in a 90% decrease in the $V_{\text{max}}$, the kinetic behavior of both the immunoprecipitated and the soluble enzymes in the presence and absence of phosphatidic acid were similar and produced similar calculated $K_a$ and $K_c$ values (Table II). Since the kinetic behavior of the immunoprecipitated enzyme was similar to the kinetic behavior of the soluble enzyme, we feel that the kinetic parameters calculated for the immunoprecipitated enzyme are valid and accurately reflect relative differences in the kinetic behavior of PLC-γ1 due to tyrosine phosphorylation or the presence of phosphatidic acid.

**Phosphatidic Acid Is an Allosteric Modifier of PLC-γ1—**To determine whether phosphatidic acid acts as an allosteric modifier of PLC-γ1 activity, PLC-γ1 was assayed at increasing mole fractions of PtdIns-4,5-P$_2$ in the presence of three phosphatidic acid mole fractions (0.0, 0.1, 0.2-mol fraction). Data represent the average of three separate experiments. Panel A, PLC-γ1 isolated from control cells. Panel B, PLC-γ1 isolated from EGF-treated cells. PA, phosphatidic acid.

**FIG. 7.** Kinetic behavior of control and tyrosine-phosphorylated PLC-γ1 assayed in the presence of various phosphatidic acid mole fractions. PLC-γ1 was isolated from control and EGF-treated A-431 cells as described under “Experimental Procedures.” PtdIns-4,5-P$_2$ hydrolysis was measured in duplicate at increasing mole fraction and at one micellar concentration (200 μM) of PtdIns-4,5-P$_2$ in the presence of three phosphatidic acid mole fractions (0.0, 0.1, 0.2-mol fraction). Data represent the average of three separate experiments. Panel A, PLC-γ1 isolated from control cells. Panel B, PLC-γ1 isolated from EGF-treated cells. PA, phosphatidic acid.
Effect on increasing phosphatidic acid mole fraction on the kinetic parameters of both control and tyrosine-phosphorylated PLC-γ1

PLC-γ1 was isolated from control and EGF-treated cells as described under “Experimental Procedures.” The ability of PLC-γ1 to hydrolyze PtdIns-4,5-P2 was measured as described in the legend to Fig. 7. Kinetic parameters were determined by fitting the data to the Hill equation using Enzfitter (Biosoft) computer program. n, cooperativity index.

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Mole fraction phosphatidic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>-EGF</td>
<td>Relative V (nmol/min/mg)</td>
</tr>
<tr>
<td></td>
<td>S0.5 (mole fraction)</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>+EGF</td>
<td>Relative V (nmol/min/mg)</td>
</tr>
<tr>
<td></td>
<td>S0.5 (mole fraction)</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
</tbody>
</table>

index was observed (Fig. 7B) (Table III). However, the relative velocity (v) of the tyrosine-phosphorylated enzyme increased, with a resulting decrease in the K_m, as the phosphatidic acid mole fraction was increased.

There have been recent reports demonstrating that phosphatidic acid activates a protein kinase (9, 42). It is possible, therefore, that the kinetic changes observed in the presence of phosphatidic acid were due to activation of a kinase in the immunoprecipitate. To determine whether phosphatidic acid could result in the phosphorylation of PLC-γ1, PLC-γ1 was incubated with [γ-32P]ATP in the presence and absence of phosphatidic acid. No phosphorylation of PLC-γ1 was observed (data not shown).

DISCUSSION

The data presented in this study further understanding of the kinetic mechanisms(s) by which PLC-γ1 activity may be controlled. Previous work (8) showed that the control enzyme displays cooperativity toward PtdIns-4,5-P2, whereas the in vivo tyrosine-phosphorylated enzyme displays apparent Michaelis-Menten kinetics. We have also performed experiments demonstrating that when PLC-γ1 is phosphorylated in vitro with EGF receptor, phosphorylation changed the kinetic profile from sigmoidal to hyperbolic. This indicates that tyrosine phosphorylation, per se, is responsible for the observed change in kinetic behavior. In this study, we demonstrate that the anionic phospholipid, phosphatidic acid, increases both control and tyrosine-phosphorylated PLC-γ1 activity. In the case of the control non-tyrosine-phosphorylated enzyme, the data show that as the phosphatidic acid mole fraction is increased in the assay mixture, the cooperative effect toward PtdIns-4,5-P2 decreases. Phosphatidic acid, therefore, increases activity by acting as an allosteric modifier of enzymatic activity. Phosphatidic acid also affects the kinetics of the tyrosine-phosphorylated form of PLC-γ1. As the mole fraction of phosphatidic acid in the assay mixture increases, a lower K_m for PtdIns-4,5-P2 is observed.

Unlike activation of control enzyme by tyrosine phosphorylation, phosphatidic acid has no effect on the ability of the enzyme to bind to substrate micelles, (K_d). There are three major tyrosine phosphorylation sites on PLC-γ1, Y771, Y783, and Y1254. Tyrosine phosphorylation at different sites may regulate different enzymatic reaction steps, whereas phosphatidic acid interaction with PLC-γ1 may only regulate one step in the reaction.

Based on the kinetic data, a model that explains how phosphatidic acid can be an allosteric modifier of PLC-γ1 activity can be proposed. The cooperativity index of an enzyme can be loosely correlated to the number of substrate binding sites (41). Control PLC-γ1 has an cooperativity index of 2.5; therefore, it appears that PLC-γ1 has at least two binding sites for phospholipid. The presence of two phospholipid binding sites on PLC-γ1 is reminiscent of the dual phospholipid model proposed by Hendrickson and Dennis (31). In the dual phospholipid model, there is one non-catalytic binding site and one catalytic binding site for phospholipid. The sigmoidal curve observed in Fig. 3 suggests that in the absence of tyrosine phosphorylation or phosphatidic acid, binding of substrate to the catalytic site does not readily occur. Binding of PtdIns-4,5-P2 or phosphatidic acid to the non-catalytic site may result in a conformational change in PLC-γ1 that lowers the binding constant for substrate to the catalytic site, so that a second molecule of PtdIns-4,5-P2 can bind more readily to the catalytic site.

Tyrosine-phosphorylated PLC-γ1 does not display allosteric enzyme kinetics; therefore, tyrosine phosphorylation may also result in lowering of the binding constant for PtdIns-4,5-P2 at the catalytic site on PLC-γ1. Since phosphatidic acid can increase the activity of this form of PLC-γ1, we conclude that phosphatidic acid also interacts with the tyrosine-phosphorylated form of PLC-γ1. Our observations that the tyrosine-phosphorylated form of PLC-γ1 has a lower S0.5 for phosphatidic acid than the control enzyme (0.1 compared with 0.2, respectively) and that phosphatidic acid lowers the K_m for PtdIns-4,5-P2 (Table I) suggest that the tyrosine-phosphorylated form of PLC-γ1 may have a lower binding constant for phosphatidic acid to the non-catalytic site also.

A site for phosphatidic acid binding to PLC-γ1 is not known, but there is a sequence in the C-terminal end of the Y region (a region of homology found in all PLC isozymes) that shares sequence homology with a regulatory domain of protein kinase C (43, 44). The function of this regulatory domain in protein kinase C is to act as a calcium and lipid binding domain. The preferred phospholipid that binds to this domain is phosphatidylserine, though phosphatidic acid also binds and stimulates protein kinase C activity (44). We find that calcium is required for phosphatidic acid to stimulate the activity of both tyrosine and control forms of PLC-γ1. It is possible, therefore, that the binding site for phosphatidic acid is contained within the calcium and lipid binding domain.

The fact that phosphatidic acid can activate the control enzyme provides a second mechanism by which PLC-γ1 can be activated in vivo. The physiological concentration of PtdIns-4,5-P2 in cells is 0.02-mol fraction (45). This is lower than the K_m of the tyrosine-phosphorylated form of the enzyme (0.3-mol fraction). If the in vitro measurements reflect in vivo activity, the control enzyme would essentially be inactive. Agents such as EGF can increase phosphatidic acid concentrations (18, 26, 27), and the time course of EGF-induced increases in phosphatidic acid levels are similar to the kinetics of EGF-induced increases in Ins-1,4,5-P3 levels (46). Essentially, increasing phosphatidic acid levels in the cell would effectively lower the K_m for PtdIns-4,5-P2 to near the physiological concentration of PtdIns-4,5-P2. Tyrosine phosphorylation and phosphatidic acid may work together to maximally activate PLC-γ1. Since many other growth factors also result in the production of phosphatidic acid, this suggests the possibility that PLC-γ1 could also be regulated by non-tyrosine kinase receptors.

Acknowledgments—We acknowledge Tanya Sorkin for mainte-

---

*G. Jones and G. Carpenter, unpublished results.*
nance of cell culture and Edna Kunkei for technical help in the preparation of figures.

REFERENCES