The Pleckstrin Homology Domains of Phospholipases C-β and -δ Confer Activation through a Common Site*

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Yuanjian Guo, Finly Philip, and Suzanne Scarlata‡

From the Department of Physiology and Biophysics, State University of New York, Stony Brook, New York 11794-8661

Mammalian inositol-specific phospholipase C (PLC)1 enzymes catalyze the hydrolysis of the signaling lipid, phosphatidylinositol 4,5 bisphosphate (PI(4,5)P$_2$) to produce the two second messengers, 1,4,5 inositol trisphosphate, which promotes an increase in intracellular calcium, and diacylglycerol, which promotes the activation of protein kinase C (for review, see Refs. 1 and 2). There are four known families of mammalian PLCs that are regulated differently. The PLCβs are regulated by G protein subunits, and each of the four members of the PLCβ family has varying sensitivities to Ga and Gβγ subunits. PLCβ$_2$ is a 134-kDa protein that can be independently activated by both Ga and Gβγ subunits, but simultaneous activation by both subunits does not appear to occur (3). The mammalian PLCs are modular proteins that contain several structural domains (see Fig. 1). PLCβ are distinguished by a long, 400-residue tail on their C termini, and deletion of this tail abolishes activation by Ga but not Gβγ (4–6). The neighboring C2 domain plays a role in the specific binding of PLCβ$_2$ to activated Ga subunits (7).

Binding and subsequent activation of PLCβ$_2$ by Gβγ subunits is regulated by the N terminus of the protein which contains a pleckstrin homology (PH) domain (8, 9). The association of the N terminus to Gβγ subunits does not appear to be highly specific because the pleckstrin homology domain of a related protein, PLCδ5, that is not activated by G proteins binds to Gβγ with only a 4-fold weaker affinity than that of PLCβ$_2$. These relatively close interaction energies between the two types of PLC can be compared with specific binding of the PLCβ$_2$ C2 domain to activated Ga$_q$, where a similar association to PLCδ$_1$ C2 cannot be detected (7).

Because PLCs attach to membranes to access their substrate, characterization of their membrane binding properties has been carried out. It has been found that unlike PLCδ$_1$, which only binds strongly to membranes if its substrate PI(4,5)P$_2$ is present (10, 11), PLCβ$_2$ binds strongly and fairly nonspecifically to lipid membranes (12, 13). However, membrane binding of both types of proteins appears to be mediated by the N-terminal PH domain (10, 14–18). Measurements of the membrane association of the isolated PH domains of PLCβ$_2$ and PLCδ$_1$ show that this region is responsible for both the affinity and specificity of membrane interaction (8).

To better understand the role of the N-terminal PH domains, we have previously constructed a chimera in which the PH domain of PLCβ$_2$ was swapped into the catalytic portion of PLCδ$_1$ to yield the chimeric protein, PHδ$_1$-PLCβ$_2$ (9). Although this protein had the same membrane and Gβγ interaction properties as wild type PLCβ$_2$, it was also activated by Gβγ, showing that the insertion of the PH of PLCβ$_2$ into PLCδ$_1$ confers G protein activation. Thus, the PH domain of PLCβ$_2$ directly confers Gβγ activation to the catalytic core. Analogously, it has been shown that the specific binding of PI(4,5)P$_2$ to the PH domain of PLCδ$_1$ resulted in activation of the catalytic core (17). Taken together, these studies imply that the PH domain of PLCβ$_2$ or PLCδ$_1$ contacts a common site in the catalytic region that transmits activation when Gβγ or PI(4,5)P$_2$ binds, respectively. One possible site may be a highly conserved site in the catalytic region that has been implicated in mediating Gβγ activation of PLCβ$_2$ (19).

In this study, we have constructed a chimeric protein consisting of the PH domain of PLCδ$_1$ and the remaining regions of PLCβ$_2$, called PHδPLCβ. We also constructed a chimera that includes the PH of PLCδ$_1$, a short (−20 residue) linker sequence, and the first helix on the neighboring EF hand (PH$^1$-δPLCδ). The purpose of this latter construct was to test whether altering this linker and EF hand would affect the interaction between the PH domain and the catalytic core. We find that both proteins have the same basal activity as wild type PLCβ$_2$ and displayed PI(4,5)P$_2$-specific binding and acti-

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‡To whom correspondence should be addressed. Tel.: 631-444-3071; Fax: 631-444-3428; E-mail: Suzanne.Scarlata@stonybrook.edu.

1. The abbreviations used are: PLC, phospholipase C; PH, pleckstrin homology; PI(4,5)P$_2$, phosphatidylinositol 4,5-bisphosphate; coumarin, 7-di-ethylaminohex-3-(4-maleimidophenyl)-4-methylcoumarin; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PI, phosphatidylinositol; PC, phosphatidylycholine; PS, phosphatidylserine.
activation as PLCδ1. As expected, the chimeric proteins are activated by PI(4,5)P2 but not Gβγ, suggesting that activation of the catalytic cores of PLCδ2 and PLCδ3 are mediated by a common site in their PH domains. By using a peptide that we predicted should inhibit the interaction between the PH and catalytic domains based on theoretical models, we found that we could inhibit both the basal activity of both enzymes and also see a decrease in PI(4,5)P2 and Gβγ activation of the PLC catalytic cores in a concentration-dependent manner. Our results thus give a molecular basis for the common activation of these two enzymes.

**MATERIALS AND METHODS**

**Protein Preparation—**Synthetic peptides were prepared and purchased from the American Peptide Company (Sunnyvale, CA) and used as received. PLCδ2 and Gβγ subunits were expressed in Sf9 cells and purified using the method described (ref. 11).

**Construction of Chimeras—**Human PLC δi was expressed in Escherichia coli and recombinant human PLCβ2 in Sf9 cells. Two chimeras were prepared. The first, PHδPLCβ2, replaced residues 1–134 of PLCδ2 with residues 1–117 of PLCδ1. The second, PHδδPLCβ2, included a 20-residue linker region and the first EF hand of PLCδ1. The coding sequence of the N-terminal half of PLCδ1, a PL Cδ1 insert, was amplified by PCR using corresponding PLCδ1 cDNA. (The primers were 5′-ATGGACTCCGGGCGGAGAC 3′ and 3′-GCTGATTTGCTGATACATTTGCCCCAGGC- GC-5′ or 3′-GCAAGCTCTGGA CATTGTTGTTTTTGTGACGCAGCGC-5′.) The chimeras were constructed by introducing SpeI and NheI restriction enzyme sites into a pVL1392 vector containing the coding sequence of these proteins. These were then ligated into the PLCδ2 vector. The obtained construct served as a template. These were then ligated into the PLCδ2 vector within the NheI and NdeISpeI sites to give the corresponding chimeric proteins. The chimeric enzymes were expressed in Sf9 cells and purified using the protocol for PLCδ2. The purified proteins were identified by Western blotting using a monoclonal antibody to the N terminus of PLCδ2 and a polyclonal antibody to the C-terminal region of PLCβ2.

**Hydrolysis of PI or PI(4,5)P2 in Phospholipid Vesicles—**Hydrolysis of [3H]PI or [3H]PI(4,5)P2 in phospholipid vesicles was conducted on membrane surfaces, the resulting bimolecular association curves yield apparent Kd values that are dependent on the total available membrane surface area. We have previously translated these apparent values of Kd to the dissociation constant that would be observed if the proteins were not membrane-bound (20). However, because all of the studies here were done at an identical lipid concentration, this analysis is not required. We have only reported the experimental values of the apparent Kd to be used for comparative purposes.

**Membrane Binding of the PH and Catalytic Domains of PLCδ2—**Models of PH-domain-XV domain and complexes of rat PLCδ3 were constructed using a protein docking program, Global Range Molecular Docking or GRAMM (21). We used docked models (obtained from SD Jigsaw) of PLCδ2, PH domain to catalytic region using high and low resolution methods.

The following parameters were used for high resolution docking of PLCδ2. PH domain to PHPLCδ3: matching mode (generic/helical) = generic, grid step = 2.1; repulsion (attraction is always 1.1); attraction double range (fraction of single range) = 0; potential range type (atom_radius, grid_step) = grid_step; projection (black-white, gray) = gray; representation (all, hydrophobic) = all; number of matches to output = 1000; angle for rotations, deg (10, 12, 15, 18, 20, and 30; 0 indicates no rotation) = 10.

**RESULTS**

**Membrane Binding Properties of the PHδPLCδ3 Chimera—**Although the isolated PH domain of PLCδ2 binds strongly to lipid membranes (12, 16), the contribution of other enzyme domains in membrane association is not clear (see ref. 6), and so the chimeras in Fig. 13 may allow us to determine the contribution of these other PLCδ2 domains in membrane association. The PH domains of PLCδ2 and PLCδ3 have different binding characteristics. The PH domain of PLCδ3 binds strongly (i.e., Kd = 50–200 μM) to membranes containing PI(4,5)P2 because of specific interactions, and also to highly negatively charged lipids because of electrostatic attraction by the positive lobe of the protein (14, 15). In contrast, the PH domain of PLCδ2 binds weakly to membranes with little specificity for charge or chemical structure (8, 14). Thus, if the PH domain solely directs membrane association of the whole enzyme, we would expect the PHδPLCδ3 chimeras to have identical characteristics to PLCδ3.

We have previously found that the emission intensity of coumarin-labeled PLCδ2 and the isolated PH domains of PLCδ2 and PLCδ3 increase upon membrane binding (8). We thus measured the binding of the proteins to large, unilamellar vesicles of varying membrane compositions by the increase in fluorescence of the probe coumarin covalently attached to the proteins (see methods). In Fig. 2a we show the association of
PLC$_{\beta_2}$, PLC$_{\delta_1}$, and the PH$_{\delta}$PLC$_{\beta}$ chimera to POPC bilayers. In accord with previous studies using both spectroscopic and sedimentation techniques, PLC$_{\delta_1}$ does not bind to POPC bilayers in this concentration range, whereas PLC$_{\beta_2}$ binds strongly (12–14, 22). Unlike PLC$_{\delta_1}$, the PH$_{\delta}$PLC$_{\beta}$ chimera bound to POPC bilayers with an affinity strong enough to be measured by fluorescence, but 20-fold weaker than PLC$_{\beta_2}$. These results support the idea that regions other than the PH domain of PLC$_{\beta_2}$ contribute to membrane binding (see Ref. 6).

When 5% PI(4,5)P$_2$ is incorporated into POPC bilayers, the membrane binding affinities of PLC$_{\beta_2}$ and PLC$_{\delta_1}$ become comparable in accord with previous work (12, 14). In Fig. 2b we show that the presence of PI(4,5)P$_2$ increases the binding affinity of the PH$_{\delta}$PLC$_{\beta}$ chimera 20-fold, bringing its value ($K_p = 20.6 \pm 2.8 \mu M$) close to that of PLC$_{\beta_2}$ ($K_p = 28.4 \pm 11.8 \mu M$) and PLC$_{\delta_1}$ ($K_p = 19.6 \pm 10 \mu M$). Thus, the characteristic PI(4,5)P$_2$ binding specificity of the PLC$_{\delta_1}$ PH domain remains intact in the chimera. This increase in membrane binding affinity of PLC$_{\delta_1}$ is not entirely because of electrostatic effects because the chimera and PLC$_{\delta_1}$ bound much more weakly to membranes containing 30% negatively charged lipids (i.e. PC:PS (2:1)) (data not shown).

**Binding of PLC Enzymes to G Protein Subunits**—We have previously characterized the lateral association between PLC$_{\beta_2}$ and G protein subunits (3) and between the isolated PH domains of PLC$_{\beta_2}$ and PLC$_{\delta_1}$ to G$_{\beta\gamma}$ subunits on membrane surfaces (8). We have found that even though PLC$_{\delta_1}$ is not activated by G$_{\beta\gamma}$ subunits, it will bind to these subunits with a 4-fold weaker affinity than the G$_{\beta\gamma}$-activable PLC$_{\beta_2}$. The comparable G$_{\beta\gamma}$ affinities between the PH domain of PLC$_{\beta_2}$ and the whole enzyme, and similarly between the PH domain of PLC$_{\delta_1}$ and the whole enzyme, suggest that G$_{\beta\gamma}$ association is mediated entirely through the PH domain (8). If this is the case, then the G$_{\beta\gamma}$ binding affinity of the PH$_{\delta}$PLC$_{\beta}$ chimera should be closer to PLC$_{\delta_1}$ than to PLC$_{\beta_2}$.

We measured the lateral association of the PLC proteins to G$_{\beta\gamma}$ subunits on membrane surfaces by labeling G$_{\beta\gamma}$ subunits with coumarin, reconstituting C-G$_{\beta\gamma}$ into lipid bilayers and adding the PLC proteins under conditions where the proteins would be entirely membrane-bound. Control studies substituted buffer for the titrating protein solution. Under our labeling conditions, the fluorescence intensity of the coumarin probe attached to G$_{\beta\gamma}$ is sensitive to PLC association, giving association constants identical to previous values obtained using fluorescence resonance energy transfer (3, 8, 20). Also, the midpoint of the titration curves shift appropriately over an 8-fold initial amount of G$_{\beta\gamma}$ to give identical values of $K_d$, which is a key indicator of a protein-protein association process. The observation that the coumarin label changes upon association with PLC$_{\beta_2}$ implies that the labeling site may be

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**Fig. 1.** Schematic diagram of the chimeras made in this study. The length of the EF hands of PLC$_{\beta_1}$ is poorly defined. Therefore only the starting residue number is given.

**Fig. 2.** Demonstration of the PI(4,5)P$_2$ specificity of the PLC proteins. a, binding of PLC$_{\beta_2}$ (●), PH$_{\delta}$PLC$_{\beta}$ (○), and PLC$_{\delta}$ (■) at 50 nM protein to large, unilamellar vesicles of POPC, which have electrically neutral surfaces ($n = 3$). b, increase in binding of the PH$_{\delta}$PLC$_{\beta}$ chimera (○), relative to PLC$_{\beta_2}$ (●) and PLC$_{\delta}$ (■) at 50 nM when 5% PI(4,5)P$_2$ is incorporated into the membranes ($n = 3$).

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**Table 1.** Binding of PLC Enzymes to G Protein Subunits

| Enzyme   | Catalytic Domain | C2 | Midpoint Kd (M) |
|----------|------------------|----|-----------------|
| PLC$_{\beta_2}$ |                 |    | 28.4 ± 11.8     |
| PLC$_{\delta_1}$ |                 |    | 19.6 ± 10       |
| PH$_{\delta}$PLC$_{\beta}$ |             |    | 20.6 ± 2.8      |

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**Table 2.** Binding of PLC Enzymes to G Protein Subunits

| Enzyme   | Catalytic Domain | C2 | Midpoint Kd (M) |
|----------|------------------|----|-----------------|
| PLC$_{\beta_2}$ |                 |    | 28.4 ± 11.8     |
| PLC$_{\delta_1}$ |                 |    | 19.6 ± 10       |
| PH$_{\delta}$PLC$_{\beta}$ |             |    | 20.6 ± 2.8      |
close to the protein-protein interaction site, although the apparent $K_d$ does not appear to be affected (see Ref. 3). Because our studies compare the affinities between the PLC enzymes and G protein subunits, perturbation of the absolute affinities because of the probe is not critical.

We reconstituted C-G$\beta_\gamma$ subunits onto PC:PS (1:2) lipid membranes because this high concentration of negatively charged lipids ensures complete membrane binding of both PLC$\beta_2$ and the PH-PLC$\delta_1$ chimeras in the absence of PI(4,5)P$_2$ (data not shown). The studies were carried out at identical lipid concentrations so that apparent affinities can be directly compared. The results of this study are shown in Fig. 3. As can be seen, the G$\beta\gamma$ affinity of the chimera is closer to PLC$\delta_1$ than to PLC$\beta_2$. We find that the ratio of the apparent G$\beta\gamma$ dissociation constant of the PH$\delta$PLC$\beta$ to wild type PLC$\beta_2$ is similar to the ratio of the PH domains of PLC$\delta_1$ and PLC$\beta_2$ and also to corresponding ratios of the whole enzymes (8). If other sites in PLC$\beta_2$ contributed to its association with G$\beta\gamma$ subunits, we
would expect these ratios to differ. Thus, these results are in accord with the idea that the PH domain is the main docking site for \( \gamma/\delta \) subunits and that other domains, if they play a role, do not significantly contribute to the binding energy.

Response of the Chimera to Activators—When the PH domain of PLC\( \beta_2 \) was swapped into PLC\( \delta_1 \), the specific activity of this chimera was much lower than PLC\( \delta_1 \) and comparable with PLC\( \beta_2 \). This result raised the question of whether the PH domain modulates the activity of the catalytic core. One factor that may contribute to the higher enzymatic activity of PLC\( \delta_1 \) as compared with PLC\( \beta_2 \) is the idea that the specific binding of PI(4,5)P\(_2\) to the PLC\( \delta_1 \)-PH domain induces activation of the

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**Fig. 5.** a, proposed PI(4,5)P\(_2\)-binding sites in the most appropriate docked model of PLC\( \delta_1 \). The PH domain (green) and the catalytic domain (orange) are on the same side of the protein to give proper membrane orientation. Residues around the D-myo-inositol 1,4,5-trisphosphate-binding site in the PH domain are highlighted in red, and residues around the D-myo-inositol 1,4,5-trisphosphate-binding site in the catalytic domain are in purple. b, left panel, PH domain (green) of PLC\( \delta_1 \) docked onto the C-terminal domains of PLC\( \delta_1 \) (orange) with catalytic domain residues Tyr\(^{314}, \) Leu\(^{315}, \) Gln\(^{319}, \) Thr\(^{361}, \) Ser\(^{360}, \) Thr\(^{381}, \) Pro\(^{365}, \) and Asp\(^{378}, \) which are predicted to interact with the PH domain in cyan. The right panel is a close up of the interaction region where residues in yellow are conserved and important for phosphoinositide hydrolysis (His\(^{311}, \) His\(^{356}, \) Lys\(^{438}, \) Ser\(^{302}, \) Arg\(^{348}, \) Lys\(^{440}, \) Asp\(^{343}, \) Glu\(^{390}, \) and Asn\(^{312} \)). c, left panel, PH domain (green) of PLC\( \beta_2 \) docked onto its XY domain (orange) with catalytic domain residues, which are predicted to interact with PH domain in cyan. The right panel is a close up of the interaction region where residues in yellow are the residues homologous to catalytic histidines 311 and 356 of PLC\( \delta_1 \).
catalytic core (see Ref. 17). If the PH domain of PLC\(\beta_1\) interacted with the catalytic region PLC\(\beta_2\) in a manner similar to that of wild type PLC\(\beta_1\), then we would expect the PH\(\beta_1\)-PLC\(\beta_2\) chimera to be activated by PI(4,5)P\(_2\) and not by G\(\beta\gamma\) subunits.

To determine the relative activity of the PLC enzymes, we compared the specific activities of PLC\(\beta_1\), PLC\(\beta_2\), and PH\(\beta_1\)PLC\(\beta_2\) by measuring the hydrolysis of [\(\text{H}\)]PI in small, unilamellar vesicles composed of PI:PC:PS (1:5:5) with increasing amounts of cold PI(4,5)P\(_2\). This substrate concentration has been previously used to show that PI(4,5)P\(_2\) produces an 8–9-fold increase in PLC\(\beta_1\) activity (17). In the absence of PI(4,5)P\(_2\) activator, we find that the specific activity of PH\(\beta_1\)PLC\(\beta_2\) lies between that of PLC\(\delta_1\) and PLC\(\beta_2\), giving a value only 17-fold less than PLC\(\delta_1\) for the chimera as opposed to a 51-fold less value obtained for PLC\(\beta_2\) relative to PLC\(\delta_1\) (data not shown). Because the chimera contains the catalytic region of PLC\(\beta_2\), its higher activity toward [\(\text{H}\)]PI indicates that the PH\(\delta_1\) has a different interaction with the PLC\(\beta_2\) catalytic core than the wild type PH domain. This observation supports the idea that the PH domain serves to inhibit the core in the basal state in the absence of activators (9).

We then tested whether PI(4,5)P\(_2\) could activate the enzymes. These studies were carried out by measuring [\(\text{H}\)]PI hydrolysis with increasing amounts cold PI(4,5)P\(_2\) in the membrane substrate (see Ref. 17). The results, presented in Fig. 4, show that PI(4,5)P\(_2\) activation is close to the level of activation seen in previous studies using PLC\(\delta_1\) (17). PI(4,5)P\(_2\) activation of the PH\(\beta_1\)PLC\(\beta_2\) chimera is only slightly lower than PLC\(\delta_1\), whereas activation of PLC\(\beta_2\) does not significantly occur.

### Sequence Comparison of PLC\(\delta_1\) and PLC\(\beta_2\) PH – Catalytic Domain Interaction Regions

| PLC\(\delta_1\) | MDSGRDFTLTGLQGDLQWLLKKSQGQGLKQDACKTFLQRWESKVRM 60 |
|----------------|------------------------------------------------------|
| PLC\(\beta_2\) | ---------------MSLLNPVLLPKVAXLQGGRFIIKWDRTSIAFVILRVDP5GYLW---HQ 52 |
| PLC\(\delta_1\) | SPSSQQLPSIEDIQEVQRMGKTEG---------------------------EKFADIPEDRCSIVVDQKQ----------------------------NT 107 |
| PLC\(\beta_2\) | SKHEKFDLVSIRDRGFLKFKPSQKLREVVNMDPUNHFLKTTTTSQGDYGLTF 112 |
| PLC\(\delta_1\) | LSSLAPSADAQMWWQLKJKHSSGM 136 |
| PLC\(\beta_2\) | HNFVESYKENVKDWALAKHPMTAH 141 |

![Sequence comparison of the PH domain residues of PLC\(\delta_1\) and PLC\(\beta_2\), in which the regions proposed to interact with their corresponding catalytic region are given in italics and the sequence of the peptide used in this study is underlined. Identical residues are denoted by asterisks, highly homologous residues are denoted by colons, and homologous residues are denoted by dots.](http://www.jbc.org/)

![Activity of PLC Enzymes with PH-PLC\(\delta_1\)(84-95) Peptide](http://www.jbc.org/)

*Fig. 6.* Sequence comparison of the PH domain residues of PLC\(\delta_1\) and PLC\(\beta_2\), in which the regions proposed to interact with their corresponding catalytic region are given in italics and the sequence of the peptide used in this study is underlined. Identical residues are denoted by asterisks, highly homologous residues are denoted by colons, and homologous residues are denoted by dots.

*Fig. 7.* Change in the normalized hydrolysis of [\(\text{H}\)]PI(4,5)P\(_2\) of PLC enzymes with increasing amounts of the PLC\(\delta_1\) (84–95) peptide whose sequence is suggested to be at the interface between the PH and catalytic domains where PLC\(\beta_2\) (black bars), PH\(\beta_1\)PLC\(\delta\) (light gray bars), and PLC\(\delta_1\) (dark gray bars) all show a decrease in activity with increasing peptide concentration.
studies show that the PH domain of PLCδ1 regulates PI(4,5)P2 activation to the catalytic cores of both PLCβ2 as well as the wild type enzyme.

The proteins were then tested for their ability to be activated by G protein subunits. Because PHββδ shows an ~3-fold weaker binding affinity to Gβγ subunits than PLCβ2, activation studies were carried out to high concentrations of Gβγ to ensure complete protein-protein association. Our previous study revealed that the catalytic core of PLCδ1 could be activated by Gβγ when the PH domain of PLCβ2 was linked to PLCδ1 (9). Based on this study, we predicted that because the PHββδ chimera contains a PH domain from a PLC that is not activated by Gβγ subunits, no changes in activity will be seen. We could not detect activation of the chimera even at concentrations well above the apparent Kd for Gβγ binding (data not shown).

Identification of a Common Site Involved in PLC-β2 and -δ1 Activation—The ability of the PH domain of PLCδ1 to confer activation to the catalytic domain of PLCβ2 and vice versa suggests a common interaction site between the catalytic and PH domains. Although the catalytic domain of the two phospholipases are highly conserved having 50% sequence identity and 82% sequence homology, their PH domains have only 15% sequence identity and 56% sequence homology (see Ref. 12). Nevertheless, we have previously made threading models of the PH domain PLCδ1 based on the known PHδ1 structure and obtained a predicted 82% structural identity (23). The high sequence homology between the catalytic domains of the two enzymes allowed us to easily make structural models of the catalytic core of PLCβ2. We used these models and the known structures of the PH and catalytic domains of PLCδ1 and docked the domains to identify potential interaction sites.

The docking procedure was carried out using GRAMM, which performs an exhaustive six-dimensional search for low intermolecular energy states of the structures by changing the atom-atom potentials of the inputted atomic coordinates of the two molecules (21). We carried out the docking procedure using GRAMM at both high and low resolution because the latter suppresses local minima (false positive fits), giving more appropriate candidates. This procedure resulted in 50 PLCδ1 models. Approximately 75% of these were similar and gave fairly consistent structures, and these were used for further investigation. Because the structures of the PH and catalytic domains of PLCδ1 are known, we present the results for this enzyme. We discarded all but one model based on the criterion that the PI(4,5)P2-binding site in the PH and catalytic domains of PLCδ1 must have the same orientation. This model was docked at high resolution and gives all configurations with a high score stearic fit and is shown in Fig. 5c. The models in Fig. 5 predict three common regions of the PH domains of PLCδ1 and PLCβ2 that interact with a common site in the catalytic core as presented in Fig. 6.

To test the model in Fig. 5, we synthesized a peptide having the PH domain sequence corresponding to residues 84–95 of PLCδ1, which lies very close to the catalytic region and has high homology to the corresponding region of PLCβ2. We then determined the effect of this peptide on the basal and Gβγ/PI(4,5)P2-stimulated activity of the PLC enzymes relative to a control peptide with a unhomologous sequence. If the PH domain peptide does mimic the common interaction site between the PH and catalytic domains of PLCβ2 and PLCδ1, then it should produce similar changes in activity in both the PLC enzymes and their chimeras.

We monitored the change in PI(4,5)P2 hydrolysis of PLCβ2, PLCδ1, and a PHβγ-PLCδ1 chimera that can be activated by Gβγ subunits (9). The addition of increasing amounts of the PLCδ1 (84–95) peptide to each of these proteins causes a pro-

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*F. Philip and S. Scarlata, unpublished results.*
gressive reduction in activity (Fig. 7), whereas the control peptides had no effect on the activity. We note that this peptide does not interact with lipid bilayers under our assay conditions. A reduction in activity of the PLC enzymes would be expected if catalysis involves productive interaction between the PH and the catalytic core.

We then focused on activation of the PH-PLCβ₂-containing enzymes by Gβγ by measuring the ability of the PLCδ₁ (84–95) peptide to inhibit Gβγ activation of wild type PLCβ₂ and the Gβγ-activatable chimera PH₂PLCδ. At partially activating concentrations of Gβγ (i.e. 25 nM Gβγ, which produces a 1.5-fold increase in activation), addition of the peptide could completely abolish Gβγ activation of both PLCβ₂ and the PH₂PLCδ chimeras (Fig. 8, left panel). However, at higher Gβγ concentrations (i.e. 200 nM, which produces a 4-fold increase in activation), the loss of activation by the peptide was greatly reduced (Fig. 8, right panel). Thus, raising the Gβγ concentration eliminates the effect of the peptide.

To determine whether the PLCδ₁ (84–95) peptide reduced Gβγ activation by competing with Gβγ with the interaction site on the PH domain of PLCβ₂, we carried out a series of fluorescence titrations where we measured the association between PLCβ₂ and Gβγ subunits at increasing levels of peptide. We find that not only that the peptide binds to isolated Gβγ and PLCβ₂ with apparent K_d of 40 and 50 nM, respectively (data not shown), but also that the peptide inhibits the association between the two proteins, indicating that it mimics both the interaction site between the PH domain and the catalytic core and between the PH domain and Gβγ subunits (Fig. 9).

We then determined whether the peptide could specifically inhibit the PH domain-mediated activation of PLCδ₁ by PI(4,5)P₂. We found that the addition of 1 μM peptide reduces the PI(4,5)P₂ activation by ~50% (Fig. 10). Taken together, these results show that a peptide that targets a homologous interaction region between the PH and catalytic regions of PLCδ₁ can inhibit the basal and stimulated activities of both this enzyme and PLCβ₂.

**DISCUSSION**

In this study, we have shown that we can transfer the activators of the catalytic core of PLCβ₂ and PLCδ₁ by transferring their PH domains. Because this activation process must involve a common interface between the PH and catalytic domains in the two enzymes, we constructed structural models that enabled us to identify this interface and tested these using a synthetic peptide inhibitor. Our results also suggest that the interface between the PH and catalytic domains is the region involved in the docking of Gβγ subunits to PLCβ₂.

We first constructed two chimeras that consist of the PH domain of PLCδ₁ and the remainder of PLCβ₂ and of the PH-EF domains of PLCδ₁ and the remainder of PLCβ₂. The resulting proteins had electrophoretic mobilities on SDS-PAGE gels matching that of PLCβ₂. Western blot analysis showed that the chimeras contain monoclonal antibody epitopes for the N-terminal region of PLCδ₁ and the C-terminal region of PLCβ₂, thus verifying that the proteins are true chimeras. The chimeras showed the same PI(4,5)P₂-dependent membrane binding behavior as PLCδ₁ and bound to Gβγ subunits with identical affinities as PLCδ₁, showing that, functionally, the N-terminal region of the protein is correctly folded. The specific activity of the chimeras fell between the high specific activity of PLCδ₁ and PLCβ₂, suggesting that the catalytic core of PLCβ₂ functions at an enhanced level when the PH domain of PLCδ₁ is present. We also note that the chimeras bound to unactivated Gα₅ with an affinity identical to PLCβ₂, strongly suggesting that the C2 and C-terminal extensions are correctly folded (data not shown).

Although the PHδPLCβ construct was designed to better define the role of the PH domain of PLCβ₂ in membrane binding and G protein activation, we also constructed a chimera in which the PH domain, linker, and first EF hand of PLCδ₁ were swapped into PLCβ₂. There is 25% sequence homology of this first EF hand in the two PLCs. Like the N-terminal PH domain, this first EF hand was unresolved in the crystal structure of PLCδ₁; however the structure of the isolated PH domain was later solved (24, 25). Although the functional role of the EF hands in PLCδ₁ are unknown, it has been suggested that the second pair of EF hands interact with the C2 domain to give structural integrity to the protein core because deletion of this region inactivates the enzyme (see Ref. 24). In all of the studies presented here, we found no significant difference in membrane binding, Gβγ binding, and PI(4,5)P₂ activation between the two chimeras, suggesting that the EF hand does not directly modulate that catalytic core of the protein. The observation that both chimeras have similar activities supports the
Inhibition PLCδ₁ Activity by PLCδ₁(84–95) peptide with Varying Amounts of PI(4,5)P₂

![Inhibition of PI(4,5)P₂ stimulation of PLCδ₁ by the PLCδ₁ (84–95) peptide where the activation of [3H]PI hydrolysis in the absence of peptide was normalized for this plot, but the raw data corresponded to the values in Fig. 4 (n = 5).](image)

Fig. 10. Inhibition of PI(4,5)P₂ stimulation of PLCδ₁ by the PLCδ₁ (84–95) peptide where the activation of [3H]PI hydrolysis in the absence of peptide was normalized for this plot, but the raw data corresponded to the values in Fig. 4 (n = 5).

The finding that the PH domains of either enzyme can confer activation to the catalytic core suggests a conserved interface between these domains. Molecular modeling and protein docking studies allowed us to identify several potential models of the domain complex. We selected the model in Fig. 5 based on the assumption that the PI(4,5)P₂-binding sites in PH-PLCδ₁ and the catalytic domain must be on the same side of the protein to give the proper membrane orientation. This model identifies a PLCδ₁-PH domain interface that has high homology to that of PLCβ₂ and correlates well to the most reasonable docking model of the PH and catalytic domains of PLCβ₂.

The model in Fig. 5 is in accord with our current knowledge of PH domain-induced activation and activation by Gβγ subunits. The model shows that the PH domain may be located close to the catalytic core, and it is reasonable to assume that subtle changes in this interface through docking of activators or binding to membrane surfaces could directly affect catalytic activity. The peptide made to this interface (i.e., residues 84–95 of PLCδ₁) not only inhibited the basal activity of both the PLCβ₂ and PLCδ₁ enzymes and inhibited PI(4,5)P₂ activation of PLCδ₁, which would be expected if both proteins shared this common site, but it also competed with Gβγ binding and inhibited Gβγ activation for the PH-PLCβ₂ enzymes. This result suggests that Gβγ subunits bind to this region and confer activation.

Previous studies also based on peptide competition suggested that Gβγ activation of PLCβ₂ proceeds through a conserved region in the catalytic domain, which is homologous to residues 516–541 in PLCδ₁ (19). The homologous region in PLCδ₁ lies in close proximity to the PH-catalytic domain interface and can easily contact bound Gβγ subunits. The high homology between PLCβ₂ and PLCδ₁ in the catalytic domain would allow Gβγ to contact both enzymes as long as the PH domain of PLCβ₂ is present. Because the strength of Gβγ association to the PH domain of PLCδ₁ is only ~4-fold lower than PLCβ₂ and because the PH-PLCδ₁ domain cannot confer activation, it is likely that the regions outside the interface.

The observation that the PH domain of PLCδ₁ displays weak binding, shows that the PH domain is a key regulator of PLCδ₁ membrane association, whereas the C-terminal region works to enhance this association.

We have found that the PHδSPLCβ chimera can be activated by the presence of PI(4,5)P₂ to a similar extent as PLCδ₁. It has been previously shown that binding of PI(4,5)P₂ to the PLCδ₁ PH domain can confer activation to the catalytic domain (17). Using a PHβSPLCδ chimera, an analogous model was proposed for the activation of PLCβ₂ by Gβγ in which the binding of Gβγ to the PH domain conferred activation of the catalytic core (9). The observation that the PH domain of PLCδ₁ could not confer Gβγ activation of the PLCβ₂ catalytic core even under conditions where the two proteins are associated correlates well with the model that the PH domain of PLCβ₂ mediates Gβγ activation (9). Thus, the PH domains play a similar role in activation of their host core proteins through the binding of an activator.

If we consider that the level of PI(4,5)P₂ activation of the chimera is similar to that seen for PLCβ₂ by Gβγ subunits, then it appears that each activator stimulates the catalytic core of PLCβ₂ to a similar extent, which is most likely an intrinsic property of the catalytic site.

The finding that the PH domains of either enzyme can confer activation to the catalytic core suggests a conserved interface between these domains. Molecular modeling and protein docking studies allowed us to identify several potential models of the domain complex. We selected the model in Fig. 5 based on the assumption that the PI(4,5)P₂-binding sites in PH-PLCδ₁ and the catalytic domain must be on the same side of the protein to give the proper membrane orientation. This model identifies a PLCδ₁-PH domain interface that has high homology to that of PLCβ₂ and correlates well to the most reasonable docking model of the PH and catalytic domains of PLCβ₂.

The model in Fig. 5 is in accord with our current knowledge of PH domain-induced activation and activation by Gβγ subunits. The model shows that the PH domain may be located close to the catalytic core, and it is reasonable to assume that subtle changes in this interface through docking of activators or binding to membrane surfaces could directly affect catalytic activity. The peptide made to this interface (i.e., residues 84–95 of PLCδ₁) not only inhibited the basal activity of both the PLCβ₂ and PLCδ₁ enzymes and inhibited PI(4,5)P₂ activation of PLCδ₁, which would be expected if both proteins shared this common site, but it also competed with Gβγ binding and inhibited Gβγ activation for the PH-PLCβ₂ enzymes. This result suggests that Gβγ subunits bind to this region and confer activation.

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cannot make the productive contacts needed for Gβγ activation. Taken together, our studies suggest that Gβγ subunits initially dock to the PH domain of PLCβ2. The docking site lies in or close to the PH and catalytic domain interface where it can then make the necessary contacts with the catalytic regions, and most likely the region encompasses residues 564–593 to induce activation (19).

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The Pleckstrin Homology Domains of Phospholipases C-β and -δ Confer Activation through a Common Site
Yuanjian Guo, Finly Philip and Suzanne Scarlata

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