Membrane-induced Allosteric Control of Phospholipase C-β Isozymes*

Received for publication, June 3, 2014, and in revised form, August 13, 2014. Published, JBC Papers in Press, September 5, 2014. DOI 10.1074/jbc.M114.586784

Thomas H. Charpentier‡, Gary L. Waldo‡, Matthew O. Barrett‡, Weigang Huang§, Qisheng Zhang§, T. Kendall Harden‡, and John Sondek†*‡

From the Departments of Pharmacology and Biochemistry and Biophysics and the Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599 and the Division of Chemical Biology and Medicinal Chemistry, University of North Carolina School of Pharmacy, Chapel Hill, North Carolina 27599

Background: Phospholipase C-β (PLC-β) isozymes hydrolyze phosphatidylinositol 4,5-bisphosphate to propagate signals for several physiological responses.

Results: Membranes are essential for the allosteric release of autoinhibition of PLC-β isozymes.

Conclusion: Activators of PLC-β release autoinhibition by orientating the isozymes at the membrane.

Significance: The model described provides a better understanding of PLC-β regulation and potential mechanisms to inhibit their activation.

All peripheral membrane proteins must negotiate unique constraints intrinsic to the biological interface of lipid bilayers and the cytosol. Phospholipase C-β (PLC-β) isozymes hydrolyze the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP₂) to propagate diverse intracellular responses that underlie the physiological action of many hormones, neurotransmitters, and growth factors. PLC-β isozymes are autoinhibited, and several proteins, including Gαq, Gβγ, and Rac1, directly engage distinct regions of these phospholipases to release autoinhibition. To understand this process, we used a novel, soluble analog of PIP₂ that increases in fluorescence upon cleavage to monitor phospholipase activity in real time in the absence of membranes or detergents. High concentrations of Gαq or Gβγγ2 did not activate purified PLC-β3 under these conditions despite their robust capacity to activate PLC-β3 at membranes. In addition, mutants of PLC-β3 with crippled autoinhibition dramatically accelerated the hydrolysis of PIP₂ in membranes without an equivalent acceleration in the hydrolysis of the soluble analog. Our results illustrate that membranes are integral for the activation of PLC-β isozymes by diverse modulators, and we propose a model describing membrane-mediated allosterism within PLC-β isozymes.

Many extracellular stimuli, including hormones, growth factors, and neurotransmitters, activate phospholipase C (PLC) isozymes to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP₂) into the second messengers, inositol 1,4,5-trisphosphate and diacylglycerol (1–3). Elevated concentrations of inositol 1,4,5-trisphosphate mobilize intracellular calcium, whereas diacylglycerol directly activates protein kinase C isozymes; these bifurcating signals are necessary for many cellular responses, including cell proliferation and migration, smooth muscle contraction, platelet aggregation, and neurotransmission (4–6). PIP₂ also directly regulates many cytosolic, cytoskeletal, and membrane proteins, and its depletion by phospholipase C isozymes regulates ion channel activity (7–12).

Based on sequence conservation, the PLC isozymes are divided into six subfamilies (PLC-β, -δ, -γ, -ε, -ζ, and -η). All PLCs except sperm-specific PLC-ζ share a core set of domains: an N-terminal PH domain, a set of four atypical EF-hands, a catalytic triose-phosphate isomerase (TIM) barrel, and a C2 domain (13, 14). The two halves of the TIM barrel are connected by a region of variable length known as the XY-linker. This poorly conserved region nonetheless functions as a major regulatory element, and its removal from most PLCs results in constitutive phospholipase activity in vitro and in cells (15, 16). We previously proposed that G protein activators recruit and orient PLC isozymes at membranes, leading to displacement of the XY-linker from the viscosity of the lipase active site (13).

Domains that are unique to certain PLC isozymes provide additional isozyme-specific regulation of activity. For instance, the PLC-β isozymes contain a unique C-terminal domain that is structurally similar to Bin-amphiphysin-Rvs domains (17, 18). This region promotes membrane association and is required for efficient activation of PLC-β isozymes by Gαq and related Gα subunits (Gα11, Gα12, and Gα13) of heterotrimeric G proteins (19). In contrast, the C-terminal domain is not required for activation of PLC-β isozymes by either Gβγ or Rac1 (20, 21). The C-terminal domain was previously thought to interact directly with Gαq in the activation of PLC-β isozymes, but our measurements using soluble proteins indicate that PLC-β3 lacking the C-terminal domain binds active Gαq with an affinity only 2-fold lower than that of full-length
Allosteric Modulation of PLC-β Isozymes by Diverse Activators

PLC-β3 (22). These affinity measurements are supported by a recent crystal structure of full-length PLC-β3 and Goα, showing minimal interaction between Goα and the C-terminal domain (18). Nevertheless, Goα-dependent activation of PLC-β isoymes at membranes clearly is supported by the C-terminal domain (23–25). Whereas it is reasonable to posit that membranes facilitate interactions between the C-terminal domain and Goα to support phospholipase activity, the nature of these potential interactions remains poorly understood. In contrast to the C-terminal domain, a conserved region that immediately follows the C2 domain and is induced to form a helix-turn-helix (HTH) upon interaction with active Goα provides the major interface with Goα in several crystal structures (18, 22, 26). This interface involves the switch regions of Goα such that only the active form of Goα engages the HTH. Despite these structural insights, how Goα or Gβγ dimers activate PLC-β isoymes remains ill-defined. In particular, the role of membranes in this process is highly speculative.

Here, we used a recently developed, soluble analog of PIP2 to monitor the phospholipase activities of PLC-β2 and -β3 in real time in the absence of lipid vesicles or detergents and compared these measurements with more conventional conditions. These studies confirm the major role of the XY-linker in mediating autoinhibition of phospholipase activity; however, this autoinhibition was greater for PLC-β isoymes operating at lipid surfaces. In contrast, neither the C-terminal domain nor the HTH modulated the capacity of PLC-β2 or -β3 to hydrolyze the soluble analog of PIP2, although the HTH exhibited a modest capacity to autoinhibit PLC-β-3 acting at phospholipid vesicles. Furthermore, high concentrations of activated Goα or Gβγ2 failed to modulate the capacity of PLC-β3 to hydrolyze the soluble substrate, whereas either component of the heterotrimeric G proteins at membranes robustly activated PLC-β-3 variants mutated to either remove the XY-linker or destabilize the HTH. Indeed, the combination of these two mutations produced a variant of PLC-β3 that was supra-activated to hydrolyze PIP2 after expression in cells in the absence of G protein activators.

Taken together, these data support a model whereby the XY-linker and the HTH cooperate to act as a “dual cap” that prevents a highly complementary interface between the membrane surface and the PLC-β isoymes. Activators such as Goα and Gβγ must move the dual cap to elevate phospholipase activity. Because they bind at a distance from at least a portion of the dual cap, they exploit membranes as an allosteric conduit to couple complex formation with movement of the dual cap, presumably by steric and electrostatic repulsion between the dual cap and the membrane surface. In this way, membranes become an integral part of the activation process of PLC-β isoymes, and activators such as Goα are functionally inert in the absence of membranes.

**EXPERIMENTAL PROCEDURES**

**Materials**—HisTrap HP affinity columns and Superdex 200 resin were supplied by GE Healthcare. Complete EDTA-free protease inhibitor was from Roche Applied Science, and WH-15 (KXTbio, Inc.) was synthesized as reported previously (27).

**Mutagenesis and Cloning**—Standard PCR-mediated mutagenesis (Stratagene; QuickChange site-directed mutagenesis manual) was used to introduce deletions and point mutations. Dideox sequencing was used to confirm the entire open reading frame for all mutated genes. For the expression of PLC isoymes in insect cells and yeast cells, the pFastBacHT C and the pPICZ B vectors were modified to include an N-terminal hexahistidine tag followed by a cleavage site for tobacco etch virus (TEV) protease. Deletion of the XY-linker of PLC-β2 (PH-C2) and PLC-β3 followed a similar method previously published for PLC-β2 (15). PLC-β2(PH-C2,ΔXY) lacks residues 470–535, and PLC-β3(ΔXY) lacks residues 472–585. In both instances, the deleted residues were replaced with a Gly-Ser-Gly-Ser insertion. The DNA encoding PLC-β3(ΔXY) also contains a stop codon that prevents translation of the C-terminal 33 residues. These residues are not conserved among PLC-β isoymes, and their removal did not affect hydrolysis of PIP2 by PLC-β3.

Goα was expressed from pFastBac1 in insect cells as described previously (28). The expressed protein was a chimera containing the first 28 residues of rat Goαf fused to mouse Goαq with an intervening TEV cleavage site and an N-terminal hexahistidine tag. Cleavage with TEV protease produced Goαq lacking seven residues from the N terminus but was otherwise intact and considered wild type. Human Gβ, DNA was cloned into a modified pFastBacHT B that includes an N-terminal hexahistidine tag and TEV cleavage site and introduced a serine to alanine substitution at the second position (S2A). The human Gγ2 DNA was cloned into pFastBac1 that included no tags. The Gγ2(C68S) DNA was cloned into pFastBac1 and contained a mutation in the CaaX box (C68S) to prevent prenylation.

The pcDNA3.1 vector was used to express proteins in COS-7 cells; all proteins were full-length human isoforms. PLC-β3(F715A) was N-terminally HA-tagged. PLC-β3(ΔXY) and PLC-β3(F715A,ΔXY) were N-terminally Myc-tagged. Gβγ2 corresponds to the co-expression of Gβγ with no tags and Gγ2 with an N-terminal HA tag.

**Purification of PLC-β Isozymes**—Modified pFastBacHT C vectors encoding PLC-β isoymes were transformed into DH10Bac cells (Invitrogen) to produce bacmid DNA, which was subsequently used to generate baculovirus in Sf9 insect cells according to the manufacturer’s protocol (Invitrogen Bac-to-Bac manual). Four liters of High Five insect cells (2–3 × 10^6 cells/ml) were infected with recombinant baculovirus (10–30 ml/liter) and harvested 48 h later by centrifugation at 750 × g for 15 min in a JA10 rotor.

Purification schemes for PLC-β3 and -β2 followed previously published protocols (15, 22). In brief, cell pellets were resuspended and lysed in buffer containing 50 mM HEPES, pH 8.0, 50 mM NaCl, 10 mM β-mercaptoethanol (β-ME), 5% (v/v) glycerol, 15 mM imidazole, 0.1 mM EDTA, 0.1 mM EGTA, and 0.1 mg/ml PMSF. The lysed cells were centrifuged for 60 min at 100,000 × g in a Type 35 rotor and Beckman L8-80 M ultracentrifuge. Supernatants were collected and passed through 0.2-μm filters prior to loading onto a 1-ml HisTrap column at 1 ml/min. Columns were subsequently washed with 30 bed volumes of buffer containing 20 mM HEPES, pH 8.0, 400 mM NaCl,
10 mM β-ME, 2% (v/v) glycerol, 15 mM imidazole, 0.1 mM EDTA, 0.1 mM EGTA, and 0.1 mg/ml PMSF. The PLC-β variants were eluted with 5 ml of 0.5 M imidazole in wash buffer. Eluted proteins were applied to a 125-ml Superdex 200 size exclusion column and eluted with buffer containing 20 mM HEPES pH 8.0, 100 mM NaCl, 2 mM dithiothreitol (DTT), 2% (v/v) glycerol, 0.1 mM EDTA, 0.1 mM EGTA, and 0.1 mg/ml PMSF. Fractions containing PLC-β variants were pooled and treated overnight at 4 °C with His6-TEV protease. The pools of PLC-β variants were flash-frozen and stored at –80 °C.

Modified pPICZ B vector encoding PLC-β2(PH-C2,ΔXY) isozyme were linearized using Pmel and then transformed into the Pichia pastoris strain SMD1163H using electroporation. PLC-β2(PH-C2,ΔXY) isozyme was expressed according to the manufacturer’s protocol (Invitrogen, EasySelect Pichia expression kit). Recombinant PLC-β2(PH-C2,ΔXY) isozyme was purified as described above.

Purification of G Proteins—Gαq was purified from High Five insect cells using previously published protocols (22) and stored in 20 mM HEPES, pH 8.0, 100 mM NaCl, 2 mM DTT, 2% (v/v) glycerol, 0.1 mg/ml PMSF augmented with 10 mM NaF, 30 μM AlCl3, and 5 mM MgCl2.

Gβ1γ2 was prepared from baculovirus-infected High Five insect cells using a modified version of a previously published protocol (29). For the purification of Gβ1γ2, cell pellets were resuspended and lysed in buffer containing 25 mM HEPES, pH 8.0, 300 mM NaCl, 10 mM MgCl2, 10 mM β-ME, 5% (v/v) glycerol, 15 mM imidazole, 10 mM NaF, 30 μM AlCl3, 10 μM GDP, and 0.1 mg/ml PMSF. The lysed cells were centrifuged for 8 min at 350 × g in a Type J6 rotor and Beckman J2-HS ultracentrifuge to remove unlysed cells and nuclei. Membranes were solubilized with 1% (w/v) CHAPS in lysis buffer and stirred on ice for 60 min. The solubilized membranes were centrifuged for 60 min at 100,000 × g in a Type 35 rotor and Beckman L8–80 M ultracentrifuge. The supernatant was collected and incubated with a 20-ml slurry of nickel-nitrilotriacetic acid beads overnight at 4 °C. The beads were subsequently washed with 250 ml of the lysis buffer augmented with 0.5% CHAPS (w/v). The beads were further washed with 50 ml of wash buffer plus 50 mM imidazole. Gβ1γ2 was eluted from the beads with wash buffer plus 250 mM imidazole. The eluted Gβ1γ2 was applied to a 125-ml Superdex 200 size exclusion column and eluted with buffer containing 25 mM HEPES, pH 8.0, 100 mM NaCl, 2 mM DTT, 2% (v/v) glycerol, 0.5 mM EDTA, 0.5% CHAPS (w/v), and 0.1 mg/ml PMSF. Fractions containing purified Gβ1γ2 were pooled and treated overnight at 4 °C with His6-TEV protease. The pool of Gβ1γ2 was adjusted to 0.4 M NaCl and 20 mM imidazole and passed over a second 1-ml HisTrap column. This time, the flow-through and wash fractions were collected. The Gβ1γ2 was concentrated with an Amicon stirred cell concentrator fitted with a PBQK10 membrane to final volumes of ~1 ml. The sample was then passed over a 23-ml Superdex 200 10/30 size exclusion column and eluted with buffer containing 20 mM HEPES, pH 7.8, 50 mM NaCl, 2 mM DTT, 1% (v/v) glycerol, 0.4% CHAPS (w/v), and 0.1 mg/ml PMSF, and fractions containing purified Gβ1γ2 were pooled and concentrated by VivaSpin 10 concentrators to final concentrations of 400–500 μM. Aliquots were flash-frozen and stored at –80 °C.

Gβ1γ2(C68S) was prepared from baculovirus-infected High Five insect cells using a modified version of a previously published protocol (30). In brief, cell pellets were resuspended and lysed in buffer containing 50 mM HEPES, pH 8.0, 50 mM NaCl, 10 mM MgCl2, 10 mM β-ME, 5% (v/v) glycerol, 15 mM imidazole, 0.1 mg/ml PMSF. The lysed cells were centrifuged for 60 min at 100,000 × g in a Type 35 rotor and Beckman L8–80 M ultracentrifuge. Supernatants were collected and passed through 0.2-μm filters prior to loading onto a 5-ml HisTrap column at 5 ml/min. Columns were subsequently washed with 30 bed volumes of buffer containing 20 mM HEPES, pH 8.0, 400 mM NaCl, 10 mM MgCl2, 10 mM β-ME, 5% (v/v) glycerol, 15 mM imidazole, 10 mM NaF, 30 μM AlCl3, and 0.1 mg/ml PMSF. The Gβ1γ2(C68S) was eluted with 25 ml of 0.5 M imidazole in wash buffer. EDTA (250 mM) was added to the elute to produce a final concentration of 1 mM, and the sample was applied to a 125-ml Superdex 200 size exclusion column and eluted with buffer containing 20 mM HEPES, pH 8.0, 2 mM DTT, 5% (v/v) glycerol, 50 mM NaCl, and 0.1 mg/ml PMSF. Fractions containing Gβ1γ2(C68S) were pooled and treated overnight at 4 °C with His6-TEV protease. The pool of Gβ1γ2(C68S) was adjusted to 0.4 M NaCl and 20 mM imidazole and passed over a second 1-ml HisTrap column. This time, the flow-through and wash fractions were collected. Proteins were concentrated with an Amicon stirred cell concentrator fitted with a PBQK10 membrane to final volumes of ~1 ml. EDTA (250 mM) was added to the elute to produce a final concentration of 1 mM, and the sample was then passed over a 23-ml Superdex 200 10/30 size exclusion column, and fractions containing purified Gβ1γ2(C68S) were pooled and concentrated by VivaSpin 10 concentrators to final concentrations of 0.5–1 mM. Aliquots were flash-frozen and stored at –80 °C.

WH-15 Fluorogenic Assay—All fluorescence assays were performed in small volume cuvettes (Starna Cells, Inc., Atascadero, CA) in a FluoroLog-3 fluorescence spectrophotometer (Horiba Scientific, Edison, NJ) with an excitation wavelength of 344 nm and an emission wavelength of 535 nm. The WH-15 reporter was dissolved in buffer containing 50 mM HEPES, pH 7.0, 70 mM KCl, 2.97 mM CaCl2, 3 mM EDTA, 2 mM DTT, 0.5% (w/v) cholate, and 1% (w/v) DMSO to make a stock concentration of 90 μM. To monitor WH-15 hydrolysis by PLC-β isozymes, 2.5 μM WH-15 was added to buffer containing 50 mM HEPES, pH 7.0, 70 mM KCl, varying amounts of calcium, 18 mM EGTA, 2 mM DTT, and 50 μg/ml fatty acid-free bovine serum albumin (BSA). WH-15 assays in the presence of phospholipid vesicles contained 220 μM 1,2-sn phosphatidylethanolamine and 40 μM 1,2-sn phosphatidylserine. Lipids were dried under N2 in chloroform solution followed by resuspension by sonication in 20 mM HEPES, pH 7.2. After 800 s, varying amounts of PLC-β...
isozymes (3–30 nM) were added to the WH-15 solution, and fluorescence was monitored for 6,000 s at 25 °C. Data were recorded at intervals of 30 s using a water-jacketed, turreted holder for four cuvettes. Experiments were repeated at least two times. Rates \((s^{-1})\) of WH-15 hydrolysis by PLC-β isozymes were calculated from initial linear data points plotted to determine the slope in Prism version 5.0 software (GraphPad, San Diego, CA). Equivalent amounts of purified PLC-β variants (1 μg) were verified by SDS-PAGE followed by staining with Coomassie Brilliant Blue (Fig. 2B). Free calcium concentrations were calculated by Ca-EGTA version 1.3 (31).

**Quantification of Phospholipase Activity of Purified PLC-β3 Isozymes**—Basal phospholipase activity was determined by combining 220 μM L-α-phosphatidylethanolamine and 20 μM L-α-phosphatidylinositol 4,5-bisphosphate and drying the lipids under N₂ in chloroform solution, followed by resuspension by sonication in 20 mM HEPES, pH 7.2. Reactions were incubated for 8 min at 30 °C in a final buffer consisting of 30 mM HEPES, pH 7.2, 70 mM KCl, 2 mM DTT, 16.7 mM NaCl, 3 mM EGTA, 200 mM free Ca²⁺, 0.17 mg/ml fatty acid-free BSA, and 20,000 dpm [³H]PIP₂ with different amounts of PLC-β3 isozymes (4 nM PLC-β3, 0.3 nM PLC-β3(F715A), and 0.02 nM PLC-β3(ΔXY)) to maintain linearity of the enzyme assay. Assays comparing basal and Gβγ-stimulated phospholipase activity contained 0.3 nM PLC-β3, 0.01 nM PLC-β3(F715A), and 0.003 nM PLC-β3(ΔXY) to maintain linearity of the enzyme assay. Assays comparing basal and Gαq-stimulated phospholipase activity included 10 mM NaF, 30 mM AlCl₃, 2.7 mM MgCl₂, and different amounts of PLC-β3 isozymes (0.1 nM PLC-β3, 0.01 nM PLC-β3(F715A), and 0.01 nM PLC-β3(ΔXY)).

**Transfection of COS-7 Cells and Quantification of [³H]Inositol Phosphate Accumulation**—COS-7 cell-based assays measured accumulation of [³H]inositol phosphates. COS-7 cells were maintained in high glucose Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in an atmosphere of 95% air, 5% CO₂. COS-7 cells were transfected with the indicated DNA vectors using Fugene-6 (Promega, Madison, WI) according to the manufacturer’s protocol. The total amount of transfected DNA was 600 ng and included empty pcDNA vector to maintain an equal amount of DNA per well. Twenty-four hours post-transfection, media were exchanged, and cells were metabolically labeled with 1 μCi of myo-[³H]inositol (American Radiolabeled Chemicals, St. Louis, MO). Metabolic labeling proceeded at 37 °C for 1 h in a water bath (Fig. 6) or for 12–18 h in a CO₂ incubator (Fig. 7) with 10 mM LiCl to inhibit inositol phosphate phosphatases. Incubations were terminated by aspiration of the culture medium and the subsequent addition of 50 mM formic acid for 20 min, followed by neutralization with 150 mM NH₄OH. [³H]-Labeled inositol phosphates were isolated and quantified using Dowex chromatography.

**Size Exclusion Chromatography-Multiangle Light Scattering**—Light scattering measurements were performed as described previously (16). In brief, measurements were made with a Wyatt DAWN EOS light scattering instrument (Wyatt Optilab refractometer and Wyatt dynamic light scattering module) coupled to a Superdex 200 10/300 GL size exclusion column (GE Healthcare) pre-equilibrated with 20 mM HEPES, pH 8.0, 2 mM DTT, 150 mM NaCl, and 0.02% NaN₃. The column was loaded with 100 μl of PLC-β3 protein at 5 mg/ml. Data collection and analysis were performed with ASTRA software (Wyatt Technologies).

**RESULTS**

**WH-15 Is a Reporter of PLC Activity**—WH-15 is a soluble, fluorogenic mimic of PIP₂ specifically designed to report the catalytic activity of PLC isozymes. For example, PLC-γ1 hydrolyzes WH-15 with \(K_m\) and \(V_{max}\) values essentially equivalent to

![Fluorogenic WH-15 reports the catalytic activity of PLC isozymes.](image-url)
those determined with PIP2 solubilized in cholate (27). Hydrolysis of WH-15 by PLC produces inositol 1,4,5-trisphosphate, quinomethide, and 6-aminoquinoline (Fig. 1A), causing the emission maxima of the fluorescent reporter to shift from below 400 nm for WH-15 to 535 nm for 6-aminoquinoline (Fig. 1B).

All PLC isozymes are calcium-dependent lipases and use a calcium ion bound within the active site to bind PIP2 and stabilize the transition state for lipid hydrolysis (32, 33). If WH-15 functionally mimics PIP2 in the active site, then WH-15 hydrolysis by PLCs should also depend on calcium concentration. This assumption was tested, and as expected, increasing concentrations of free calcium led to increased rates of hydrolysis of WH-15 by PLC-β (Fig. 1C). Therefore, similar to PIP2, the hydrolysis of WH-15 by PLC-β is highly dependent upon the fraction of calcium bound within the active site of the phospholipase. However, in contrast to the endogenous substrate, WH-15 provides a sensitive readout of lipase activity in solution without the need for mem-

**TABLE 1**

Rates of WH-15 hydrolysis by PLC-β isozymes

| Construct       | PLC-β2 | PLC-β3 |
|-----------------|--------|--------|
| WT              | $6 \pm 4 \,(n = 2)$ | $8 \pm 3 \,(n = 11)$ |
| ΔXY             | $83 \pm 7 \,(n = 2)$ | $5 \pm 1 \,(n = 3)$ |
| F715A           | $6 \pm 3 \,(n = 6)$ | $7 \pm 3 \,(n = 6)$ |
| PH-C2           | $6 \pm 3 \,(n = 2)$ | $3 \pm 3 \,(n = 3)$ |
| PH-C2-HTH       | $200 \pm 3 \,(n = 3)$ | $8 \pm 3 \,(n = 2)$ |
| WT + Gβ1γ2      | $3.3 \pm 0.1 \,(n = 2)$ | $3 \pm 3 \,(n = 2)$ |
| WT + Gαs,αa     | $1.0 \pm 0.1 \,(n = 2)$ | $1.0 \pm 0.1 \,(n = 2)$ |

*30 nm PLC-β3 + AlF4.
Allosteric Modulation of PLC-β Isozymes by Diverse Activators

Autoinhibition at Membranes—WH-15 is a unique substrate for quantification of PLC activity independent of potential allosteric mediators by membranes. We previously showed that a portion of the XY-linker inserted within the catalytic TIM barrel of PLC-β isozymes directly occludes the active site (15, 22). Deletion of this “plug” elevates phospholipase activity at membranes, and activity is further increased by removal of the remaining portion of the XY-linker. As predicted from these previous results, deletion of the entire XY-linker also markedly increased the capacity of PLC-β3 to hydrolyze WH-15 (Fig. 2, A–C, and Table 1), and similar robust activation of PLC-β2 was observed after the removal of its XY-linker (Fig. 2D and Table 1). In contrast, hydrolysis of WH-15 was not increased by removal of all portions C-terminal to the C2 domain of PLC-β3 (Fig. 2E) or PLC-β2 (Fig. 2F), indicating that the carboxyl-terminal regions of these PLC-β isoforms do not directly block access of substrate to the active site. Moreover, these data provide strong evidence that regions C-terminal to the C2 domain do not allosterically modulate the active site of these PLCs in the absence of membranes because no change in basal lipase activity was observed between the full-length and truncated forms. In the same vein, the addition of either 2 μM activated Gaq (Fig. 3A), 30 μM soluble Gβγ (Fig. 3B), or 2 μM prenylated Gβγ (inset) did not increase the capacity of PLC-β3 to hydrolyze WH-15, strongly suggesting that these modulators do not directly activate PLC-β isozymes in the absence of membranes. Based on their relative Ka values for binding to PLC-β3, these concentrations of G proteins are sufficient to complex ~90% of PLC-β3 with Gaq (Ka ~200 nM (22)); ~30% of PLC-β3 will be in complex with soluble Gβγ at 30 μM (Ka ~60 μM (34)); and ~3% will be in complex at 2 μM prenylated Gβγ.

These results contrast with studies measuring activation of PLC-β isozymes at membranes. For example, the C-terminal domain of PLC-β isozymes facilitates phospholipase activity at membranes, as evidenced by the approximately 3-fold higher activity of full-length, wild-type PLC-β3 relative to the equivalent fragment truncated immediately following the C2 domain (PH-C2) observed in assays with PIP2-containing vesicles (Fig. 4A). The observation that both wild-type PLC-β3 and the truncated form exhibited essentially identical phospholipase activities measured with WH-15 suggests that the C-terminal domain functions as a membrane anchor to increase the effective concentration of PLC-β isozymes near its substrate. More interestingly, a purified form of PLC-β3 that extends 40 residues past the C2 domain (PH-C2-HTH) had reduced phospholipase activity relative to the PH-C2 fragment under identical membrane assay conditions (Fig. 4A). These 40 residues are the major site of interaction with activated Gaq and form a HTH when Gaq-bound. In two structures of cephalopod PLC-β isoforms without bound Gaq, this region folds back onto the TIM barrel and was shown to allosterically regulate phospholipase activity on membranes. However, the presence of the HTH did not modulate the capacity of PLC-β3 to hydrolyze WH-15 (Fig. 4B). Therefore, it seems reasonable to propose that the HTH, like portions of the XY-linker (15, 22), protrudes above the main body of PLC-β isozymes to hinder productive engagement with membranes (Fig. 4C).

Membranes as an Allosteric Conduit—In the structures of the cephalopod PLC-β isoforms (35), the equivalent of Phe-715 in PLC-β3 resides within the TIM barrel and forms a substantial part of the interface with the HTH. Mutation of Phe-715 was postulated to disrupt this interface, and indeed, consistent with previous work (35), purified full-length PLC-β3 harboring a substitution of Phe-715 to alanine (F715A) exhibited a 20-fold higher increase in basal phospholipase activity at membranes relative to the wild-type isoform (Fig. 5A). However, this increased phospholipase activity is dwarfed by the 200-fold elevation observed with PLC-β3 lacking its XY-linker determined under identical conditions, further supporting the major role of the XY-linker in autoinhibition of PLC-β isozymes (15) (Fig. 5A and Table 2). Furthermore, no increase in phospholipase activity was observed with PLC-β3(F715A) relative to the activity of the wild-type isoform in assays with WH-15, indicating that this region does not directly modulate the lipase active site through allosterism (Fig. 5B). This result also indicates that interactions between Phe-715 and the HTH of PLC-β3 do not directly modulate phospholipase activity in solution, which is consistent with the data presented above illustrating that removal of the entire C-terminal region, including the HTH, does not lead to increased hydrolysis of WH-15 by either PLC-β3 (Fig. 2E) or β2 (Fig. 2F). In accord with the activities observed in phospholipid vesicle assays, overexpression of PLC-β3(F715A) in COS-7 cells resulted in negligible increases in lipase activity relative to the
large increase observed with overexpression of low amounts of PLC-β3(ΔXY) (Fig. 6). Interestingly, introduction of the F715A mutation into PLC-β3(ΔXY) (all at 10 nM, final concentration) was incubated with 2.5 μM WH-15. Fluorescence was monitored at 335 nm after excitation at 344 nm and normalized to initial fluorescence. C, model of PLC-β3 at membranes. The fragment of PLC-β3 spanning the PH to C2 domains derives from Protein Data Bank entry 3OHM; helix 2 is from an asymmetric unit of this structure; and the distal C-terminal domain (Protein Data Bank entry 1JAD) was oriented based on its electrostatic potential relative to membranes. Disordered regions are indicated with dotted lines. Error bars, S.D.

FIGURE 4. The helix-turn-helix inhibits the basal capacity of PLC-β3 to hydrolyze PIP2 in vesicles but not WH-15 in solution. A, initial rates of hydrolysis of [3H]PIP2 were measured with reconstituted phospholipid vesicles for purified PLC-β3(WT), PLC-β3(PH-C2), or PLC-β3(PH-C2-HTH) (all at 4 nM, final concentration). B, purified PLC-β3(WT), PLC-β3(PH-C2), or PLC-β3(PH-C2-HTH) (all at 10 nM, final concentration) was incubated with 2.5 μM WH-15. Fluorescence was monitored at 335 nm after excitation at 344 nm and normalized to initial fluorescence. C, model of PLC-β3 at membranes. The fragment of PLC-β3 spanning the PH to C2 domains derives from Protein Data Bank entry 3OHM; helix 2 is from an asymmetric unit of this structure; and the distal C-terminal domain (Protein Data Bank entry 1JAD) was oriented based on its electrostatic potential relative to membranes. Disordered regions are indicated with dotted lines. Error bars, S.D.
As a corollary of these observations, we might also expect $G_{\alpha_1}\gamma_2$ or $G_{\alpha_q}$ in lipid vesicles to enhance the capacity of PLC-$\beta_3$ to hydrolyze WH-15 by recruiting the PLC to membranes and relieving autoinhibition. Indeed, lipid vesicles containing phosphatidylserine in place of PIP$_2$ enabled wild-type $G_{\alpha_1}\gamma_2$ (270 nM) to enhance the hydrolysis of WH-15 by PLC-$\beta_3$ ($\approx 2.5$ fold; Fig. 9). This fold activation is much lower than equivalent measurements using [$^3$H]PIP$_2$-containing vesicles (Fig. 8D and Table 2) and can be attributed to either of two likely scenarios. First, only a small portion of WH-15 incorporated into the vesicles where it is hydrolyzed. Alternatively, we suggest that recruitment of PLC-$\beta_3$ to the membrane displaces the XY-linker, and a fraction of WH-15 is able to negotiate the membrane surface and gain access to the active site of the PLC.

**DISCUSSION**

PLC-$\beta$ isozymes hydrolyze PIP$_2$ embedded in cellular membranes. This environment places intrinsic constraints on catalysis; the isozymes must maintain a specific spatial relationship and complementarity relative to the membrane surface. In fact, the PLC-$\beta$ isozymes must partially penetrate the bilayer to gain access to substrate because the substrate cannot protrude from the membrane surface (36). To overcome these entropic and enthalpic constraints requires work, and we previously proposed a general mechanism for the activation of PLC-$\beta$ isozymes through stabilization of productive orientations of these isozymes with respect to the membrane surface (37, 38). This simple model is useful to explain how diverse activators, including $G_{\alpha_1}\gamma_2$ and Rac1, which bind to distinct portions of the PLC-$\beta$ isozymes, are capable of activating these enzymes to hydrolyze PIP$_2$. Indeed, we previously proposed a modification of this model to explain the low basal activity of PLC-$\beta$ isozymes (13, 15, 22). In this case, distinct portions of the XY-linker work as either a “plug” or a “cap” to the active site of the PLC.

**TABLE 2**

Rates of PIP$_2$ hydrolysis by PLC-$\beta_3$ constructs

| PLC construct | Basal | With $G_{\alpha_1}\gamma_2$ | With $G_{\alpha_1}\gamma_2$ |
|---------------|-------|---------------------------|---------------------------|
| PLC-$\beta_3$ | 0.5 ± 0.3 (n = 4) | 20 (n = 1) | 15 (n = 1) |
| PLC-$\beta_3$(F715A) | 10 ± 5 (n = 4) | 133 (n = 1) | 346 (n = 1) |
| PLC-$\beta_3$(XY) | 100 ± 50 (n = 4) | 237 (n = 1) | 618 (n = 1) |

As a corollary of these observations, we might also expect $G_{\alpha_1}\gamma_2$ or $G_{\alpha_q}$ in lipid vesicles to enhance the capacity of PLC-$\beta_3$ to hydrolyze WH-15 by recruiting the PLC to membranes and relieving autoinhibition. Indeed, lipid vesicles containing phosphatidylserine in place of PIP$_2$ enabled wild-type $G_{\alpha_1}\gamma_2$ (270 nM) to enhance the hydrolysis of WH-15 by PLC-$\beta_3$ ($\approx 2.5$ fold; Fig. 9). This fold activation is much lower than equivalent measurements using [$^3$H]PIP$_2$-containing vesicles (Fig. 8D and Table 2) and can be attributed to either of two likely scenarios. First, only a small portion of WH-15 incorporated into the vesicles where it is hydrolyzed. Alternatively, we suggest that recruitment of PLC-$\beta_3$ to the membrane displaces the XY-linker, and a fraction of WH-15 is able to negotiate the membrane surface and gain access to the active site of the PLC. **DISCUSSION**

PLC-$\beta$ isozymes hydrolyze PIP$_2$ embedded in cellular membranes. This environment places intrinsic constraints on catalysis; the isozymes must maintain a specific spatial relationship and complementarity relative to the membrane surface. In fact, the PLC-$\beta$ isozymes must partially penetrate the bilayer to gain access to substrate because the substrate cannot protrude from the membrane surface (36). To overcome these entropic and enthalpic constraints requires work, and we previously proposed a general mechanism for the activation of PLC-$\beta$ isozymes through stabilization of productive orientations of these isozymes with respect to the membrane surface (37, 38). This simple model is useful to explain how diverse activators, including $G_{\alpha_1}\gamma_2$ and Rac1, which bind to distinct portions of the PLC-$\beta$ isozymes, are capable of activating these enzymes to hydrolyze PIP$_2$. Indeed, we previously proposed a modification of this model to explain the low basal activity of PLC-$\beta$ isozymes (13, 15, 22). In this case, distinct portions of the XY-linker work as either a “plug” or a “cap” to the active site of the PLC.

**TABLE 2**

Rates of PIP$_2$ hydrolysis by PLC-$\beta_3$ constructs

| PLC construct | Basal | With $G_{\alpha_1}\gamma_2$ | With $G_{\alpha_1}\gamma_2$ |
|---------------|-------|---------------------------|---------------------------|
| PLC-$\beta_3$ | 0.5 ± 0.3 (n = 4) | 20 (n = 1) | 15 (n = 1) |
| PLC-$\beta_3$(F715A) | 10 ± 5 (n = 4) | 133 (n = 1) | 346 (n = 1) |
| PLC-$\beta_3$(XY) | 100 ± 50 (n = 4) | 237 (n = 1) | 618 (n = 1) |
plug corresponds to the C-terminal end of the XY-linker that directly interacts and blocks the active site. The cap corresponds to the negatively charged and disordered portion of the XY-linker located in proximity to the active site, presumably directly above it, and that decreases affinity of the PLC-β isozymes for membranes. In this scenario, an activator such as G\(_\alpha_q\) engages and orients a PLC-β isozyme at a lipid bilayer; the cap and plug are pushed out of the way of the active site by steric repulsion with the membrane surface; and the active site is freed to engage and hydrolyze PIP\(_2\). To further exemplify this, PLC-β3 lacking its XY-linker had a modestly elevated capacity in comparison with wild-type PLC-β3 to hydrolyze WH-15 (10-fold), whereas the hydrolysis of PIP\(_2\) in vesicles was greatly increased (200-fold) (Fig. 5 and Tables 1 and 2). This difference in rate enhancements reflects the preferential capacity of the XY-linker to mediate autoinhibition that is dependent on the vesicle surface, probably through a combination of electrostatic repulsion created by the cap and steric occlusion formed by the plug of the XY-linker.

Recent structural and biochemical work suggests that this model is overly simple (39). Structures of two cephalopod PLC-β isozymes highlighted a short extension of the C2 domain that interacts with the catalytic TIM barrel but not within the lipase active site (35). Mutations designed to disrupt this interaction in mammalian PLC-β3 led to increased phospholipase activity, indicating that this region also engenders autoinhibition through an allosteric mechanism yet to be defined (35).

The data presented here strongly suggest that this allosterism is only operational at membranes, and we propose a “dual cap” model (Fig. 10) to account for this additional regulation. The second cap derives from the short extension (HTH) of the C2 domain and functions similarly to the cap provided by the XY-linker in preventing productive engagement of mem-
Allosteric Modulation of PLC-β Isozymes by Diverse Activators

In the absence of the membrane, no conduit exists, and hence no allosterism occurs. The biochemical data described here are consistent with this model. First and foremost, activated \( \text{G}_{\alpha q} \) is incapable of elevating the phospholipase activity of full-length PLC-β3 in solution as monitored by the hydrolysis of the fluorescent reporter, WH-15 (Fig. 3A). This result is perhaps superficially surprising given the fact that complex formation is guaranteed to disrupt the interface between the HTH and the TIM barrel previously shown to mediate autoinhibition (18, 22). However, if we posit that the extension results in autoinhibition of the phospholipase solely by colliding with the membrane surface, then autoinhibition is only operative within the context of membrane-resident \( \text{PIP}_2 \) and no contradiction arises from the enzymology carried out with soluble WH-15 and \( \text{PIP}_2 \) in vesicles or cellular membranes. Also highly supportive of this model are the differences in behavior of the fragments of PLC-β3. The fragment spanning from the PH domain to the HTH has reduced phospholipase activity for \( \text{PIP}_2 \) in vesicles relative to the equivalent fragment lacking the HTH (Fig. 4A). In contrast, the two fragments have equal capacity to hydrolyze \( \text{WH-15} \) (Fig. 4B). Therefore, the HTH is only autoinhibitory in the context of membranes, presumably because it protrudes above the plane of a membrane surface in the basally autoinhibited PLC-β isoymes and must be displaced to allow complementary and productive engagement of the membrane surface. The XY-linker also imparts autoinhibition in the context of membranes, and the data indicate that the magnitude of this effect is much greater than that contributed through the HTH. It is unclear if the displacement of the HTH is a prerequisite for the movement of the XY-linker leading to full phospholipase activity. However, it is certain that displacement of the XY-linker is an obligatory step for full activation of PLC-β isoymes because the plug must be removed from the active site for unfettered access of \( \text{PIP}_2 \).

It is also important to note that this allosterism is general. Because communication between an activator and autoinhibitory site is dependent on the membrane acting as a conduit, the activator merely needs to orient a PLC-β isozyme at the membrane surface such that the surface and autoinhibitory site overlap. This generality also is presumably operative in the regulation of PLC-β isoymes by \( \text{G}_{\beta \gamma} \). Interestingly, Lyon et al. (35) argued that the interface between the HTH and the TIM barrel shown to mediate autoinhibition was important for \( \text{G}_{\alpha q} \)-dependent activation but did not report measurements of the capacity of \( \text{G}_{\beta \gamma} \) to activate mutants of PLC-β that destabilize the basal position of the HTH. However, we show here that, similar to \( \text{G}_{\alpha q} \), \( \text{G}_{\beta \gamma} \) also supra-activated PLC-β3 mutated within either portion of the dual cap (Figs. 7 and 8). This marked activation occurs despite the fact that both activators

\[ \text{PLC-β + vesicles} \]

\[ \begin{align*}
\text{normalized fluorescence} & \quad \text{time (sec)} \\
\text{+G}_{\beta \gamma} & \quad 1.0 \pm 0.4 \\
\text{-G}_{\beta \gamma} & \quad 2.5 \pm 0.2
\end{align*} \]

FIGURE 9. Phospholipids enhance the activation of PLC-β3 in the presence of \( \text{G}_{\beta \gamma} \). Purified PLC-β3 WT (30 nm) was added to lipid vesicles that contained 2.5 \( \mu \text{M WH-15} \) in the presence (red) or absence (black) of 270 nm \( \text{G}_{\beta \gamma} \).
engage distinct surfaces of PLC-β3 and that neither activator enhanced the phospholipase activity of PLC-β3 in solution. Indeed, Gβγ does not appear to engage directly either of the autoinhibitory portions of PLC-β isozymes (41–45). Finally, the Rac GTPases also directly bind and activate PLC-β2 and -β3 (21, 24, 46, 47). Because the site of Rac1 binding is far removed from the phospholipase active site, we presume that the Rac GTPases also activate the PLC-β isozymes using allosterism mediated by the membrane surface (37, 48). These studies do not address whether Gβγ or Rac1 displace the HTH in addition to the XY-linker.

Roles for the C-terminal domain in the regulation of PLC-β isozymes remain unclear and continue to be debated. Undoubtedly, this situation stems from the disparate properties of the C-terminal domain. For example, this highly elongated coiled-coil has been reported to be dispensable for phospholipase activation by Gβγ and Rac1 but appears to be critical for activation mediated by Gαq (19, 21, 34, 49). The C-terminal domain also enhances the propensity of PLC-β isozymes to associate with membranes. This property was suggested by early cell fractionation studies showing shifts from particulate to soluble fractions of PLC-β isozymes that lacked an intact C-terminal domain (24, 25). More recent fluorescent microscopy studies highlight the intrinsic propensity of this region to associate with cellular membranes, which is consistent with the observation that the C-terminal domain is highly electrostatically polarized along its long axis (50).

The C-terminal domain clearly supports basal phospholipase activity because full-length PLC-β3 hydrolyzed PIP2 in vesicles about 3 times more efficiently than PLC-β3 truncated immediately after its C2 domain and lacking its C-terminal domain (Fig. 4A). Although it is hypothetically possible that the C-terminal domain directly interacts with the active site to elevate phospholipase activity, this possibility is not supported by biochemical data and lacks simplicity. Indeed, it is more compelling to attribute the elevated phospholipase activity to a C-terminal domain-mediated increase in effective concentration of PLC-β3 near its membrane-resident substrate. Conversely, if the C-terminal domain directly impacted the active site, one would expect the C-terminal domain to modulate the capacity of PLC-β3 to hydrolyze WH-15 in solution, and this is clearly not the case (Fig. 2E). A corollary to this proposition is that the C-terminal domain probably does not substantially stabilize the XY-linker to support autoinhibition, as previously suggested by electron microscopy studies of full-length PLC-β3 (18).

Nevertheless, activation of PLC-β isozymes by Gαq is strongly dependent on the C-terminal domain, although this activation does not depend on a high affinity interaction between Gαq and the C-terminal domain per se. Based on binding studies using surface plasmon resonance and soluble proteins, we originally reported that activated Gαq binds with similar affinity to full-length PLC-β3 and a form of PLC-β3 that lacked the C-terminal domain (22). This result was initially contradicted by results from Lyon et al. (35) indicating that
full-length PLC-β bound to Goαq with 45-fold higher affinity than PLC-β lacking the C-terminal domain. However, recent results by the same group are more consistent with the original 2-fold difference in Goαq-binding affinities for the two forms of PLC-β (18). The lack of high affinity interactions between the C-terminal domain of PLC-β and activated Goαq is also consistent with the inability to model one of the C-terminal domains in the asymmetric unit of the low resolution structure of the complex between full-length PLC-β3 and Goαq (18). Because the C-terminal domain does not engage Goαq with high affinity in solution, perhaps it is more productive at a membrane where even transient interactions might serve to orient Goαq to better activate the phospholipase. How this process might occur remains unclear, as are the ramifications of potential dimerization mediated by the C-terminal domain (17). Approximately 4% of PLC-β3 is dimeric in solution based on size exclusion chromatography coupled to multiangle light scattering (Fig. 11). Although the extent of dimerization is low, the membrane might increase propensity for dimer formation.

Goαq and GBγ can simultaneously activate PLC-β isozymes, and this activation may be important for the integration of signals downstream of multiple, activated G protein-coupled receptors (51). For PLC-β2 and especially PLC-β3, this activation can be synergistic and requires low basal phospholipase activity (52). We show here that low basal phospholipase activity requires both the XY-linker and HTH. These two autoinhibitory regions cooperate to prevent the productive association of PLC-β isozymes with membranes. Membranes act as a conduit for Goαq and GBγ to cooperate with each other and these autoinhibitory elements. Finally, this model is sufficiently general that additional activators of PLC-β isozymes, such as Rac1, may also synergize with heterotrimeric G proteins to integrate multiple signals that impact PLC-β isozymes.

Acknowledgments—We acknowledge S. Hicks for cloning and purifying several PLC-β isozymes. We are thankful to N. Hajicek for revisions to the manuscript. We thank T. Kozasa for providing the Goαq construct. We acknowledge A. Tripathy for assistance with the size exclusion chromatography-multiangle light scattering measurements.

REFERENCES

1. Berridge, M. J., and Irvine, R. F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312, 315–321.
2. Michell, R. H. (1975) Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta 415, 81–147.
3. Nishizuka, Y. (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258, 607–614.
4. Rhee, S. G. (2001) Regulation of phosphoinositide-specific phospholipase C. Annu. Rev. Biochem. 70, 281–312.
5. Suh, P. G., Park, J. I., Manzoli, L., Coccol, L., Peak, J. C., Katan, M., Fukami, K., Katoaka, T., Yun, S., and Ryu, S. H. (2008) Multiple roles of phosphoinositide-specific phospholipase C isozymes. BMB Rep. 41, 415–434.
6. Gresset, A., Sondek, J., and Harden, T. K. (2012) The phospholipase C isozymes and their regulation. Subcell. Biochem. 58, 61–94.
7. Gamper, N., Reznikov, V., Yamada, Y., Yang, J., and Shapiro, M. S. (2004) Phosphatidylinositol 4,5-bisphosphate signals underlie receptor-specific Goαq-mediated modulation of N-type Ca2+ channels. J. Neurosci. 24, 10980–10992.
8. Horowitz, L. F., Hirdes, W., Suh, B. C., Hilgemann, D. W., Mackie, K., and Hille, B. (2005) Phospholipase C in living cells: activation, inhibition, Ca2+ requirement, and regulation of M current. J. Gen. Physiol. 126, 243–262.
9. Kobrinsky, E., Mirshahi, T., Zhang, H., Jin, T., and Logothetis, D. E. (2000) Receptor-mediated hydrolysis of plasma membrane messenger PIP2 leads to K+ current desensitization. Nat. Cell Biol. 2, 507–514.
10. Suh, B. C., and Hille, B. (2005) Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. Curr. Opin. Neurobiol. 15, 370–378.
11. Yue, G., Malik, B., Yue, G., and Eaton, D. C. (2002) Phosphatidylinositol 4,5-bisphosphate (PIP2) stimulates epithelial sodium channel activity in A6 cells. J. Biol. Chem. 277, 11965–11969.
12. Zhang, H., Craciun, L. C., Mirshahi, T., Robics, T., Lopes, C. M., Jin, T., and Logothetis, D. E. (2003) PIP2 activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. Neuron 37, 963–975.
13. Harden, T. K., Waldo, G. L., Hicks, S. N., and Sondek, J. (2001) Mechanism of activation and inactivation of Goq-phospholipase C-β signaling nodes. Chem. Rev. 111, 6120–6129.
14. Kadamur, G., and Ross, E. M. (2013) Mammalian phospholipase C. Annu. Rev. Physiol. 75, 127–154.
15. Hicks, S. N., Jezyk, M. R., Gershburg, S., Seifert, J. P., Harden, T. K., and Sondek, J. (2008) General and versatile autoinhibition of PLC isozymes. Mol. Cell 31, 383–394.
16. Gresset, A., Hicks, S. N., Harden, T. K., and Sondek, J. (2010) Mechanism of phosphorylation-induced activation of phospholipase C-γ isozymes. J. Biol. Chem. 285, 35836–35847.
17. Singer, A. U., Waldo, G. L., Harden, T. K., and Sondek, J. (2002) A unique fold of phospholipase C-β mediates dimerization and interaction with Goαq. Nat. Struct. Biol. 9, 32–36.
18. Lyon, A. M., Dutta, S., Boguth, C. A., Skiniotis, G., and Tesmer, J. J. (2013) Full-length Goαq-phospholipase C-β3 structure reveals interfaces of the C-terminal coiled-coil domain. Nat. Struct. Mol. Biol. 20, 355–362.
19. Kim, C. G., Park, D., and Rhee, S. G. (1996) The role of carboxyl-terminal basic amino acids in Goα-dependent activation, particulate association, and nuclear localization of phospholipase C-β1. J. Biol. Chem. 271, 21187–21192.
20. Schnabel, P., and Camps, M. (1998) Activation of a phospholipase Cβ2 deletion mutant by limited proteolysis. Biochem. J. 330, 461–468.
21. Illenberger, D., Walliser, C., Nurnberg, B., Diaz Lorente, M., and Gierschik, P. (2003) Specificity and structural requirements of phospholipase C-β stimulation by Rho GTPases versus G protein βγ dimers. J. Biol. Chem. 278, 3006–3014.
22. Waldo, G. L., Ricks, T. K., Hicks, S. N., Cheever, M. L., Kawanoto, T., Tsuboi, K., Wang, X., Montell, C., Kozasa, T., Sondek, J., and Harden, T. K. (2010) Kinetic scaffolding mediated by a phospholipase C-β and Gγ signaling complex. Science 330, 974–980.
23. Paulissen, R. H., Woodson, J., Liu, Z., and Ross, E. M. (1996) Carboxy-terminal fragments of phospholipase C-β1 with intrinsic Gγ GTPase-activating protein (GAP) activity. J. Biol. Chem. 271, 26622–26629.
Allosteric Modulation of PLC-β Isozymes by Diverse Activators

24. Gutman, O., Walliser, C., Piechulek, T., Gierschik, P., and Henis, Y. I. (2010) Differential regulation of phospholipase C-β2 activity and membrane interaction by Goq, Gβγ, and Rac2. J. Biol. Chem. 285, 3905–3915

25. Zhang, W., and Neer, E. J. (2001) Reassembly of phospholipase C-β2 from separated domains: analysis of basal and G protein-stimulated activities. J. Biol. Chem. 276, 2503–2508

26. Lyon, A. M., Taylor, V. G., and Tesmer, J. J. (2014) Strike a pose: Goq complexes at the membrane. Trends Pharmacol. Sci. 35, 23–30

27. Huang, W., Hicks, S. N., Sondek, J., and Zhang, Q. (2011) A fluorogenic, small molecule reporter for mammalian phospholipase C isozymes. ACS Chem. Biol. 6, 223–228

28. Nance, M. R., Kreutz, B., Tesmer, V. M., Sterne-Marr, R., Kozasa, T., and Tesmer, J. J. (2013) Structural and functional analysis of the regulator of G protein signaling 2-Goq complex. Structure 21, 438–448

29. Kozasa, T., and Gilman, A. G. (1996) Protein kinase C phosphorylates G12α and inhibits its interaction with Gβγ. J. Biol. Chem. 271, 12562–12567

30. Iniguez-Lluhi, J. A., Simon, M. I., Robishaw, J. D., and Gilman, A. G. (1992) Dependence of the activity of phospholipase C-β1 and phospholipase C-β2: role of the C-terminal domain in its interaction with G-αq. J. Biol. Chem. 267, 23409–23417

31. Schoenmakers, T. J., Visser, G. J., Flik, G., and Theuvenet, A. P. (1992) CHELATOR: an improved method for computing metal ion concentrations in physiological solutions. BioTechniques 12, 870–874, 876–879

32. Grobler, J. A., and Hurley, J. H. (1998) Catalysis by phospholipase Cα1 requires that Ca2+ bind to the catalytic domain, but not the C2 domain. Biochemistry 37, 5020–5028

33. Jenco, J. M., Becker, K. P., and Morris, A. J. (1997) Membrane-binding properties of phospholipase C-β1 and phospholipase C-β2: an electrostatic analysis of the C-terminal region of phospholipase C-β. Biochim. J. 327, 431–437

34. Runnels, L. W., and Scarlata, S. F. (1999) Determination of the affinities between heterotrimeric G protein subunits and their phospholipase C-β effectors. Biochemistry 38, 1488–1496

35. Lyon, A. M., Tesmer, V. M., Dhamsania, V. D., Thal, D. M., Gutierrez, J., Chowdhury, S., Suddala, K. C., Northup, J. K., and Tesmer, J. J. (2011) An autoinhibitory helix in the C-terminal region of phospholipase C-β mediates Goq activation. Nat. Struct. Mol. Biol. 18, 999–1005

36. James, S. R., Paterson, A., Harden, T. K., Demel, R. A., and Downes, C. P. (1997) Dependence of the activity of phospholipase Cβ on surface pressure and surface composition in phospholipid monolayers and its implications for their regulation. Biochemistry 36, 848–855

37. Jezyk, M. R., Snyder, J. T., Gershberg, S., Worthylake, D. K., Harden, T. K., and Sondek, J. (2006) Crystal structure of Rac1 bound to its effector phospholipase C-β2. Nat. Struct. Mol. Biol. 13, 1135–1140

38. Harden, T. K., and Sondek, J. (2006) Regulation of phospholipase C isoforms by Ras superfamily GTPases. Annu. Rev. Pharmacol. Toxicol. 46, 355–379

39. Lyon, A. M., and Tesmer, J. J. (2013) Structural insights into phospholipase C-β function. Mol. Pharmacol. 84, 488–500

40. Kenakin, T., and Miller, L. J. (2010) Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. Pharmacol. Rev. 62, 265–304

41. Dowal, L., Elliott, J., Popov, S., Wilkie, T. M., and Scarlata, S. (2001) Determination of the contact energies between a regulator of G protein signaling and G protein subunits and phospholipase Cβ1. Biochemistry 40, 414–421

42. Wang, J., Dowal, L., El-Maghrabi, M. R., Rebecchi, M., and Scarlata, S. (2000) The pleckstrin homology domain of phospholipase C-β2 links the binding of Gβγ to activation of the catalytic core. J. Biol. Chem. 275, 7466–7469

43. Bonacci, T. M., Ghosh, M., Malik, S., and Smrcka, A. V. (2005) Regulatory interactions between the amino terminus of G-protein βγ subunits and the catalytic domain of phospholipase Cβ2. J. Biol. Chem. 280, 10174–10181

44. Nguyen, Y., Wu, Y., Smrcka, A., Jiang, H., and Wu, D. (1996) Identification of a structural element in phospholipase Cβ2 region that interacts with Gβγ. Proc. Natl. Acad. Sci. U.S.A. 93, 2964–2968

45. Sankaran, B., Osterhout, J., Wu, D., and Smrcka, A. V. (1998) Identification of a structural element in phospholipase C β2 that interacts with G protein βγ subunits. J. Biol. Chem. 273, 7148–7154

46. Bourdon, D. M., Wing, M. R., Edwards, E. B., Sondek, J., and Harden, T. K. (2006) Quantification of isozyme-specific activation of phospholipase C-β2 by Rac GTPases and phospholipase C-ε by Rho GTPases in an intact cell assay system. Methods Enzymol. 406, 489–499

47. Snyder, J. T., Singer, A. U., Wing, M. R., Harden, T. K., and Sondek, J. (2003) The pleckstrin homology domain of phospholipase C-β2 as an effector site for Rac. J. Biol. Chem. 278, 21099–21104

48. Illenberger, D., Walliser, C., Strobel, J., Gutman, O., Niv, H., Gaidzik, V., Kloog, Y., Gierschik, P., and Henis, Y. I. (2003) Rac2 regulation of phospholipase C-β2 activity and mode of membrane interactions in intact cells. J. Biol. Chem. 278, 8645–8652

49. Ilkaeva, O., Kinch, L. N., Paulsen, R. H., and Ross, E. M. (2002) Mutations in the carboxyl-terminal domain of phospholipase C-β1 delineate the dinner interface and a potential Gαq interaction site. J. Biol. Chem. 277, 4294–4300

50. Advibo-Hermans, M. I., Crosby, K. C., Putyrski, M., Bhageloe, A., van Weeren, L., Schultz, C., Goedhart, J., and Gadella, T. W., Jr. (2013) PLCβ isoforms differ in their subcellular location and their CT-domain dependent interaction with Gαq. Cell. Signal. 25, 255–263

51. Rebres, R. A., Roach, T. I., Fraser, I. D., Philip, F., Moon, C., Lin, K. M., Liu, J., Santat, L., Cheadle, L., Ross, E. M., Simon, M. I., and Seaman, W. E. (2011) Synergistic Ca2+ responses by Gαq and GαqGβγ-coupled G-protein-coupled receptors require a single PLCβ isoform that is sensitive to both Gβγ and Gαq. J. Biol. Chem. 286, 942–951

52. Philip, F., Kadamura, G., Silos, R. G., Woodson, J., and Ross, E. M. (2010) Synergistic activation of phospholipase C-β3 by Gαq and Gβγ describes a simple two-state coincidence detector. Curr. Biol. 20, 1327–1335