Differential Regulation of Phospholipase C-β2 Activity and Membrane Interaction by Gαq, Gβ1γ2, and Rac2*

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We combined fluorescence recovery after photobleaching (FRAP) beam-size analysis with biochemical assays to investigate the mechanisms of membrane recruitment and activation of phospholipase C-β2 (PLCβ2) by G protein αq and βγ dimers. We show that activation by αq and βγ differ from activation by Rac2 and from each other. Stimulation by αq enhanced the plasma membrane association of PLCβ2, but not of PLCβ2Δ, which lacks the αq-interacting region. Although αq resembled Rac2 in increasing the contribution of exchange to the FRAP of PLCβ2 and in enhancing its membrane association, the latter effect was weaker than with Rac2. Moreover, the membrane recruitment of PLCβ2 by αq occurred by enhancing PLCβ2 association with fast-diffusing (lipid-like) membrane components, whereas stimulation by Rac2 led to interactions with slow-diffusing membrane sites. On the other hand, activation by βγ shifted the FRAP of PLCβ2 and PLCβ2Δ to pure lateral diffusion 3- to 5-fold faster than lipids, suggesting surfing-like diffusion along the membrane. We propose that these different modes of PLCβ2 membrane recruitment may accommodate contrasting functional needs to hydrolyze phosphatidylinositol 4,5-biphosphate (PtdInsP2) in localized versus dispersed populations. PLCβ2 activation by Rac2, which leads to slow lateral diffusion and much faster exchange, recruits PLCβ2 to act locally on PtdInsP2 at specific domains. Activation by αq leads to lipid-like diffusion of PLCβ2 accompanied by exchange, enabling the sampling of larger, yet limited, areas prior to dissociation. Finally, activation by βγ recruits PLCβ2 to the membrane by transient interactions, leading to fast “surfing” diffusion along the membrane, sampling large regions for dispersed PtdInsP2 populations.

Phospholipase C-β (PLCβ)⁴ isozymes hydrolyze phosphatidylinositol 4,5-biphosphate (PtdInsP2) to produce inositol 1,4,5-trisphosphate and diacylglycerol (1–3). They are activated to different extents by heterotrimeric G protein αq subunits (αq) and βγ dimers (βγ) (1–3). PLCβ2 is also activated by the Rho GTPases Rac and Cdc42 (4–7). Activation of Rac has also been demonstrated for PLCγ2 (8). PLCβ2 has long been known to be expressed in hematopoietic cells (2, 3) but is also encountered in a variety of other cell types and tissues, including smooth muscle cells (9) and several brain regions (10, 11). Moreover, PLCβ2 was shown to be essential for taste perception via certain G protein-coupled oral taste receptors (12).

Activation of PLCβ2 by αq and related α subunits requires the C-terminal region of the enzyme; mutants with deletions in this region (e.g. PLCβ2Δ, which lacks the Phe⁸¹⁹–Glu¹¹⁶⁶ segment) are resistant to stimulation by αq but undergo activation by βγ and Rac/Cdc42 (7, 13–15). Recent results show that βγ and Rac/Cdc42 activate PLCβ2 by interacting, at least in part, with different regions of the effector enzyme. Thus, although the pleckstrin homology (PH) domain of PLCβ2 is dispensable for activation of the enzyme by βγ dimers (6), this domain also interacts with βγ, suggesting that the latter binds to at least two sites on the enzyme (16). In contrast, direct interaction of the PH domain with activated Rho GTPases is both necessary and sufficient for their stimulatory function (6, 17, 18). Functional evidence for a connection between PLCβ2 and Rho GTPases in cells is provided by the chemoattractant receptor system, which activates Rac/Cdc42 and PLCβ2 (19–22).

The mechanisms by which heterotrimeric G proteins and Rho GTPases regulate PLCβ isozymes are only partially understood (16, 23, 24), especially in live cells. Because both PLCβ substrate(s) and stimulators are membrane-bound (16, 23, 24), recruitment of PLCβ from the cytoplasm to the membrane is clearly required for effective enzyme activation. This view is consistent with the loss of PLCβ activation following mutations that interfere with the membrane association of αq or βγ (25, 26). PtdInsP2, the substrate of PLCβ enzymes, is located at the internal plasma membrane leaflet in both dispersed and localized populations (27–33) and diffuses in the cytoplasmic leaflet of cell membranes with a lateral diffusion coefficient (D) of 0.5–1 μm²/s (32, 34). The PH domains of several proteins (e.g. PLCδ1, and the Dictostelium discoideum CRAC protein) were found to undergo dynamic membrane-cytosol exchange along with lateral diffusion over short distances (35, 36). Interestingly, we found a similar behavior for PLCβ2 (7), although this protein is likely to interact with the membrane through several distinct sites; these include the catalytic triosephosphate isomerase barrel, the C-terminal region, and possibly the EF hands motif and the C2 domain, as well as its PH domain,
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which, unlike that of PLCδ, appears to be unable to bind phosphoinositides (16, 18).

We have formerly studied the interactions of green fluorescent protein (GFP)-tagged PLCβ2 (PLCβ2-GFP) and PLCβ2Δ-GFP with the plasma membrane in live cells (7). Using FRAP (i) In mouse pcDNA3.1(Δ) encoding the N-terminal half (residues 1–155) of human PLCβ2 (Clontech) to generate the plasma membrane via binding to slow diffusing membrane proteins. However, the effects of the G protein subunits αq and βγ on the membrane interactions of PLCβ2 and their potential relevance to PLCβ2 stimulation were not explored. Here, we show that αq and βγ recruit PLCβ2 to the plasma membrane by distinct mechanisms, which differ from the mechanism employed by Rac2 and from each other. Each stimulator leads to a specific ratio between the rates of exchange and lateral diffusion characterizing the interaction of PLCβ2 with the membrane, and this in turn allows the enzyme to act preferentially on localized or dispersed PtdIns(2)P populations.

EXPERIMENTAL PROCEDURES

Materials and Plasmids—Murine anti-GFP antibodies were from Roche Applied Science, and peroxidase-conjugated goat anti-mouse IgG was from Sigma. The cDNAs of wild-type (wt) and G12V mutant human Rac2 (Rac2(wt) and Rac2(G12V), respectively), mouse αq(wt) and αq(R183C), human β1, bovine γ2(wt) and γ2(C68S), and human PLCβ2 were ligated into pcDNA3.1(+) or pcDNA3.1(–) (Invitrogen). Bovine and human γ2 have identical amino acid sequences, and mouse αq differs from human αq in but one residue (S141A). There are several indications that this residue has no role in αq function: (i) In mouse αq, Ser141 is in a long loop connecting helices αE and αF of the helical domain (PDB accession codes 2BC and 2RGN); it does not reside in any of the three switch domains or in regions known to interact with PLCβ isoforms and is not among the residues that contact the bound guanine nucleotide and Gβγ (38, 39). (ii) Secondary structure prediction algorithms do not predict a structural difference between mouse and human αq αE-loop-αF region (40, results not shown). (iii) Mouse αq has been successfully reconstituted with many signaling proteins from several other species, including human, for functional and structural analyses (e.g. 41–43). The αq(wt) and αq(R183C) cDNAs were a gift from R. Conklin and H. R. Bourne (University of California, San Francisco, CA). The cDNAs of PLCβ2 and the deletion mutant PLCβ2Δ, which lacks a C-terminal region necessary for stimulation by αq (Phe819–Glu1166), were inserted in-frame with the cDNA of GFP into the EcoRI/Sall site of pEGFP-N1 (Clontech) to generate the plasmids encoding PLCβ2-GFP and PLCβ2Δ-GFP (7). Plasmids (pcDNA3.1) encoding the N-terminal half (residues 1–155) of the venus fluorescent protein fused to the N terminus of human γ2 (venus 1–155–γ2) or venus residues 156–239 fused to the N terminus of human β1 (venus 156–239–β1) were derived earlier (44) and donated by N. A. Lambert (Medical College of Georgia, Augusta, GA). A vector encoding the Salmonella typhimurium SigD protein cloned in pEGFP-N1 lacking the EGFP-encoding sequences (45), with SigD starting at residue 28 to increase stability, was a gift from B. Brett Finlay (University of British Columbia, Vancouver, Canada).

Cell Culture and Transfection—Cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum (7). For FRAP experiments, COS-7 cells grown on glass coverslips in 35-mm dishes for 24 h were transfected using DEAE-dextran (46) with 150 ng of plasmid DNA encoding one of the PLCβ2-GFP derivatives together with 850 ng of empty vector or expression vectors encoding the various activating proteins: human β1 along with bovine γ2(wt) (β1 γ2(wt)), β1 plus the isoprenylation-defective γ2(C68S) (β1 γ2(C68S)), murine αq(wt), constitutively active αq(R183C), and human Rac2(wt) or Rac2(G12V). After 24 h, the cells were taken for the FRAP studies.

For studies of inositol phosphate formation, COS-7 cells (1.5 × 10⁵/well) were seeded into 12-well plates. After 24 h, the cells were incubated with fresh medium (1 ml, 1 h), and co-transfected with vector encoding a GFP-tagged PLCβ2 derivative (250 ng) together with 750 ng of vectors encoding the various activating proteins. Transfection was done with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer’s instructions. At 48 h post-transfection, the cells were taken for experiments on inositol phosphate formation. For experiments on subcellular fractionation of PLCβ2, COS-7 cells (2.5 × 10⁵/10-cm dish) were co-transfected as above with PLCβ2-GFP or PLCβ2Δ-GFP (7 μg of DNA) together with 7 μg of vectors encoding one of the PLCβ2 stimulators or empty vector.

FRAP—FRAP studies (47, 48) were conducted as described (7). The experiments were performed 24–26 h post-transfection on COS-7 cells transfected with PLCβ2-GFP derivatives as described above. All experiments were conducted at 22 °C, in Hank’s balanced salt solution supplemented with 20 mM HEPES, pH 7.2. The monitoring argon ion laser beam (488 nm and 1.2 microwatts) was focused through the microscope (Zeiss Universal, Carl Zeiss MicroImaging) to a Gaussian spot with a radius ω = 0.85 ± 0.02 μm (63 × 1.4 numerical aperture (NA) oil-immersion objective) or 1.36 ± 0.04 μm (40 × 0.75 NA objective). Experiments were conducted with each beam size (beam-size analysis; described previously (7, 37)). The ratio between the illuminated areas (ω²(40×)/ω²(63×)) was 2.56 (n = 39). After a brief measurement at the monitoring intensity, a 5-millisecond pulse (4–6 ms or 10–20 ms for the 63× and 40× objectives, respectively) bleached 50–70% of the fluorescence in the spot. Fluorescence recovery was followed by the monitoring beam. The apparent characteristic fluorescence recovery time (τ) and the mobile fraction (Rm) were derived from the FRAP curves by nonlinear regression analysis, fitting to a lateral diffusion process with a single τ value (49).

Statistical Analysis of FRAP Data—The significance of differences between τ values measured with the same laser beam size was evaluated by Student’s t test. To compare ratio measurements (τ(40×)/τ(63×)) and ω²(40×)/ω²(63×)), we employed bootstrap analysis, which is preferable for comparison between ratio values (50). The τ(40×) and τ(63×) values were resampled with replacement using Excel, and average values from each group of resampled data (τ(40×) Boot and τ(63×) Boot) were derived. For each beam size, 1000 averaged samples were generated, followed by calculation of the bootstrap ratio dividing
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FIGURE 1. Regulation of wild-type PLCβ2, PLCβ2-GFP, and PLCβ2Δ-GFP by heterotrimeric G protein subunits and Rac2 in intact cells. COS-7 cells were co-transfected as indicated at the abscissa with 250 ng per well of either empty vector (Mock) or vector encoding GFP, PLCβ2, PLCβ2-GFP, or PLCβ2Δ-GFP together with empty vector (Control) or vectors encoding the activating proteins as shown in the inset (750 ng in all cases, composed of 375 ng each for β1 and γ2; Rac2-expressing vectors were added at 50 ng, completed to 750 ng of DNA by empty vector). At 24-h post-transfection, the cells were incubated for 20 h in the presence of myo-[2-3H]inositol (10 μCi/ml) and 10 mM LiCl, and the levels of inositol phosphates were determined as described under "Experimental Procedures." The values shown correspond to the means ± S.D. of triplicate determinations. Co-transfection with αq(R183C) (data not shown) yielded saturating levels of inositol phosphate formation in all cases (25,000–34,000 cpm), including the mock- and GFP-transfected controls, because high levels of activated αq stimulate the activity of PLCβ isoforms endogenously present in COS-7 cells.

RESULTS

PLCβ2 Activation by Different Stimulators Can Occur at Varying Extents of Membrane Recruitment—PLCβ2 can be activated by G protein subunits (α, βγ) and by Rac with different orders of efficacy and potency (1, 2, 6). Because the different stimulators interact with distinct regions of the enzyme, they may differ in their ability to recruit PLCβ2 to the membrane, as well as in the resulting mode of membrane interactions. We therefore compared, in cells and under identical conditions, the activation of PLCβ2 and the PLCβ2Δ mutant by the different stimulators (Fig. 1). Because a major part of the studies involves PLCβ2-GFP chimeric proteins, we validated that the GFP tagging does not interfere with the activation of the enzyme by all the stimulators. To this end, we compared side-by-side (Fig. 1) the ability of β1γ2, αq, and Rac2 (wt or mutant) to stimulate inositol phosphate formation by PLCβ2, PLCβ2-GFP, and PLCβ2Δ-GFP. Inositol phosphate formation was measured in COS-7 cells co-transfected with vectors encoding one of the above PLCβ2 constructs (replaced by empty or GFP-encoding vectors as controls) together with: (i) β1γ2 (β1 along with γ2 (wt)); (ii) β1γ2(C68S) (β1 along with the isoprenylation mutant γ2(C68S)); (iii) αq(wt); (iv) Rac2(wt); or (v) Rac2(G12V). Comparison between the inositol phosphate formation levels in cells transfected with PLCβ2 versus PLCβ2-GFP (third and fourth bar groups in Fig. 1) clearly demonstrates that PLCβ2-GFP was activated by all stimulators to an extent similar to that of untagged PLCβ2. These results confirm the earlier demonstration that PLCβ2-GFP is effectively stimulated by activated Rac2 (7), and extend them to show that attachment of GFP to the C terminus of PLCβ2 does not affect the regulation of the enzyme by the heterotrimeric G protein subunits. For both PLCβ2 and PLCβ2-GFP, the degree of stimulation was Rac2(G12V) > αq(wt) > β1γ2. Considering that only a portion of αq(wt) is activated under the conditions used here, this is consistent with the rank order of potencies (which cannot be directly determined in cells) of the three stimulators in vitro assays: activated αq > activated Rac2 > β1γ2 (6, 14, 54). As shown in Fig. 1, stimulation by β1γ2 required membrane anchorage of γ2, as suggested by the lack of PLCβ2 activation...
upon replacement of $\gamma_2$ by the isoprenylation-resistant mutant $\gamma_2(C68S)$. Rac2(wt) had only a weak effect, in accord with the notion that it should undergo activation to stimulate PLC$\beta_2$ (7). It should be noted that $\alpha_q(wt)$ induced a marked stimulatory response in cells expressing PLC$\beta_2$ or PLC$\beta_2$-GFP, but not in cells expressing PLC$\beta_2$-GFP, in line with the inability of the latter to bind $\alpha_q$. However, PLC$\beta_2$-GFP remained responsive to Rac2(G12V) or $\beta_1$,$\gamma_2$, in accord with earlier in vitro studies on the untagged form of this mutant (6). Constitutively active $\alpha_q(R183C)$ gave a very high stimulation of inositol phosphate formation (up to ~60-fold; not shown in Fig. 1 due to the out-of-range value); this robust activation was obtained already in mock- or GFP-transfected cells, suggesting that it reflects activation of endogenous PLC$\beta$ isozymes other than PLC$\beta_2$, which are present in COS-7 cells (13, 55). Constitutively active Rac2(G12V) did not stimulate inositol phosphate formation in the absence of a co-transfected PLC$\beta_2$ construct (Fig. 1), because it specifically activates PLC$\beta_2$, whose endogenous expression level in these cells is very low.

PLC$\beta_2$ must be recruited to the membrane to interact with its substrate, PtdInsP$_2$, which is membrane-associated. We therefore investigated the ability of the different stimulators to translocate PLC$\beta_2$-GFP and PLC$\beta_2$-GFP to the membrane fraction (Fig. 2). To this end, COS-7 cells were co-transfected with a vector encoding PLC$\beta_2$-GFP or PLC$\beta_2$-GFP together with either empty vector or with vectors encoding various PLC$\beta_2$ activators, and the relative distribution of PLC$\beta_2$-GFP or PLC$\beta_2$-GFP between the cytosolic (S) and membrane (P) fractions was determined by cell fractionation as described under “Experimental Procedures.” Fig. 2A shows that singly expressed PLC$\beta_2$-GFP was mostly in the cytosol (S fraction). Co-expression with the different PLC$\beta_2$ activators increased the proportion (%) of PLC$\beta_2$-GFP in the particulate fraction to varying degrees (Fig. 2A), to extents that correlated with their abilities to stimulate the enzymatic activity of PLC$\beta_2$ (cf. Fig. 1): Rac2(G12V) $> \alpha_q(wt) > \beta_1\gamma_2$. Because the fractionation experiment specifically detects the localization of PLC$\beta_2$-GFP, it is not masked by activation of endogenous PLC$\beta$ enzymes (unlike the stimulation of PLC$\beta$ activity), enabling us to measure the effects of constitutively active $\alpha_q(R183C)$ on PLC$\beta_2$-GFP membrane association. This mutant had a slightly higher effect than $\alpha_q(wt)$, which was hard to detect due to the limited sensitivity of the fractionation assay, but is supported by the more sensitive biophysical FRAP studies of PLC$\beta_2$ membrane interactions (see Fig. 4). Interestingly, even though both Rac2(G12V) and $\alpha_q(R183C)$ are both constitutively active, Rac2(G12V) recruited PLC$\beta_2$-GFP to the particulate fraction to a higher extent than $\alpha_q(R183C)$. Together with the lower membrane recruitment and lower activation of PLC$\beta_2$ by $\beta_1\gamma_2$ (Figs. 1 and 2A), this raises the possibility that the extent and/or mode of membrane recruitment mediated by the distinct stimulators are different. This view gains strong support from the FRAP beam-size analysis experiments (Fig. 4), which detect with high sensitivity the membrane association dynamics of PLC$\beta_2$-GFP in live cells; it is corroborated by fractionation studies on cells expressing PLC$\beta_2$-GFP (Fig. 2B). Here, $\beta_1\gamma_2$ did not induce a measurable increase in the percentage of PLC$\beta_2$-GFP in the membrane pellet, contrasting with its ability to induce mild, but

FIGURE 2. Effect of G protein subunits and Rac2 on the subcellular distribution of PLC$\beta_2$-GFP and PLC$\beta_2$-GFP. COS-7 cells grown in 10-cm dishes were co-transfected with 7 µg per dish of vector encoding PLC$\beta_2$-GFP (A) or PLC$\beta_2$-GFP (B) together with either empty vector (Control) or vectors encoding the activating proteins shown in the absissa (7 µg of DNA, composed of 3.5 µg each for $\beta_1$ and $\gamma_2$). After 20 h, the cells were serum-starved for another 24 h, homogenized, and fractionated into postnuclear particulate (P) and soluble (S) fractions as described under “Experimental Procedures.” The two fractions were well separated, as controlled by immunoblotting for RhoGDI (cytosolic) and G$\beta_1\gamma_2$ (membrane-bound, not shown). Equal proportions (v/v) of the P and S fractions of each sample were subjected to SDS-PAGE and quantified by immunoblotting and densitometry using anti-GFP antibodies. The regions shown are those around 160 kDa (A) and 125 kDa (B); no other immunoreactive bands were detected. The immunoblots shown (upper panels) are of a representative experiment, whereas the bar graphs (lower panels) depict the means ± S.E. ($n = 3$) of multiple experiments quantified by densitometry. Asterisks indicate a significant increase in the percentage of PLC$\beta_2$-GFP (A) or PLC$\beta_2$-GFP (B) in the membrane fraction (P) relative to the control (**, $p < 0.01$; *, $p < 0.05$; Student’s t test).
clear stimulation of PLC\(\beta_2\)-GFP enzymatic activity (Fig. 1). The ability of \(\beta_1\gamma_2\) to activate PLC\(\beta_2\) or PLC\(\beta_2\Delta\) despite its weak effect on their recruitment to the particulate fraction is likely to reflect transient (as opposed to stable) membrane recruitment, enabling significant dissociation of the enzyme from the membrane during fractionation. The notion that \(\beta_1\gamma_2\) elicits transient association of the enzyme with the membrane was validated by the biophysical studies described later (see Figs. 4 and 5). In line with the inability of PLC\(\beta_2\Delta\) to bind \(\alpha_q\), neither \(\alpha_q\) (wt) nor \(\alpha_q\) (R183C) affected its membrane association. On the other hand, Rac2(G12V) was highly effective in recruiting PLC\(\beta_2\Delta\) to the particulate fraction, in accord with the concept that activated Rac/Cdc42 GTPases interact with the N-terminal PH domain also present in the mutant (6, 7, 17, 18).

**FRAP Studies Demonstrate Distinct Modes of PLC\(\beta_2\) Membrane Recruitment by the Different Stimulators**—The subcellular fractionation experiments (Fig. 2) provide a measure for the population of PLC\(\beta_2\) molecules that exhibit relatively stable association with the total membrane fraction. To investigate the effects of the various PLC\(\beta_2\) activators on the mode and dynamics of PLC\(\beta_2\) interactions with the plasma membrane of live cells, we expressed PLC\(\beta_2\)-GFP in COS-7 cells and employed FRAP to measure its lateral diffusion and membrane association dynamics in the presence or absence of the various stimulators. Typical FRAP experiments are depicted in Fig. 3; quantitative results on multiple cells using two different sizes of a Gaussian laser beam (FRAP beam-size analysis) are shown in Fig. 4. The beam-size analysis (7, 37) explores the membrane interaction mode of proteins capable of both lateral diffusion in the membrane and of exchange between membrane-associated and cytoplasmic pools. If FRAP occurs exclusively by diffusion, the characteristic fluorescence recovery time \(\tau\) is identical to the characteristic diffusion time \(\tau_D\), which is proportionally to the bleached area (\(\tau = \tau_D = \omega^2/4D\), where \(\omega\) is the Gaussian radius of the beam, and \(D\) is the lateral diffusion coefficient) (49). In the current studies, the ratio between the recovery times obtained with the two beam sizes generated using the 40 × and 63 × objectives, \(\tau(40\times)/\tau(63\times)\), should be 2.56 (the measured ratio between the illuminated areas). When FRAP occurs by exchange, \(\tau\) reflects the chemical relaxation time, which is independent of the bleached area; i.e., \(\tau(40\times)/\tau(63\times)\) should equal 1. Intermediate \(\tau\) ratios suggest mixed recovery, where the faster process has a higher contribution (7, 37).

Because PLC\(\beta_2\)-GFP (and PLC\(\beta_2\)Δ-GFP) have a significant cytoplasmic fraction (cf. Fig. 2), we focused on the laser beam on flat cell regions near the cell periphery, resulting in low contribution of cytoplasmic fluorescence due to the thin cell volume in such regions. Moreover, the FRAP rate of free PLC\(\beta_2\)-GFP in the cytoplasm is very fast, showing immediate recovery on the time scale of the current experiments, ensuring no contribution of cytoplasmic diffusion to the FRAP curves (7). The results shown in Figs. 3 and 4 demonstrate that the different stimulators each induce a distinct effect on the membrane interactions mode of PLC\(\beta_2\). Prior to stimulation, FRAP beam-size analysis of PLC\(\beta_2\)-GFP yielded a \(\tau(40\times)/\tau(63\times)\) ratio of 2.0, intermediate between the ratios characterizing FRAP by lateral diffusion (2.56 with the current beam sizes used) and by exchange (\(\tau\) ratio = 1), suggesting a mixed contribution of the two mechanisms (7, 37). The contribution of exchange does not allow an accurate calculation of the lateral diffusion coefficient; yet, an estimate of \(D\) can be calculated from \(\tau(63\times)\) and the beam size with the 63 × objective, because the recovery at this smaller beam size contains a higher contribution of lateral diffusion (56). This yields \(D = \omega^2/4\tau_D = (3.2 \pm 0.2) \, \mu m^2/s\), faster than \(D\) of the lipid probe DiIC16 (1,1003) (1 µm²/s) in the same cells (7) or the 0.5–1 µm²/s value reported for PtdInsP₂ (32, 34), in accord with the lack of stable binding of the enzyme to the plasma membrane and the significant contribution of exchange to the FRAP measurements.

Stimulation of PLC\(\beta_2\)-GFP by co-expression with Rac2(G12V) led to a large and highly significant increase in the FRAP times (\(\tau\), slower recovery rates) of PLC\(\beta_2\)-GFP (Fig. 4A), in line with the marked increase in its membrane-associated fraction (Fig. 2A). Importantly, this was accompanied by a shift of the FRAP mechanism to recovery by nearly pure exchange
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FIGURE 4. FRAP beam-size analysis suggests activator-dependent distinct modes of PLCβ₂-GFP interactions with the plasma membrane. FRAP experiments were conducted at 22 °C as in Fig. 3, on COS-7 cells transfected with PLCβ₂-GFP, and an excess of empty vector (Control) or vectors encoding the indicated proteins as described under "Experimental Procedures." Two beam sizes were generated using a 63× and 40× objectives (see "Experimental Procedures"), and the τ values were determined with each. The ratio between the areas illuminated by the two beams, ω²(40×)/ω²(63×), was 2.56 (n = 39). This ratio is expected for FRAP by lateral diffusion, whereas a ratio of 1 is expected for recovery by exchange (37). The τ values were high in all cases (r < 0.93), A, τ values. Bars are means ± S.E. of 40–60 measurements, each conducted on a different cell. Comparing τ values measured with the same beam size, Rac2(G12V) and αq(R183C) induced significant increases in τ of PLCβ₂-GFP relative to the control (***, p < 0.1; **, p < 0.005; Student’s t-test). β₁, γ₂ has no significant effect on τ(40×), but reduced τ(63×) (**, p < 0.02). β₂, τ(40×)/τ(63×) ratios. The ratio values (τ ratios and the beam-size ratio) and their S.E. were calculated from the experimentally measured values (τ(40×)) and τ(63×) for τ ratio, ω²(40×) and ω²(63×) for the beam-size ratio) using bootstrap analysis. The bootstrap analysis (see "Experimental Procedures") showed that the τ ratios of PLCβ₂ differ significantly from the 2.56 beam-size ratio predicted for FRAP by lateral diffusion in all cases (***, p < 10⁻⁶; **, p < 0.02), except for co-expression with β₁, γ₂ (p > 0.3). Comparison of the τ ratios to 1 (the value expected for FRAP by exchange) using bootstrap analysis shows that in the presence of Rac2(G12V) the τ ratio of PLCβ₂-GFP is not significantly different from 1 (p > 0.4).

(τ(40×)/τ(63×) ≈ 1; Fig. 4B), suggesting that the characteristic diffusion time (τex) must be at least an order of magnitude slower than τ for exchange (τex), resulting in a negligible contribution of the lateral diffusion to the fluorescence recovery. Thus, in the presence of Rac2(G12V), τex of PLCβ₂-GFP is at least 2 s, 10-fold slower than the measured τ(63×) (0.2 s, reflecting exchange), providing an upper limit of D = 0.09 μm²/s. This D value is much lower than D of lipid probes in the plasma membrane, indicating that the enhanced association of PLCβ₂ with the plasma membrane following activation by Rac2(G12V) is due to interactions with membrane proteins and/or localized protein/lipid clusters. In addition, because the dissociation rate governs fluorescence recovery due to exchange (57, 58), τ measured in the FRAP experiment under conditions where the recovery is due to exchange (τex) reflects the time constant for dissociation from the plasma membrane. This allows the calculation of the distance that a given fraction of the membrane-associated protein will diffuse laterally prior to dissociation into the cytoplasm (travel range); for a fraction comprising 63% of the protein population, this range (τ) is given by r = (2 × D × τex) (59). For Rac2(G12V)-activated PLCβ₂-GFP, this calculation yields r = (2 × 0.09 × 0.2) = 0.19 μm, suggesting that recruitment of PLCβ₂ to the membrane by Rac2(G12V) targets the activated PLCβ₂ mainly to laterally restricted small ranges, where it acts until it dissociates back to the cytoplasm.

To examine whether PtdInsP₂ hydrolysis following PLCβ₂ activation is involved in altering the membrane interaction dynamics of PLCβ₂-GFP upon activation, we co-transfected COS-7 cells with PLCβ₂-GFP (150 ng of DNA) together with a 7-fold excess of a plasmid encoding SigD, a bacterial inositol phosphatase shown to hydrolyze PtdInsP₂ and reduce its cellular level (60, 61). FRAP studies conducted 24 h post-transfection showed no change in PLCβ₂-GFP FRAP parameters, suggesting that PtdInsP₂ hydrolysis per se does not significantly affect the dynamics of PLCβ₂ membrane interactions. This notion is further supported by the finding that each PLCβ₂ activator has a distinct effect on the FRAP kinetics of the stimulated PLCβ₂ (Figs. 4 and 5; see below), although PtdInsP₂ hydrolysis by PLCβ₂ is elicited in all cases (Fig. 1).

Unlike the robust effect of Rac2(G12V) on the FRAP dynamics of PLCβ₂-GFP, co-expression with αq (wt or constitutively active) induced modest, albeit significant, effects on the FRAP parameters of the enzyme. This correlated with the biochemical fractionation experiments (Fig. 2A), where Rac2(G12V) induced a higher increase in the percentage of PLCβ₂-GFP associated with the membrane fraction. Accordingly, αq(R183C) and αq(wt) modulated the FRAP parameters of PLCβ₂ toward the same direction as Rac2(G12V), but to a lower extent (Fig. 4). They mildly increased the τ values of PLCβ₂-GFP and shifted its τ(40×)/τ(63×) ratio to lower values, albeit still higher than 1. Note that the sensitivity of the FRAP beam-size analysis demonstrates that αq(R183C) has a stronger effect than αq(wt) on the τ ratio (Fig. 4B), a difference that was too mild to detect by the fractionation studies. Because the exchange rates of PLCβ₂-GFP stimulated by αq (wt or mutant) were distinctively faster than after stimulation by active Rac2, the increase in the τ values was very mild. This is especially valid for τ(40×), which contains a higher contribution of exchange (due to the larger beam size) and was only weakly affected by αq(wt) and even less by αq(R183C). Therefore, the τ ratio can detect the stronger effect of αq(R183C) with higher sensitivity. The results depicted in Fig. 4 suggest that, although stimulation by αq(R183C) or αq(wt) enhances the mobility-retarding interactions of PLCβ₂-GFP with the plasma membrane, the interactions are weaker than those induced by Rac2(G12V) (7; see Fig. 4), and may involve association with different targets in the membrane. Estimation of D for PLCβ₂-GFP co-expressed with αq(R183C) or αq(wt) from the τ(63×) values (which are very similar for the two αq proteins) yields D = (2.1 ± 0.2) μm²/s, somewhat higher than lipid probe diffusion, most likely due to the residual contribution of exchange. Because the τ(40×)/τ(63×) ratio of PLCβ₂-GFP upon co-expression with an αq protein is intermediate between the values expected for recovery by diffusion and exchange, the characteristic exchange time τex should be in the same range as τex and can be
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estimated from τ(40X) (τ measured with the larger beam size, where the relative contribution of exchange is higher). The τ(40X) values for αq(R183C) and αq(wt) are 0.12 and 0.15 s, respectively, suggesting that the travel range of PLCβ2 following activation by αq is roughly r = (2 × D × ταq)0.5 = (2 × 2.1 × 0.135)0.5 = 0.75 μm, 4-fold larger than after stimulation with Rac2(G12V). Thus, activation by αq leads to a less confined recruitment of PLCβ2 to the plasma membrane, because the active enzyme can diffuse laterally a longer distance prior to dissociation to the cytoplasm.

Unexpectedly, the effects of β1γ2 on the FRAP parameters of PLCβ2-GFP were highly different from those of Rac2(G12V) and αq. Unlike the marked increase in τ of PLCβ2-GFP induced by the latter two stimulators, activation by β1γ2 had only very subtle effects on the τ values (Fig. 4A). Concomitantly, β1γ2 shifted the τ(40X)/τ(63X) ratio of PLCβ2 in a direction opposite to that mediated by Rac2(G12V) and αq, resulting in a τ ratio indistinguishable from lateral diffusion (Fig. 4B). This suggests that, following stimulation by β1γ2, the exchange rate of PLCβ2-GFP between the plasma membrane and the cytoplasm becomes much slower than its lateral diffusion rate, indicating enhanced membrane interactions. Yet, these interactions are highly transient. This notion is supported by the biochemical fractionation studies, which showed a markedly weaker recruitment of PLCβ2 to the membrane fraction by β1γ2 relative to Rac2(G12V) or αq (Fig. 2A). The D value of PLCβ2-GFP co-expressed with β1γ2 can be accurately calculated from the FRAP experiments, because in the presence of β1γ2, the τ ratio between the two beam sizes is as expected for lateral diffusion. Interestingly, the D value thus obtained, (3.6 ± 0.2) μm²/s, is markedly higher than D of lipid probes such as DiIC16 (1 μm²/s) or PtdInsP₂ (0.5–1 μm²/s) (7, 32, 34). It is much higher than the lateral diffusion of β1γ2 at the plasma membrane of COS-7 cells, which we measured by FRAP on cells co-transfected with 850 ng DNA (1:1 ratio) of venus 1–155-PLCβ2 and venus 155–239-β1. The two halves of the venus protein are not fluorescent separately and form a stable fluorescent complex due to bifunctional fluorescence complementation upon association (44, 62). These measurements yielded D = (0.21 ± 0.2) μm²/s (n = 31). This value, which is similar to the 0.23 μm²/s value reported for β1γ2 in HEK293 cells (62), was not altered by co-expressing the venus-tagged β1γ2 constructs with PLCβ2 (150 ng of plasmid DNA). This suggests that β1γ2-stimulated PLCβ2 exhibits a surfing-like diffusion along the plasma membrane, spending some of the time transiently bound to membrane lipids and/or fatty acid-anchored proteins, including the β1γ2 complex itself. The latter notion is supported by the failure of β1γ2(C68S), where γ2 cannot undergo isoprenylation, to modulate the FRAP parameters of PLCβ2 (Fig. 4). These findings have important implications for the travel range of PLCβ2 following stimulation by β1γ2. A lower limit for this travel range can be derived based on the assumption that the exchange time (ταq) of PLCβ2-GFP co-expressed with β1γ2 is at least 10-fold slower than the diffusion time τex which is essentially equal to the measured τ value (e.g. τ(63X) = τex(63X) = 0.045 s, and thus τex is at least 0.45 s). Therefore, the lower limit of the travel range is r = (2 × D × τex)0.5 = (2 × 3.6 × 0.45)0.5 = 1.8 μm, ~10-fold larger than following stimulation with Rac2(G12V). We conclude that activation of PLCβ2 by β1γ2 involves a mechanism that recruits the enzyme to the plasma membrane by inducing interactions that enable it to roam relatively large membrane regions prior to detachment to the cytoplasm, as required for hydrolysis of dispersed PtdInsP₂ populations.

To validate the specificity of the effects of the various stimulators on the FRAP parameters of PLCβ2, we conducted analogous FRAP studies to measure their effects on PLCβ2-∆GFP (Fig. 5), which does not respond to αq due to the F819-E1166 C-terminal deletion. In line with the loss of the response of this mutant to αq as measured by both activation and recruitment to the membrane (Figs. 1 and 2), neither αq(R183C) nor αq(wt) modulated the τ values or the τ(40X)/τ(63X) ratios of PLCβ2-∆GFP (Fig. 5). On the other hand, the effects of β1γ2 on the FRAP
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The mechanisms regulating the membrane recruitment and activation of PLCβ isoforms by their activators are not fully understood. Here, we investigated these issues in live cells for PLCβ₂ activated by several stimulators (Rac2, αq, and β₂γ₂). Our findings demonstrate that each activator causes a distinct mode of PLCβ₂ membrane association, ranging between recruitment to confined regions (activation by Rac2, and to a lesser degree by αq) and fast, surfing-like diffusion of the enzyme along the cytoplasmic leaflet of the plasma membrane (activation by β₂γ₂). The diversity of these mechanisms has important implications for the PtdInsP₂ populations targeted by PLCβ₂, because the first mechanism targets the activated enzyme to act on discrete PtdInsP₂ populations localized at or near the recruitment sites, while the second directs the enzyme to act on dispersed PtdInsP₂ populations.

In the current study, we combined FRAP beam-size analysis with biochemical and signaling assays to investigate the mechanisms by which Rac2, αq, and β₂γ₂ mediate membrane recruitment and activation of PLCβ₂. Because the FRAP studies and some of the biochemical studies employed GFP-tagged PLCβ₂ or PLCβ₂Δ, we first validated that the GFP-tagged enzymes are as responsive as their untagged counterparts to the various PLCβ₂ activators (Fig. 1). Moreover, the specificity of the activation was kept in the GFP-tagged constructs, as shown by the loss of their response to αq upon mutational removal of the γ₂ membrane anchor site (C68S) and the loss of αq activation in the PLCβ₂Δ mutant (Fig. 1).

The fractionation studies demonstrate that PLCβ₂-GFP and PLCβ₂Δ-GFP are mainly cytosolic prior to activation (Fig. 2). However, transient association can be overlooked in such studies, which require relatively stable association with the membrane. Indeed, the FRAP beam-size analysis shows that unstimulated PLCβ₂ and PLCβ₂Δ do interact transiently with the plasma membrane, as indicated by the mixed contribution of lateral diffusion and exchange to their FRAP kinetics, which are much slower than that of free cytoplasmic GFP (Figs. 3–5; see also Ref. 7). Thus, although mainly cytoplasmic, unstimulated PLCβ₂ (and PLCβ₂Δ) experiences some mobility-retarding interactions with plasma membrane constituents, which are insufficient for stable association. The D values estimated from τ(63×) for PLCβ₂ and PLCβ₂Δ prior to activation (3.2 and 4.8 μm²/s, respectively) are ~4-fold higher than those of lipids and of PtdInsP₂ (7, 32, 34), most likely due to contributions of exchange and possibly of surfing-like diffusion along the cytoplasmic face of the membrane, a diffusion mode discussed later in the context of β₂γ₂-activated PLCβ₂. It should be noted that the lack of significant enzymatic activity in the unstimulated enzymes (Fig. 1), despite their transient interactions with the plasma membrane, suggests that such interactions per se are not sufficient to activate PLCβ₂, and that transient recruitment to the membrane (as observed following β₂γ₂ stimulation) should be accompanied by an additional event (e.g. a conformational change) to induce activation.

A striking finding of the current studies is that, although all the stimulators enhance the membrane interactions of PLCβ₂, they induce these effects by different mechanisms. Expression dynamics of PLCβ₂Δ closely resembled their effects on PLCβ₂, inducing only a very minor effect on the τ values, while increasing the τ(40×)/τ(63×) ratio very close to the ratio expected for FRAP by pure lateral diffusion. These findings support the notion that β₂γ₂ expression induces highly transient interactions of PLCβ₂ and PLCβ₂Δ with the plasma membrane, in line with its inability to measurably enhance the fraction of PLCβ₂Δ-GFP in the membrane pellet (Fig. 2B). Accordingly, the very fast lateral diffusion of β₂γ₂-stimulated PLCβ₂Δ (D = (5.2 ± 0.3) μm²/s, calculated from the τ(63×) in Fig. 5A) suggests surfing-like diffusion (even faster than that of β₂γ₂-stimulated PLCβ₂), resulting in a high travel range prior to dissociation to the cytoplasm; assuming that τex for PLCβ₂Δ-GFP is at least 10-fold slower than τ(63×), the lower limit of β₂γ₂-stimulated PLCβ₂Δ travel range is r = (2 × D × τex)½ = (2 × 5.2 × 0.33)½ = 1.9 μm, similar to β₂γ₂-stimulated PLCβ₂. Finally, activation of PLCβ₂Δ-GFP by Rac2(G12V) markedly increased the τ values (Fig. 5A), as in the case of PLCβ₂Δ-GFP, supporting the notion that activation by Rac2 enhances the membrane association of PLCβ₂Δ, in accord with the fractionation studies (Fig. 2). However, the effect of Rac2(G12V) on the τ(40×)/τ(63×) ratio differed markedly between PLCβ₂ and PLCβ₂Δ (cf. Figs. 5B and 4B), shifting the τ ratio of PLCβ₂Δ toward recovery by pure lateral diffusion. As shown and discussed by us earlier (37), when recovery occurs by lateral diffusion and exchange, their contribution to the measured FRAP is determined by the relative rates of the two processes, with the faster process prevailing. Thus, the shift of Rac2-stimulated PLCβ₂Δ to FRAP by lateral diffusion directly demonstrates that its exchange rate is at least 10-fold slower than its lateral diffusion rate. This situation differs from that observed for Rac2-stimulated PLCβ₂, where exchange becomes the dominant mechanism, reflecting a much slower lateral diffusion rate for the full-length, Rac2-stimulated PLCβ₂ (compare Figs. 5B and 4B). This difference, discussed by us extensively earlier (7), is in line with the suggestion (7) that the C-terminal region missing in PLCβ₂Δ has a role in the membrane interactions of full-length PLCβ₂, mainly with membrane proteins that diffuse slower than lipid probes. In its absence (PLCβ₂Δ), the interactions with slow diffusing membrane proteins become weaker, resulting in a loss of the diffusion-restricting interactions with the above proteins and in faster, lipid-like diffusion of Rac2-stimulated PLCβ₂Δ (D = (1.3 ± 0.2) μm²/s, calculated from τ(63×) of Rac2(G12V)-stimulated PLCβ₂Δ in Fig. 5A). Under these conditions, the diffusion of PLCβ₂Δ becomes fast relative to its exchange rate, as indicated by the diffusion-dominated FRAP mechanism (Fig. 5B). Thus, τex of Rac2-stimulated PLCβ₂Δ should be much higher than the measured τ(63×) by at least 10-fold (i.e. τex = 1.4 s). This would increase the travel range of Rac2-stimulated PLCβ₂Δ, with a lower limit estimate of r = (2 × D × τex)½ = (2 × 1.3 × 1.4)½ = 1.9 μm. This in turn indicates that, unlike the limited travel range of Rac2(G12V)-activated PLCβ₂, the travel range on the plasma membrane of the Rac2-activated PLCβ₂Δ mutant is 10-fold higher, strongly compromising the localized nature of the membrane recruitment.
of constitutively active Rac2(G12V) induced a robust recruitment of PLCβ2-GFP to the membrane fraction (Fig. 2A), accompanied by a major modulation of its membrane interaction dynamics (significantly longer τ values and a drastic shift of the \(\tau(40) / \tau(63)\) ratio to \(\sim 1\), indicative of exchange-dominated FRAP (Fig. 4)). The significantly slower τ values at both laser beam sizes indicate that both the lateral diffusion and exchange of PLCβ2-GFP are retarded following activation by Rac2(G12V), while the simultaneous shift to recovery dominated by exchange suggests that the lateral diffusion of PLCβ2 is inhibited at least 10-fold more than its exchange, resulting in a negligible contribution of lateral diffusion to the FRAP. Such slow diffusion (the upper limit estimate of \(D\) is 0.09 \(\mu m^2/s\); see “Results”) is well below the typical \(D\) values of lipid probes, which are in the 1 \(\mu m^2/s\) range (7, 32, 34), indicating that Rac2(G12V) recruits PLCβ2 to the plasma membrane by enhancing its interactions with slow diffusing entities such as transmembrane proteins or protein-lipid clusters. This has important implications for the PtdInsP2 populations targeted by Rac2-stimulated PLCβ2: calculation of the resulting travel range prior to dissociation of the majority (63%) of the Rac-stimulated PLCβ2 molecules from the membrane (59) yields 0.19 \(\mu m\) (see “Results”), indicating that the enzyme is recruited to act on substrate populations localized in distinct limited regions or clusters. Interestingly, the localized nature of PLCβ2 recruitment and activation by Rac2(G12V) is disrupted in the PLCβ2Δ mutant, which is effectively recruited by activated Rac2 to the membrane fraction (Fig. 2B), but shifts to FRAP by lateral diffusion (τ ratio very close to the ratio expected for pure lateral diffusion (Fig. 5)). The \(D\) value obtained for Rac2-stimulated PLCβ2Δ is 1.3 \(\mu m^2/s\), similar to the typical values for lipid probe diffusion. These results indicate that the modulation of PLCβ2 membrane interactions by activated Rac2 involve not only the enzyme’s PH domain, which was shown to interact with activated Rho GTPases (6, 17, 18), but also the C-terminal region, a major portion of which is missing in PLCβ2Δ. The C-terminal region appears to contribute to the interactions of full-length PLCβ2 with the slow diffusing membrane constituents, and its deletion in PLCβ2Δ interferes with these interactions, leading to faster lateral diffusion relative to exchange. Due to the higher \(D\) (1.3 \(\mu m^2/s\)) and slower exchange time (\(\tau_{ex} \geq 1.4 s\)), the travel range of Rac2-stimulated PLCβ2Δ increases by at least an order of magnitude (lower estimate, 1.9 \(\mu m\)).

The C-terminal region of PLCβ2 is essential for activation by \(\alpha_q\) (7, 13–15), in line with the failure of \(\alpha_q\) or \(\alpha_q(R183C)\) to activate PLCβ2Δ and to modulate its membrane interactions (Figs. 1, 2B, and 5). This contrasts with the ability of activated Rac2 and \(\beta_1\gamma_2\) to activate PLCβ2Δ, suggesting that the recruitment and activation mechanisms of PLCβ2 by \(\alpha_q\) may be different. Indeed, although \(\alpha_q(R183C)\) and \(\alpha_q\) enhanced the recruitment of PLCβ2Δ-GFP to the membrane fraction (Fig. 2A), this effect was weaker than that mediated by activated Rac2, and the ability of the \(\alpha_q\) proteins to modulate the membrane interaction dynamics of PLCβ2 was much milder (Fig. 4). Thus, the effect of \(\alpha_q\) on prolonging the τ values was evident but much weaker, and the shift toward a τ ratio of 1 (recovery by exchange) was partial, indicating that, although stimulation by \(\alpha_q\) increases the contribution of exchange relative to diffusion, the latter still has a contribution to the fluorescence recovery. This suggests that the retardation of the lateral diffusion of PLCβ2 following stimulation by \(\alpha_q\) is less than that induced by Rac2(G12V); indeed, the \(D\) value estimated for \(\alpha_q\)-stimulated PLCβ2Δ (which may contain some contribution of exchange) is 2.1 \(\mu m^2/s\), close to but somewhat higher than \(D\) of lipid probes. This indicates that \(\alpha_q\)-stimulated PLCβ2 molecules interact with membrane constituents different from those targeted by Rac2 activation, possibly including lipids (e.g. via the PH and/or C2 domains) and/or lipid-anchored proteins. Direct association with \(\alpha_q\), which can interact with the membrane via its single fatty-acyl residue, may also contribute to these interactions. Nevertheless, the association of \(\alpha_q\)-stimulated PLCβ2 with membrane constituents (including \(\alpha_q\) itself) must be dynamic, as indicated by the contribution of exchange to the FRAP kinetics (Fig. 4) and by the lower \(D\) value (0.47 \(\mu m^2/s\)) measured for a \(\alpha_q\) subunit (\(\alpha_{oA}\)) (63). Based on the \(D\) and \(\tau_{ex}\) values, the travel range of \(\alpha_q\)-stimulated PLCβ2 is estimated to be 0.75 \(\mu m\), suggesting that PLCβ2 recruited by \(\alpha_q\) stimulation is less constrained than the Rac2-stimulated enzyme, roaming larger (albeit still limited) membrane regions.

\(\beta_1\gamma_2\) dimers can interact with the PH domain of PLCβ2 (16) but also appear to interact with other portions of the enzyme (6). In line with the latter, distinct interactions, the modulation of the membrane interactions of PLCβ2 by \(\beta_1\gamma_2\) are very different from those mediated by Rac2 or \(\alpha_q\). Unlike activation by the latter two, activation by \(\beta_1\gamma_2\) induced highly transient association with the membrane fraction, as evidenced by the fractionation experiments (Fig. 2). Yet, despite this highly transient nature, the FRAP of \(\beta_1\gamma_2\)-activated PLCβ2 (or PLCβ2Δ) is strongly dominated by fast lateral diffusion (Figs. 4 and 5), characterized by \(D\) values 3- to 5-fold faster than lipids or lipid-anchored proteins such as G protein α or \(\gamma\) subunits (7, 32, 34, 62, 63). Combining the highly transient interactions and the very fast diffusion along the plasma membrane, we propose that stimulation by \(\beta_1\gamma_2\) induces surging-like diffusion of PLCβ2 enzymes along the cytoplasmic face of the plasma membrane. Thus, when a \(\beta_1\gamma_2\)-stimulated PLCβ2 molecule dissociates from the membrane, it diffuses a short distance and quickly re-associates with another membrane component, remaining in the juxtamembrane vicinity and diffusing along the membrane. Juxtamembrane diffusion was recently found for Paxillin and vinculin above focal adhesions (58). This unique mechanism enables diffusion of \(\beta_1\gamma_2\)-stimulated PLCβ2 along the membrane at rates much faster than those of lipids or lipidated proteins, including the \(\beta_1\gamma_2\) dimers, which diffuse with \(D = 0.2 \mu m^2/s\) (62). The diffusion of \(\beta_1\gamma_2\)-stimulated PLCβ2 (or PLCβ2Δ) is also much faster than that of PtdInsP2, which has \(D\) values of 0.5–1 \(\mu m^2/s\) (32, 34), enabling very fast spatial dispersal of the activated enzyme, not limited by the lateral diffusion of either lipidated protein targets or the substrate. This mechanism is likely relevant under physiological conditions, because PLCβs are typically much less abundant (at least 100-fold) in cells than \(\gamma\) dimers (64, 65), enabling sequential interaction of a single PLCβ molecule with multiple \(\gamma\) dimers by dissociation and fast re-association. An important consequence of this fast diffusion is a dramatic increase in the travel range of \(\beta_1\gamma_2\)-activated PLCβ2 (or PLCβ2Δ) to at least 1.9 \(\mu m\). This implies
that stimulation by $\beta_1\gamma_2$ recruits PLC$\beta_2$ to act on dispersed PtdInsP$_2$ populations.

The divergent mechanisms described above may have evolved for stimulation of PLC$\beta_2$ (and possibly other PLC$\beta$ isozymes) to accomplish different tasks of cellular regulation. PtdInsP$_2$ has long been known to occur in cells not only in dispersed populations, but also to form gradients and locally enriched regions (27–33). We propose that activation by Rac/Cdc42 recruits PLC$\beta_2$ to hydrolize PtdInsP$_2$ in discrete, spatially restricted zones. On the other hand, activation by $\beta_1\gamma_2$ dimers results in signals that rapidly propagate along the plasma membrane, because the activated PLC$\beta_2$ can roam large areas along the membrane, hydrolyzing PtdInsP$_2$ with low spatial resolution. Activation by $\alpha_q$ yields an intermediate situation, which may fit conditions where relatively shallow gradients of PtdInsP$_2$ hydrolysis are beneficial.

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