Identification of a Structural Element in Phospholipase C β2 That Interacts with G Protein βγ Subunits*

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To delineate the specific regions of phospholipase C β2 (PLC β2) involved in binding and activation by G protein βγ subunits, we synthesized peptides corresponding to segments of PLC β2. Two overlapping peptides corresponding to Asn-564–Lys-583 (N20K) and Glu-574–Lys-593 (E20K) inhibited the activation of PLC β2 by βγ subunits (IC₅₀ 50 and 150 μM, respectively), whereas two control peptides did not. N20K and E20K, like some conflicting results, but in general PLC β1 and β4 are regulated primarily by Gαq, PLC β2 is regulated primarily by βγ subunits, and PLC β3 is regulated by both βγ and α₉ subunits.

Based on sequence alignments between PLC isoforms and other proteins, some domain structure has been predicted. The first 100 amino acids are predicted to form a pleckstrin homology domain (5). This domain in PLC β1, when expressed in isolation, binds to PIP₂ and IP₃ and when removed from PLC β1, inhibits anchoring to PIP₂-containing membranes but does not inhibit catalysis (6–8). The role of the pleckstrin homology domain in the other PLC isoforms is unclear but is likely to be distinct from PLC β1, since PLC β2 membrane binding is unaffected by IP₃ (9). Two highly conserved regions have been identified in all mammalian PLCs and designated X and Y. In PLC β and δ isoforms, these regions are adjacent in the primary sequence, whereas in PLC γ, the X and Y are separated by intervening src-homology SH2 and SH3 domains. PLC β isoforms are unique in that there is an extended (40 kDa) C-terminal domain that extends beyond the Y domain (4).

A three-dimensional crystal structure has been solved for PLC β1 that contains the X and Y domains but is missing the N-terminal pleckstrin homology domain (10). The structure shows that the N-terminal region between the pleckstrin homology domain and the X domain has a structural fold that is very similar to the EF-hand domain found in Cu²⁺-binding proteins, including calmodulin. The entire X domain and two-thirds of the N terminus of the Y domain fold to form a catalytic core similar in structure to the β barrel found in triose phosphate isomerase (TIM barrel). The C-terminal one-third of the Y domain forms a domain similar to the C2 domains found in PKC and PLAA where they function as calcium-dependent phospholipid binding domains. In PLC β1, the C2 domain primarily interacts with the N-terminal EF-hand domain, and its function is unclear.

Some progress has been made mapping the regions in the overall sequences of the PLCs that are involved in interaction with G proteins. Removal of the C-terminal third of PLC β1 abolishes activation by Gαq, but Ca²⁺-dependent activity remains intact (11, 12). Further analysis of this region has served with the toxin from Bordatella pertussis (PTX). βγ subunits released during activation of Gα/o proteins are thought to activate PLC in a manner that is inhibited by PTX, since this toxin blocks interaction of the Gα/o heterotrimer with receptors.

PLC enzymes consist of at least nine different isoforms that have been classified into three groups; β, γ, and δ (2, 4). All of these enzymes require Ca²⁺ for activity. PLC β isoforms are the primary enzymes that hydrolyze PIP₂ in response to activation by G proteins. Four isoforms of the PLC β class have been identified and designated PLCβ1, β2, β3, and β4. Each isoform is regulated differently by G protein βγ or α subunits. In in vitro enzyme assays and in co-transfection assays there are some conflicting results, but in general PLC β1 and β4 are regulated primarily by Gαq, PLC β2 is regulated primarily by βγ subunits, and PLC β3 is regulated by both βγ and α₉ subunits.

Many transmembrane receptors coupled to heterotrimeric G proteins can initiate the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂)1 to produce inositol trisphosphate (IP₃) and diacylglycerol. Multiple experimental paradigms provide convincing evidence that increased PIP₂ hydrolysis occurs as a result of enzymatic activation of phosphatidylinositol-specific phospholipase C (PLC) due to direct interactions of PLC with G protein βγ subunits. Removal of the N terminus of the Y domain fold to form a catalytic core similar in structure to the β barrel found in triose phosphate isomerase (TIM barrel). The C-terminal one-third of the Y domain forms a domain similar to the C2 domains found in PKC and PLAA where they function as calcium-dependent phospholipid binding domains. In PLC β1, the C2 domain primarily interacts with the N-terminal EF-hand domain, and its function is unclear.

Some progress has been made mapping the regions in the overall sequences of the PLCs that are involved in interaction with G proteins. Removal of the C-terminal third of PLC β1 abolishes activation by Gαq, but Ca²⁺-dependent activity remains intact (11, 12). Further analysis of this region has served

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1 The abbreviations used are: PIP₂, phosphatidylinositol 4,5-bisphosphate; PTX, pertussis toxin; IP₃, inositol 1,4,5-triphosphate; SMCC, succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate.
to define important regions for interaction with α subunits (13). Removal of the C-terminal third of PLC β2 does not affect its ability to be stimulated by βγ subunits (14, 15). In a series of experiments by Kuang et al. (16), segments of the PLC β2 X and Y domains when coexpressed with PLC β2 in COS-7 cells blocked activation by βγ subunits. When expressed as GST fusion proteins, a 116-amino acid polypeptide from this region bound tightly to G protein βγ subunits, whereas a 60-amino acid sequence of this same region bound weakly to βγ subunits (16).

In this paper we further define the regions of PLC β2 that interact with G protein βγ subunits. Synthetic peptides corresponding to sections of the 116-amino acid region previously identified (16) were tested for their ability to inhibit activation of PLC β2 by βγ subunits in a reconstituted, purified system. The peptides were designed based on sequence alignment of PLC β2 and PLC δ1 and referral to the crystal structure of PLC δ1. This allowed us to identify regions within Gln-526-Val-641 that were on the surface and potentially accessible to βγ subunit binding. Based on these studies we propose a model for the structural features of PLC β2 involved in βγ-PLC interactions.

**EXPERIMENTAL PROCEDURES**

**Materials**

Peptides were purchased from Coast Scientific or from Biosynthesis and had a purity of greater than 90% based on high performance liquid chromatography analysis and mass spectrometry. Phosphatidylinositol-lamine (bovine liver) and phosphatidylinositol (bovine liver) (PI) were obtained from Avanti Polar Lipids. PIP2 was prepared from bovine brain lipids and had a purity of greater than 90% based on high performance liquid chromatography analysis and mass spectrometry. Phosphatidylethanolamine and PI 3-kinase proteins were pre pared using a baculovirus expression system. Construction of baculovirus-directed expression of PLC δ1 was cut with Bst RI and Eco RI of Escherichia coli coexpressing α1 and N-myristoyltransferase according to published procedures (20). Protein concentrations were determined with an amido black protein assay (21).

**Phospholipase C and PI 3-Kinase Assays—**Phospholipase C assays were conducted as described previously (9) and in the figure legends. PI 3-kinase activity was assayed using sonicated micelles containing 600 pmol phosphatidyl ethanolamine and 300 μM bovine liver PI as in Parish et al. (22).

**RESULTS**

**Peptide Design—**There is significant homology between PLC β2 and PLC δ1 in the 116-amino acid region of PLC β2 that was found to bind to βγ subunits (16): 33% identity and 47% similarity overall with a 70-amino acid region having 54% identity and 67% similarity (Fig. 1). Based on this high level of homology, we predicted that PLC β2 might have a very similar fold as PLC δ1 in this region. Using this assumption, we examined the structure of PLC δ1 to determine which regions of PLC β2 would be likely to be on the surface of the protein and accessible to G protein βγ subunits. Three peptides were chosen for our initial studies, corresponding to amino acids 564–583 (N20K), 584–609 (A20G), and 575–594 (E20K) of PLC β2 shown in (Fig. 1B). Also tested as a control was a peptide composed of the same amino acids as N20K, except the primary sequence was randomized (YVLSKHRSDLFTKAYISSEL).

**Inhibition of βγ-stimulated PLC β2 Activity—**The peptides were tested for their ability to inhibit the stimulation of PLC β2 activity by βγ subunits (Fig. 2). In the absence of peptide, βγ subunits stimulated PLC β2 approximately 10-fold over basal Ca2⁺-dependent activity. The addition of N20K inhibited βγ-stimulated activity with an IC₅₀ of 50 μM with 95% inhibition of activity occurring at 200 μM. The adjacent peptide, A20G, and the scrambled peptide had no effect on stimulation of PLC activity by βγ subunits at concentrations up to 300 μM. The peptide that overlaps the C terminus of N20K and the N terminus of A20G–E20K (E20K) inhibited βγ-stimulated activ-
ity with an IC_{50} of 150 \mu M. Both peptides inhibited the basal Ca^{2+}-stimulated activity of PLC \beta_2, with N20K inhibiting activity by 58 \pm 10\% and E20K inhibiting activity by 43 \pm 10\% (n = 4 experiments). Importantly, \beta_\gamma-stimulated activity was inhibited to a greater extent than was basal activity. To quantify this effect, data were normalized by determining the fold stimulation of activity over basal activity by \beta_\gamma subunits (activity with \beta_\gamma divided by activity without \beta_\gamma). The percent inhibition of the fold stimulation by \beta_\gamma subunits in the presence of 200 \mu M peptides was then calculated. N20K inhibited fold stimulation over basal by 67 \%, whereas E20K inhibited fold stimulation by 42 \%. We also tested a peptide corresponding to N20K where Tyr-15 in the region that overlaps \beta_2 with PLC \beta_2 activity in the absence of \beta_\gamma subunits (data not shown). The significance of this is unclear, but it did not allow us to measure effects of N20K(Y15Q) peptide on \beta_\gamma-stimulated PLC \beta_2 activity. To prove that the inhibition of \beta_\gamma-stimulated PLC \beta_2 activity was attributable, at least in part, to binding of the peptides to \beta_\gamma subunits, several other assays were performed to demonstrate binding of the peptides to \beta_\gamma subunits.

**Inhibition of ADP-ribosylation**—G protein \alpha subunits block the ability of \beta_\gamma subunits to activate PLC by sequestering the \beta_\gamma subunits in the heterotrimeric form. This is thought to work because the \alpha subunits sterically hinder interaction between the \beta_\gamma subunits and the PLC. This predicts that peptides mimicking PLC \beta_2 binding to \beta_\gamma subunits could block interaction between \alpha and \beta_\gamma subunits. One way to measure this is to measure the \beta_\gamma-dependent enhancement of ADP-ribosylation of \alpha subunits by pertussis toxin. Since \beta_\gamma binding to \alpha subunits is required for ADP-ribosylation of \alpha, peptides that block interaction between \alpha and \beta_\gamma subunits will inhibit ADP-ribosylation.

N20K or E20K inhibited incorporation of ADP-ribose into purified recombinant myristoylated \alpha_1 in the presence of \beta_\gamma subunits. \beta_\gamma-stimulated ADP-ribosylation was inhibited by close to 100\% by 100 \mu M N20K or E20K with an IC_{50} of 8 \mu M (Fig. 3A). Neither A20G nor the scrambled peptide (100 \mu M each) had an effect on ADP-ribosylation. This indicates that N20K and E20K are binding to the \beta_\gamma subunits at a site that may overlap the binding sites for \alpha, or that the peptides are binding at a site on \beta that prevents binding of PTX. We also tested the N20K(Y15Q) peptide. This peptide was much less potent in inhibiting \beta_\gamma-dependent ADP-ribosylation (Fig. 3B), indicating that this tyrosine 15 in the peptide is important for \beta_\gamma binding.

**Effects of Peptides on Stimulation of PI 3-Kinase by \beta_\gamma Sub-
To further confirm that these peptides inhibit various effectors by binding to $\beta\gamma$ subunits, we tested the effects of these peptides on the stimulation of PI 3-kinase by $\beta\gamma$ subunits. We utilized a p110$\gamma$/p101 heterodimer purified from sf9 cells as described by Stephens et al. (18). The activity of the p110/p101 heterodimer was increased 5-fold by 150 nM $\beta\gamma$ subunits. N20K or E20K inhibited the stimulation of this enzyme by 150 nM $\beta\gamma$ subunits, with an IC$_{50}$ of 50–100 μM (Fig. 4). A20G and scrambled peptide had no effect. The peptides did not have a significant effect on basal PI 3-kinase activity.

Effects of Peptides on Stimulation of PLC $\beta_3$ by $\beta\gamma$ Subunits—PLC $\beta_3$ is activated by G protein $\beta\gamma$ subunits at a similar potency as PLC $\beta_2$ (25). Activation of PLC $\beta_3$ by $\beta\gamma$ subunits and Ca$^{2+}$ was measured in the presence of E20K and N20K. Surprisingly, E20K had little effect on the activation of PLC $\beta_3$ by G protein $\beta\gamma$ subunits, whereas N20K inhibited activity but not to the same extent as for PLC $\beta_2$ (Fig. 5). Basal, Ca$^{2+}$-dependent activity of PLC $\beta_3$ was not measurable in these assays. We have reported previously that basal activity of PLC $\beta_3$ is much lower than for PLC $\beta_2$ under these assay conditions (25).

Cross-linking of Peptides to $\beta\gamma$ Subunits—To directly demonstrate that these peptides bind to $\beta\gamma$ subunits, we developed a cross-linking assay that uses a heterobifunctional cross-linker to covalently link the peptides to purified $\beta\gamma$ subunits. The cross-linker (SMCC) has a succinimide ester group that reacts with primary amines and a maleimido group that reacts with SH moieties. Since the peptides have no cysteine residues, the only way to cross-link the peptides to $\beta$ or $\gamma$ subunits is via a primary amine on the peptide and SH groups on either $\beta$ or $\gamma$ subunits. Peptide cross-linking was monitored by immunoblotting for $\beta$ or $\gamma$ subunits after electrophoresis to resolve cross-linked subunits from the unmodified subunits.

The specificity for peptide cross-linking to $\beta\gamma$ was tested by incubating purified $\beta\gamma$ subunits with N20K, E20K, N20K(Y15Q), A20G, or scrambled peptides and cross-linker. Only in the presence of N20K or E20K (30 or 150 μM each) was there an increase in the apparent molecular weight of $\gamma$ after incubating with cross-linker (Fig. 6A). Some cross-linking of N20K(Y15Q) to $\gamma$ is observed at 150 μM peptide, consistent with the data demonstrating that N20K(Y15Q) is less potent in inhibiting ADP-ribosylation of $\alpha_{i1}$.
FIG. 6. Cross-linking of peptides to γγ. A, specificity of cross-linking to γγ. βγγ was mixed with no peptide (lane 1) or N20K (lane 2), N20K/Y15Q (lane 3), E20K (lane 4), A20G (lane 5), or scrambled peptide (lane 6) (30 or 150 μM each) at room temperature for 1 min. B, effect of various competitors on cross-linking of E20K and N20K peptides to γγ. βγγ was incubated alone (lanes 1 and 2), with 250 (for 30 μM peptide treatments), or with 500 μM (for 150 μM peptide treatments) PLC β2 (lane 3), PLC β3 (lane 4), boiled PLC β2 (lane 5), boiled PLC β3 (lane 6), or α1 (lane 7) for 10 min room temperature. 30 or 150 μM peptide was then added to the samples (lanes 2–7). The mixtures were all treated with 200 μM SMCC for 30 min at room temperature. Reactions were stopped by the addition of 10 mM Tris, pH 8.6, and 10 mM 2-mercaptoethanol. Proteins were then resolved by SDS-polyacrylamide gel electrophoresis in 17% acrylamide gels containing 6 M urea. The gels were run at a constant current of 35 mA for 2.5 h. The proteins were transferred to a nitrocellulose membrane and blotted with anti-γ antibody. Positions of uncross-linked γγ and peptides cross-linked to γγ (γγ xlink) are shown. Each experiment was repeated three times and yielded similar results.

FIG. 7. Cross-linking of peptides to βγ. A, specificity of peptide cross-linking. βγγ was incubated without any peptide (lane 1) or mixed with 30 μM N20K (lane 2), N20K/Y15Q (lane 3), E20K (lane 4), A20G (lane 5), or scrambled (lane 6) at room temperature. B, effect of competitors on cross-linking of E20K and N20K peptides to βγγ. γγ was incubated alone (lanes 1 and 2) or with 250 nM PLC β2 (lane 3), PLC β3 (lane 4), boiled PLC β2 (lane 5), boiled PLC β3 (lane 6), or α1 (lane 7) for 10 min at room temperature. 30 μM peptide was then added to the samples (lanes 2–7). Cross-linking reactions were performed as in Fig. 6 except only for 5 min. Proteins were then resolved by SDS-polyacrylamide gel electrophoresis in 12% acrylamide mini-gels. The gels were run at a constant voltage of 150 volts for 1 h. The proteins were transferred to a nitrocellulose membrane and blotted with anti-βγ antibody. Positions of uncross-linked βγ and molecular weight markers in kDa are shown. Each experiment was repeated three times and yielded similar results.

Only in the presence of N20K or E20K and cross-linker does a prominent cross-linked species appear. A number of minor higher molecular weight species are also seen only in the presence of cross-linker and peptide. It is unclear what the higher molecular weight bands correspond to, although all can be visualized with β subunit antibodies, and some are visualized with γ subunit antibodies. These could represent more than one peptide cross-linked to each β subunit or peptide cross-linked to β that is cross-linked to γ. The higher molecular weight band observed in the absence of peptides (Fig. 7B, lane 1) corresponds to β cross-linked to γ, because the upper band could be visualized with a γ subunit antibody (data not shown).

To further demonstrate the relevance of the cross-linking, the βγ subunits were incubated with excess PLC β2, PLC β3, or α11 before incubation with peptide and cross-linker to determine if they could compete for peptide cross-linking, PLC β2, PLC β3, and α11 all inhibited cross-linking of N20K and E20K to the γ subunit, although PLC β3 was less effective than PLC β2 (Fig. 6B). PLC β2 and PLC β3 both inhibited cross-linking of the peptides to the β subunit, although PLC β2 was again consistently less effective than PLC β2 (Fig. 7B). Interestingly, GDP-ligated α1 could not inhibit cross-linking of peptides to the β subunit.

DISCUSSION

Two synthetic 20-amino acid overlapping peptides that mimic a region of PLC β2 bind specifically to G protein βγ subunits and prevent interaction with different biochemical partners. An adjacent peptide and scrambled N20K had no effect in any of the assays we have examined. All the peptides are very similar with respect to amino acid chemistry. Although the peptides have some effect on the basal activities of PLC β2, this cannot explain the extent of the inhibition of βγ stimulation of PLC β2 activity. It is clear based on the other assays involving βγ subunits that these peptides bind to the βγ subunits and that this is responsible, at least in part, for the observed inhibition of βγ stimulated PLC β2.

The N20K and E20K peptides inhibit the βγ-dependent activation of PLC β2 with IC50 of 50 and 150 μM, respectively. These IC50 are similar to what has been reported for a 27-amino acid peptide derived from adenylyl cyclase type II (QEHA peptide) for inhibiting a number of βγ subunit-regulated effectors (26). N20K and E20K do not contain the βγ binding consensus sequence identified in the QEHA peptide. However, in the common 10 amino acids of the N20K and E20K there is a short stretch of seven amino acids where 4 amino acids are identical to a sequence at the C terminus of QEHA and a 5th amino acid is conservatively substituted. When we changed one of these amino acids in N20K, binding to βγ subunits was dramatically inhibited (Figs. 3B, 6A, and 7A).

The N20K and E20K peptides are more potent in inhibiting βγ-dependent ADP-ribosylation of α11 (IC50 8 μM) than for inhibition of PLC β2. This is surprising if we assume that the peptides block ADP-ribosylation of α11 by blocking α11 interactions with βγ because the affinity of βγ for α11 (Kd ~ 1 nM) is much greater than the affinity for PLC β2 (Kd ~ 100 nM). The exact mechanism for how βγ subunits stimulate ADP-ribosylation of α subunits is unclear but is known to involve a process where βγ subunits must cycle catalytically among the stoichiometric excess of α subunits. Since the mechanism is not entirely understood, it is possible that the peptides interfere with this catalytic ADP-ribosylation in a way that is not directly
related to the known high affinity of βγ subunits for α subunits. One possibility is that the peptides are not blocking a binding to βγ but are occupying a binding site on βγ required for PTX interaction. This possibility is supported by the cross-linking data that shows that cross-linking of the peptides to the β subunit is not blocked by α3-GDP.

Both N20K and E20K must cross-link directly to cysteine residues in either the β and γ subunits due to the nature of the cross-linker. The site(s) of cross-linking of the peptide to the β subunit is unclear, but there are 14 cysteine residues in the β subunit where cross-linking could have occurred. Cross-linking of E20K and N20K to both β and γ was prevented by incubation with PLC β2. PLC β3 also prevented cross-linking of N20K and E20K but was not as effective as PLC β2, suggesting that the binding sites for these two enzymes on βγ subunits do not entirely overlap. This is consistent with the observation that the peptides were not as effective or as potent at inhibiting PLC β2 as PLC β1 sequence.

The cross-linking of the peptides to the β subunit was not prevented by preincubation of the α subunit with the β subunit. This suggests that the peptide binding site on βγ does not entirely overlap with the α subunit. Although it is known that α-GDP blocks activation of PLC β by βγ subunits, it is probable that the binding sites for PLC β and α subunits overlap but do not match. Thus the binding site represented by the peptide may lie outside the overlap region. This idea is supported by a recent study by Blumil et al. (27) where it was demonstrated that a peptide from phosducin binds to βγ subunits. Visualization of the location of this peptide in the crystal structure of βγ complexed with phosducin (28) shows that this peptide would bind at a region on the β subunit that does not overlap the α subunit binding site, whereas the N-terminal domain of phosducin does overlap the α subunit binding site. That α-GDP inhibits cross-linking to the γ subunit may result from steric interference with the cross-linking reaction without interfering directly with peptide binding.

The data we have presented allows us to narrow down in the primary sequence of PLC β2 a region of 20 amino acids that may be involved in βγ subunit binding. The N terminus of the E20K peptide overlaps the C terminus of N20K peptide by 10 amino acids. The C-terminal 10 amino acids of E20K overlaps with the N-terminal 10 amino acids of A20G, which does not inhibit βγ activation in any assay. This suggests that the potential region that is binding to βγ subunits can be narrowed down to 10 amino acids, Glu-574–Lys-583. We have tested a 10-amino acid peptide corresponding to this region and found no evidence for inhibition. A possible explanation is that this 10-amino acid peptide may be too short to adopt the appropriate secondary structure. This has been seen for other peptides including the peptide from adenylyl cyclase type II (26).

Visualization of the regions homologous to our peptides in the PLC δ structure supports the idea that the 10-amino acid overlap region is the critical region of the peptides involved in binding to βγ subunits. The catalytic domain of PLC δ is composed of parts of the conserved X and Y domains that form a TIM barrel constructed from of a series of β sheets α helix repeats. The β strands line the inside of the barrel, and side chains from these strands project into the core of the structure to provide the chemistry for substrate binding and catalysis. The α helices are on the outside of this barrel structure and in some cases are exposed to solvent. When the sequence of the N20K is aligned with the PLC δ1 sequence, the N-terminal 10 amino acids align with a small amount of linker sequence and the Tβ6 strand of the barrel (nomenclature of Essen et al. (10)).

The sequence in Tβ6 is very conserved between the various PLC isoforms with two amino acids, serine 571 and phenylalnine 572 (β2 sequence), being conserved in all known PLC isoforms. For this reason we predict that this sequence in PLC β2 will occupy a similar position to that observed in the PLC δ sequence. Since this region is on the inside of the TIM barrel in PLC δ and is directly involved in substrate binding, it would be unlikely to be accessible to interaction with βγ subunits. The region homologous to Glu-574–Lys-583 of PLC β2 (overlap region between N20K and E20K) forms an α helix on the surface of PLC δ1, and because of the significant sequence homology in this region, would likely form the same type of structure on PLC β2. The surface exposure of just this 10-amino acid region of N20K further suggests that this overlap region corresponds to a portion of PLC β2 that is important for interaction with and regulation by G protein βγ subunits. The location of this binding site directly adjacent to structures that contribute amino acids to substrate binding and catalysis suggests a mechanism by which βγ subunits could activate PLC β. If the binding of βγ subunits to the helix on the surface caused movement of the adjacent β strands, this could position amino acids so they are more favorable for catalysis there by increasing enzymatic activity.

The region we have defined only overlaps the portion of PLC β2 (Leu-580–Val-641) originally defined by Kuang et al. (16) as a βγ binding domain by 4 amino acids (580–583). Using two-hybrid analysis, Yan and Gautam (29) used the N-terminal 100-amino acid region of the β subunit to demonstrate interactions with this 62-amino acid domain. One peptide (A584–G604) overlaps extensively with this domain yet had no effect in any of our assays for βγ subunit interactions. This suggests that either only a very short 4-amino acid region is necessary for some βγ binding or that other regions within a 62-amino acid region are also involved in PLC β2–βγ interactions.

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