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Biochemistry

Identification of a phospholipase C β_2 region that interacts with G$\beta\gamma$

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ABSTRACT **To delineate the phospholipase C (PLC; EC 3.1.4.3) β_2 sequences involved in interactions with the $\beta\gamma$ subunits of G proteins, we prepared a number of mammalian expression plasmids encoding a series of PLC β_2 segments that span the region from the beginning of the X box to the end of the Y box. We found the sequence extending from residue Glu-435 to residue Val-641 inhibited G$\beta\gamma$-mediated activation of PLC β_2 in transfected COS-7 cells. This PLC β_2 sequence also inhibited ligand-induced activation of PLC in COS-7 cells cotransfected with cDNAs encoding the complement component C5a receptor and PLC β_2 but not in cells transfected with the α_1B-adrenergic receptor, suggesting that the PLC β_2 residues (Glu-435 to Val-641) inhibit the G$\beta\gamma$-mediated but not the Gα-mediated effect. The inhibitory effect on G$\beta\gamma$-mediated activation of PLC β_2 may be the result of the interaction between G$\beta\gamma$ and the PLC β_2 fragment. This idea was confirmed by the observation that a fusion protein comprising these residues (Glu-435 to Val-641) of PLC β_2 and glutathione *S*-transferase (GST) bound to G$\beta\gamma$ in an *in vitro* binding assay. The G$\beta\gamma$-binding region was further narrowed down to 62 amino acids (residues Leu-580 to Val-641) by testing fusion proteins comprising various PLC β_2 sequences and GST in the *in vitro* binding assay.**

Many hormones and neurotransmitters function by activating phospholipase C (PLC; EC 3.1.4.3). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate to generate two important second messengers: diacylglycerol and inositol 1,4,5-trisphosphate. Molecular cloning has revealed at least three classes of PLC; β, γ and δ. Each of these classes occurs in a number of isoforms. Comparison of the deduced amino acid sequences derived from the cDNAs corresponding to PLC molecules in different classes has revealed two homologous regions, designated the X and Y boxes (see Fig. 1). These two regions have been suggested to comprise the catalytic domain of PLC (1). The γ isoforms of PLC were found to be activated by tyrosine kinases (2). This activation is attributed to phosphorylation at tyrosine residues between the X and Y boxes (2). The β isoforms of PLC, consisting of four isoforms, PLC β_1–β_4, can be activated by G proteins. We and others found that the G_q class of G proteins, including $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$, can activate all four PLC β isoforms (3–8). Furthermore, we found that the G$\beta\gamma$ subunits can efficiently activate PLC β_2 but not PLC β_1 or β_4 in cotransfected COS-7 cells (9, 10). Similar results were also observed in reconstitution experiments using purified proteins, where clear activation of PLC β_3 and slight activation of PLC β_1 by G$\beta\gamma$ were also observed (11–16).

The consensus was that the α subunits of G proteins were responsible for signal transduction by directly interacting with effectors. However, a series of recent findings indicate that G$\beta\gamma$ plays more active roles in signal transduction than previously thought. G$\beta\gamma$ is involved in modulation of activities of adenylyl cyclases (17), PLC β (9, 11), potassium channels (18–20), phosphatidylinositol 3-kinase (21), and mitogen-activated protein (MAP) kinases (22). The activation of PLC β_2 by G$\beta\gamma$ may account for the pertussis toxin (PTX)-sensitive activation of PLC by a number of chemokine receptors in leukocytes (23). Therefore, it is of great interest to understand the molecular basis involved in the interactions between G$\beta\gamma$ and its effectors. We have previously determined that the C termini of PLC β_1 and PLC β_2 are required for activation by $G\alpha_q$ and identified two sequences at the C-terminal part of PLC β_1 that may be involved in direct interactions with $G\alpha_q$ (7). Recently, we found that the N-terminal half of PLC β_2 contains the sequences required for activation by G$\beta\gamma$ by using various chimeras comprising PLC β_1 and PLC β_2 (10). Moreover, our observation that the chimera comprising the N-terminal 280 amino acids of PLC β_2 and the rest of PLC β_1 cannot be activated by G$\beta\gamma$ despite its activation by $G\alpha_q$ indicated that the N-terminal part of PLC β_2 cannot confer the ability to be activated by G$\beta\gamma$ (10). Thus, we suggest that the sequence extending from the beginning of the X box to the end of the Y box is most likely to contain the sequences involved in interaction with G$\beta\gamma$.

In this report we further delineate the PLC β_2 sequences that our previous results indicated are involved in interaction with G$\beta\gamma$. By using the combination of two approaches (inhibition of G$\beta\gamma$-mediated activation of PLC β_2 by various PLC β_2 fragments in cotransfected COS-7 cells, and direct binding of GST-PLC β_2 fusion proteins to purified G$\beta\gamma$), we have narrowed down the G$\beta\gamma$-binding sequence to about 60 amino acids.

MATERIALS AND METHODS

Materials. The anti-Gβ antibody (BN1) was raised against the N-terminal 14 residues of $G\beta_1$ (9). Restriction endonucleases, T4 DNA ligase, Dulbecco's modified Eagle's medium, fetal calf serum, Lipofectamine, and antibiotics (Life Technologies); deoxynucleoside triphosphates and complement component C5a (Sigma); *Pfu* DNA polymerase (Stratagene); and [^{3}H]inositol (DuPont/New England Nuclear) were obtained from the sources indicated.

Transfection of COS-7 Cells and Analysis of Inositol Phosphates (IPs). COS-7 cells were cultured and transiently transfected as described previously (5). In brief, COS-7 cells (5×10^4) were seeded in one well of a 24-well plate (Falcon). A mixture of plasmid DNA (0.5 μg) and Lipofectamine (1.7 μl) was added, and the cells were incubated for 24 h. Then the cells were labeled with 2.5 μCi (1 μCi = 37 kBq) of [^{3}H]inositol that had been added to 0.25 ml of inositol-free medium for another 24 h. The levels of IPs were determined on the third day as described previously (5). Cotransfection of cDNAs was also done in 24-well plates. DNAs (0.6 μg per well), including 0.2 μg each of the cDNAs corresponding to the C5a receptor, PLC β_{2_2} fragment or LacZ cDNA as indicated in the figure legends

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Abbreviations: PLC, phospholipase C; PTX, pertussis toxin; GST, glutathione *S*-transferase; IP, inositol phosphate.

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were premixed with 1.7 μl of Lipofectamine and added to the cells. C5a (250 nM)-dependent release of IPs was measured as described previously (23). In Gβγ-dependent activation of PLC-β_2 experiments, cDNAs (0.15 μg each of PLC β_2, PLC β_2 fragment, $G\beta_1$ and $G\gamma_2$) were added to the cells with 1.7 μl of Lipofectamine as indicated in the figures.

Construction of PLC-β_2 Fragments for Expression in COS-7 Cells. The cDNAs corresponding to PLC-β_2 fragments, as illustrated in Fig. 1, were synthesized by polymerase chain reaction (PCR) with the high-fidelity polymerase *Pfu*, using primers designed from the human PLC-β_2 sequence and the human PLC β_2 cDNA as template. The PCR products were cloned in the pCMV vectors (5). F1 encodes residues E1070–S1179 of the PLC-β_2 sequence, F2 encodes residues E435–V641, F3 encodes residues E435–L786, F4 encodes residues H320–G478, and F5 encodes residues H320–L433.

Expression and Isolation of GST-Fusion Proteins. The GST gene fusion vector pGEX-3X (Pharmacia) was used to make fusion protein constructs. F6 is GST-PLC β_2 (E435–G478), F7 is GST-PLC β_2 (E435–V641), F8 is GST-PLC β_2 (E435–T552), F9 is GST-PLC β_2 (Q526–V641), F10 is GST-PLC β_2 (E435–Q526), and F11 is GST-PLC β_2 (L580–V641). The fusion protein constructs were used to transform the *Escherichia coli* strain M15. A single colony containing the recombinant pGEX plasmid was inoculated into LB medium (Life Technologies) containing ampicillin at 100 μg/ml and incubated overnight at 37°C with vigorous shaking. Then the culture was diluted 25-fold and incubated at 30°C with vigorous shaking until OD_{600} reached approximately 0.6. Isopropyl β-D-thiogalactoside (1 mM) was then added and the culture was incubated for additional 30 min. The bacteria were pelleted by centrifugation and resuspended with ice-cold phosphate-buffered saline (PBS) containing 1% Triton X-100, lysozyme (1 mg/ml), and protease inhibitors: phenylmethanesulfonyl fluoride (7 μg/ml), aprotonin (1 μg/ml), leupeptin (1 μg/ml), and pepstatin A (1 μg/ml). After a 15-min incubation on ice, the cells were sonicated on ice with five short bursts (10 sec) and centrifuged at 10,000 × *g* for 20 min at 4°C. The supernatant was incubated with preequilibrated glutathione-Sepharose 4B gel for 1 h at 4°C with gentle tumbling. Then the gels were washed with the washing buffer [1 mM dithiothreitol/0.1% polyoxyethylene 10 lauryl ether (Sigma)/0.05% Tween-20 in PBS] and were ready for the Gβγ-binding assay.

Binding of GST-Fusion Proteins to Gβγ. Glutathione-Sepharose 4B gel slurry containing GST or GST-fusion proteins was prepared as described above. The gel slurry (10 μl) was incubated with 1 μl of 2 μM native or heat-inactivated Gβγ on ice. After 1 h, 50 μl of the washing buffer described above was added to the gel slurry, which was then centrifuged briefly at 2000 × *g* and resuspended in 50 μl of the washing buffer. This procedure was repeated four times. After the final centrifugation, the supernatant was removed and the gels were resuspended in SDS sample buffer and boiled. The samples were divided into two parts and run on two separate electrophoretic gels; one was subjected to staining with Coomassie blue and the other to Western blotting analysis. The Western blot was analyzed with an anti-Gβ antibody, which recognizes the common N termini of $G\beta_{1-3}$.

SDS/Polyacrylamide Gel Electrophoresis and Western Analysis. COS-7 cells were lysed in SDS sample buffer 48 h after transfection and subjected to electrophoresis. The proteins were electroblotted onto nitrocellulose membranes and detected with specific antibodies as indicated in the figures.

RESULTS

By using chimeric molecules comprising various parts of PLC β_1 and PLC β_2, we have previously demonstrated that the N-terminal half of the PLC β_2 molecule contains the sequences required for activation by Gβγ, whereas the N-terminal 280 amino acids of PLC β_2 cannot confer the ability to be activated by Gβγ (10). To delineate further the PLC β_2 sequences required for interaction with Gβγ, we constructed three mammalian expression plasmids encoding three segments (F1,

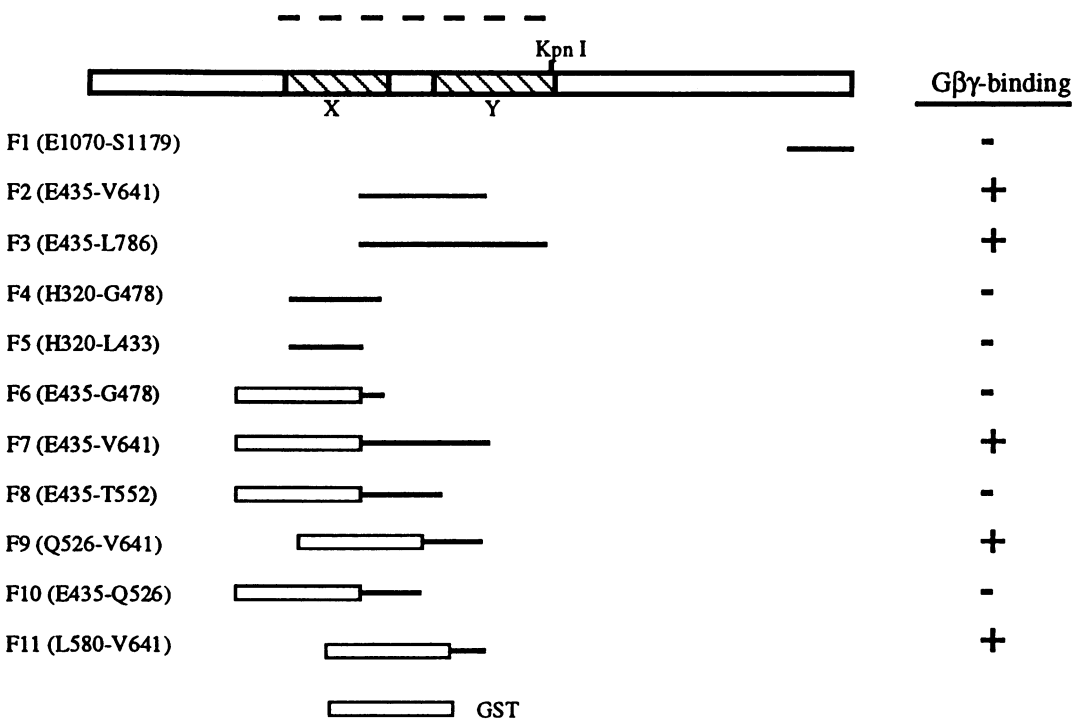

FIG. 1. Schematic representation of PLC β_2 molecule, its fragments, and glutathione *S*-transferase (GST)-fusion proteins. The X and Y boxes represent the most homologous sequences among all cloned PLC molecules, and they may comprise the catalytic domain. The broken line represents the region that we have previously identified as required for Gβγ activation. A summary of Gβγ-binding abilities is tabulated on the right. See Figs. 2–5 for the data.

F3, and F5; Fig. 1) of PLC β_2. F3 and F5 span the region containing the X and Y boxes, which we believed to contain the G$\beta\gamma$-binding sequences on the basis of our previous results (10). F1 is derived from the C terminus of PLC β_2, which is known not to interact with G$\beta\gamma$ (10). These three segments of PLC β_2 were tested for their abilities to inhibit G$\beta\gamma$-mediated activation of PLC β_2 in cotransfected COS-7 cells. As we have previously shown, cells cotransfected with cDNAs encoding G$\beta_1\gamma_2$ and PLC β_2 accumulated a higher level of IPs than those transfected with the plasmid expressing only PLC β_2 or G$\beta_1\gamma_2$ (Fig. 2*A*), suggesting that G$\beta_1\gamma_2$ can activate PLC β_2. However, when cells were cotransfected with the cDNA encoding F3 in addition to cDNAs encoding G$\beta\gamma$ and PLC β_2, they accumulated little more IPs than the cells transfected with cDNAs encoding PLC β_2 or G$\beta\gamma$ alone (Fig. 2*A*). This result indicates that the F3 segment inhibits G$\beta_1\gamma_2$-induced activation of PLC β_2, probably through binding to G$\beta_1\gamma_2$. Expression of the F1 or F5 segments showed no inhibitory effects on G$\beta_1\gamma_2$-mediated activation of PLC β_2, suggesting that the effect of F3 is specific. To delineate further the region involved in G$\beta\gamma$ binding, we constructed two additional plasmids encoding the F2 and F4 segments of PLC β_2 (Fig. 1). When cotransfected with G$\beta\gamma$ and PLC β_2, F2 but not F4 inhibits G$\beta\gamma$-mediated activation of PLC β_2 (Fig. 2*A*). Therefore, we conclude that the sequence extending from residues Gly-478 to Val-641 of PLC β_2 interferes with G$\beta\gamma$-mediated activation of PLC β_2.

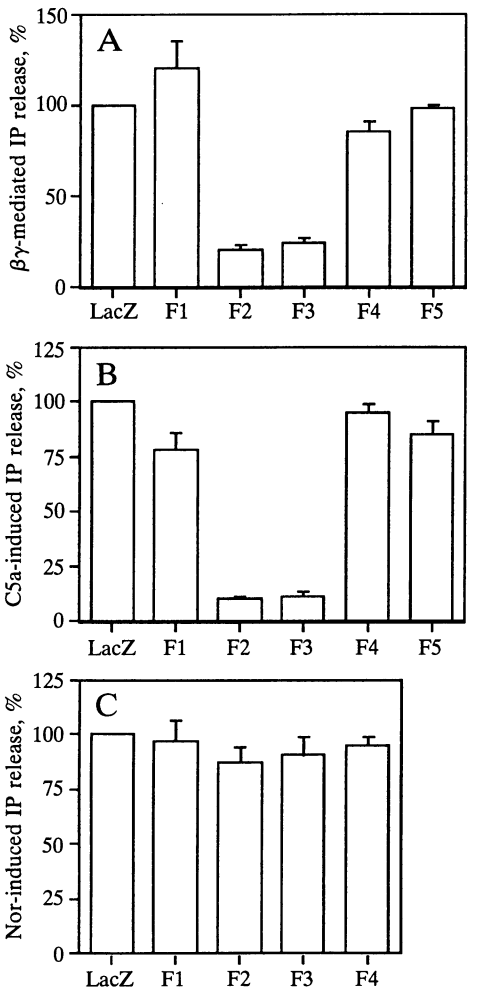

FIG. 2. (*A*) Effects of PLC β_2 fragments on G$\beta\gamma$-mediated activation of PLC β_2. COS-7 cells were cotransfected with the cDNAs encoding PLC β_2, G$\beta_1\gamma_2$, and various PLC β_2 fragments or β-galactosidase (LacZ) as a control. The levels of IPs were determined 48 h after transfection. The level of IPs (10,000 dpm) in cells transfected with cDNAs corresponding to PLC β_2 and G$\beta_1\gamma_2$ is taken as 100%. Cells transfected with the LacZ or PLC β_2 cDNA showed about 1500 and 2000 dpm. (*B*) Effects of PLC β_2 fragments on C5a receptor-mediated activation of PLC β_2. COS-7 cells were cotransfected with the cDNAs encoding PLC β_2, the C5a receptor, and various PLC β_2 fragments or β-galactosidase. The levels of IPs were determined 30 min after the addition of C5a (250 nM). C5a-induced accumulation of IPs (4000 dpm) in cells transfected with cDNAs corresponding to the C5a receptor PLC β_2 is taken as 100% (the background was 2500 dpm). (*C*) Effects of PLC β_2 fragments on $\alpha_1\beta$-adrenergic receptor-mediated activation of PLC. COS-7 cells cotransfected with the cDNAs encoding the $\alpha_1\beta$-adrenergic receptor and various PLC β_2 fragments or β-galactosidase. The levels of IPs were determined 30 min after the addition of norepinephrine (Nor; 1 μM). Norepinephrine-induced accumulation of IPs (7500 dpm; the background was 1200 dpm) in cells transfected with the cDNA corresponding to the α_1 β-adrenergic receptor is taken as 100%.

The PLC β_2 segments (F1–F5) were also subjected to a different test: they were tested for their abilities to inhibit receptor-mediated activation of PLC. We have previously demonstrated that the recombinant C5a receptor can activate recombinant PLC β_2 by releasing G$\beta\gamma$ from endogenous Gi proteins in cotransfected COS-7 cells (COS-7 cells contain endogenous G_i2 protein, but not the PLC β_2 protein or the C5a receptor) (23). To test whether the PLC β_2 segments can inhibit C5a-mediated activation of PLC β_2, we cotransfected COS-7 cells with cDNAs encoding the C5a receptor, PLC β_2, and each of the PLC β_2 segments (F1–F5). As shown in Fig. 2*B*, F2 and F3 but not F1, F4, or F5 inhibited C5a-mediated activation of PLC β_2. In contrast, F2 and F3 did not show significant inhibitory effects on norepinephrine-mediated activation of PLC in cells cotransfected with the $\alpha_1\beta$-adrenergic receptor cDNA (Fig. 2*C*), which activates PLC through $G\alpha_q$. These results indicate that F2 and F3 affect only the G$\beta\gamma$-mediated activation of recombinant PLC β_2 and not $G\alpha_q$-mediated activation of endogenous PLC β_1 or β_3. Therefore, our data support the idea that the sequence from residue Gly-478 to Val-641 inhibits G$\beta\gamma$-mediated activation of PLC β_2 and that the inhibitory effect may be the result of direct interaction between the PLC β_2 fragment and G$\beta\gamma$. The expression levels of these PLC segments (F1–F5) were examined with an antibody that specifically recognizes an epitope-tag (nine amino acids derived from hemagglutinin, which we engineered into the C termini of F1–F5). Western analysis indicated that F2 and F3 are expressed at lower levels than F1 or F4 (Fig. 3). This result, together with our previous findings that coexpression of one protein usually does not affect the

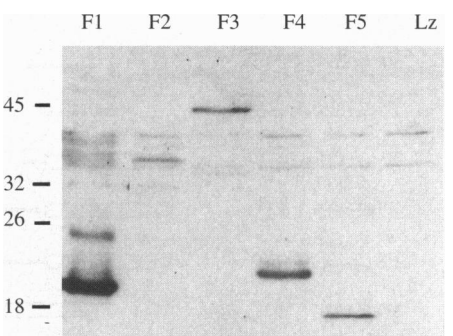

FIG. 3. Western analysis of the expression of PLC β_2 fragments in COS-7 cells. Cells were transfected with the expression plasmids encoding various PLC β_2 fragments and β-galactosidase (Lz) and were solubilized in the SDS sample buffer 48 h after transfection. The proteins were separated by SDS/15% polyacrylamide electrophoresis gels and electroblotted onto nitrocellulose membranes. The proteins were detected with an antibody that specifically recognizes a nine-amino acid epitope tag derived from hemagglutinin which was engineered into the C termini of F1–F5. Positions of marker proteins are indicated on the left in kDa.

expression of others (5–10, 23), indicates the inhibitory effects of F2 and F3 are not the results of higher expression levels.

To obtain evidence for direct interaction between F2 and Gβγ and to delineate further the Gβγ-binding region, we prepared a series of bacterial expression constructs (F6–F10, Fig. 1). These constructs encode fusion proteins comprising GST and various PLC β_2 fragments that span the F2 segment. The fusion proteins were expressed in bacteria in the presence of isopropyl β-thiogalactoside, and the bacterial extracts were incubated with glutathione-Sepharose beads, which specifically bind GST. After washing, the beads were incubated with Gβγ purified from bovine brain. Finally the beads were washed, and the SDS/PAGE sample buffer was added. The samples were divided into two parts, which were subjected to separate electrophoresis; one gel was analyzed by Western blotting with the Gβ-specific antibody (BN1) and the other was stained with Coomassie blue for quantitation of the fusion proteins. Western analysis revealed that the F7 fusion protein, which contains the same PLC β_2 residues as F2, was able to retain the Gβγ proteins (Fig. 4*A*), thus supporting our idea that the sequence extending from residue Gly-478 to Val-641 directly interacts with Gβγ. The inabilities of F6, F8, and F10 and the ability of F9 to retain Gβγ in the assay indicate that the sequence extending from residue Thr-552 to Val-641 is involved in interaction with Gβγ. The amounts of various fusion proteins present in the assay were quantitated by Coomassie blue staining and shown in Fig. 4*B*. There is slightly more protein for those constructs (GST, F6, F8, and F10) showing no interactions with Gβγ.

To delineate the Gβγ-binding region further, we prepared an additional construct that encodes a fusion protein, F11. F11 contains PLC β_2 residues from Leu-580 to Val-641. The F11 fusion protein was subjected to the same assay as F6–F10. We found that F11 could retain Gβγ in the binding assay (Fig. 5*A*). To test whether the interaction is specific, we heat-treated the Gβγ proteins by boiling the Gβγ proteins for 5 min and then

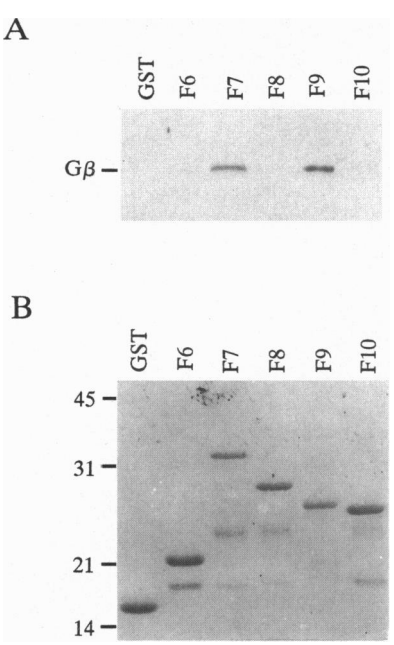

FIG. 4. The binding of GST-fusion proteins to Gβγ. Glutathione-Sepharose 4B beads which contained GST or GST-fusion proteins were incubated with bovine Gβγ on ice for 1 h. After washing, the beads were resuspended in SDS sample buffer, boiled, and divided into two parts, which were electrophoresed on two separate 12% polyacrylamide gels. One gel was subjected to Western blotting (*A*). The bound Gβγ was detected by BN1, an antibody which recognizes the common N termini of $G\beta_1$–$G\beta_3$. The other gel was stained by Coomassie blue for quantitation of the GST-fusion proteins (*B*).

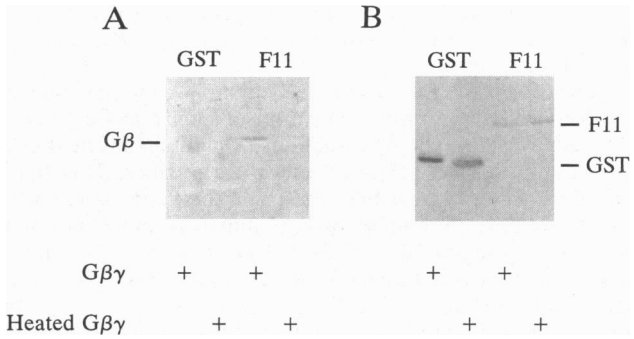

FIG. 5. Binding of F11 to Gβγ. GST- or F11-bound glutathione-Sepharose beads were incubated with either native or heat-treated Gβγ. (*A*) Bound Gβγ was detected by Western analysis with the BN1 antibody. (*B*) Levels of GST and F11 present in the Gβγ-binding assay were quantitated by Coomassie blue staining.

adding them back to the assay. We found that the heat-treated Gβγ hardly bound to F11 (Fig. 5*A*), suggesting that native conformation of Gβγ may be required for the interaction with the PLC β_2 fragments. The GST protein was used as a control in this experiment. The levels of F11 and GST present in the assay are shown in Fig. 5*B*, and there are no significant variations. Therefore, we conclude that the PLC β_2 sequence extending from residue Leu-580 to Val-641 shows a high affinity for Gβγ.

DISCUSSION

We have employed a combination of two methods to delineate the Gβγ-binding sequence on PLC β_2. We first identified a long stretch of PLC β_2 sequence (F2) as the region containing the putative Gβγ-binding site on the basis of its ability to inhibit specifically the Gβγ-mediated activation of PLC β_2. Subsequently, we confined the Gβγ-binding site to 62 amino acid residues extending from Leu-580 to Val-641 (F11) by determining the abilities of a series of fusion proteins comprising GST and various PLC β_2 sequences to bind to purified bovine Gβγ.

The sequence extending from residue Leu-580 to Val-641 appears to have the highest affinity for Gβγ in the region extending from the beginning of the X box to the end of Y box. In this report we focused only on this region, because we have previously identified it as the region that may contain the Gβγ-regulatory sequences. Thus, it is possible that there are other sequences on PLC β_2 beyond the X and Y boxes that can bind to Gβγ. It also remains unclear what exact role this Gβγ-binding sequence plays in Gβγ-mediated activation of PLC β_2—i.e., whether this sequence merely provides an anchor point for the interaction between Gβγ and PLC β_2 or also plays a role in induction of conformational changes required for activation of PLC β_2. The proximity of this sequence to the catalytic center suggests that it may be involved in the activation of PLC β_2.

Gβγ-binding sequences of βARKs (24), phosducin (25, 26), and adenylyl cyclases (27) have been characterized. Although certain homology between βARK and phosducin has been indicated (25, 26), there is no remarkable homology between the Gβγ-binding regions of adenylyl cyclases and those of βARK and phosducin (27). However, an amino acid sequence motif, QXXEK, was proposed on the basis of comparison of the Gβγ-binding regions of βARKs and adenylyl cyclases types 2, 4, and 7 (adenylyl cyclases types 2 and 4 were known to be regulated by Gβγ) (27). This motif cannot be found in phosducin, nor can it be found in the PLC β_2 sequence that we have characterized in this report. In addition, the Gβγ-binding sequence of PLC β_2 shares no significant homology with any of the known Gβγ-binding sequences. Thus, the Gβγ-binding

sequences might be diverse in their primary structures, and there may be certain requirements for the secondary or tertiary structures.

Activation of PLC β_2 by G$\beta\gamma$ may be the mechanism involved in PTX-sensitive elevation of cytosolic Ca^{2+} levels induced by many chemoattractants, including interleukin 8, fMet-Leu-Phe, C5a (23), and other chemokines. This signal transduction pathway is believed to be the main signal transduction pathway for these proinflammatory molecules. The G$\beta\gamma$-binding region of PLC β_2 that we have identified in this report, may provide targets for developing drugs to intervene specifically the interaction between G$\beta\gamma$ and PLC β_2, thus attenuating cytokine-induced inflammatory responses.

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1. Rhee, S.-G. & Choi, K. D. (1992) *J. Biol. Chem.* **267,** 12393–12396.
2. Kim, H. K., Kim, J. W., Zilberstein, A., Kim, J. G. & Rhee, S. G. (1991) *Cell* **65,** 435–441.
3. Smrcka, A., Hepler, J., Brown, K. & Sternweis, P. (1991) *Science* **251,** 804–807.
4. Taylor, S., Chae, H., Rhee, S. & Exton, J. (1991) *Nature (London)* **350,** 516–518.
5. Wu, D., Lee, C.-H., Rhee, S. G. & Simon, M. I. (1992) *J. Biol. Chem.* **267,** 1811–1817.
6. Wu D., Katz, A., Lee, C.-H., Jiang, H. & Simon, M. I. (1992) *J. Biol. Chem.* **267,** 25798–25802.
7. Wu, D., Jiang, H. A., Katz, A. & Simon, M. I. (1993) *J. Biol. Chem.* **268,** 3704–3709.
8. Lee, C. H., Park, D., Wu, D., Rhee, S.-G. & Simon, M. I. (1992) *J. Biol. Chem.* **267,** 16044–16047.
9. Katz, A., Wu, D. & Simon, M. I. (1992) *Nature (London)* **360,** 686–689.
10. Wu, D., Katz, A. & Simon, M. I. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 5297–5301.
11. Camps, M., Carozzi, A., Scheer, A., Park, P. J. & Giershik, P. (1992) *Nature (London)* **360,** 683–686.
12. Boyer, J. L., Waldo, G. L. & Harden, T. K. (1992) *J. Biol. Chem.* **267,** 25451–25456.
13. Blank, J. L., Brattain, K. A. & Exton, J. H. (1992) *J. Biol. Chem.* **267,** 23069–23075.
14. Smrcka, A. & Sternweis, P. (1993) *J. Biol. Chem.* **268,** 9663–9674.
15. Lee, C. W., Park, D. J., Lee, K. H., Kim, C. G. & Rhee, S.-G. (1993) *J. Biol. Chem.* **268,** 21318–21327.
16. Park, D., Jhon, D.-Y., Lee, C.-W., Lee, K.-H. & Rhee, S.-G. (1993) *J. Biol. Chem.* **268,** 4573–4576.
17. Tang, W. J. & Gilman, A. G. (1991) *Science* **254,** 1500–1503.
18. Logothetis, D. E., Kurache, Y. Galper, J., Neer, E. J. & Clapham, D. E. (1987) *Nature (London)* **325,** 321–326.
19. Reuveny, E., Slesinger, P. A., Ingleses, J., Morales, J. M., Iniguez-Lluhi, J. A., Lefkowitz, R. J., Bourne, H. R., Jan, Y. N. & Jan, L. Y. (1994) *Nature (London)* **370,** 143–146.
20. Wickman, K. D., Iniguez-Lluhi, J. A., Davenport, P. A., Taussig, R., Krapivinsky, G. B., Linder, M. E., Gilman, A. G. & Clapham, D. E. (1994) *Nature (London)* **368,** 255–257.
21. Stephens, L., Smrcka, A., Cook, F. T., Jackson, T. R., Sternweis, P. C. & Hawkins, P. T. (1994) *Cell* **77,** 83–93.
22. Crespo, P., Xu, N.-Z., Simonds, W. F. & Gutkind, J. S. (1994) *Nature (London)* **369,** 418–420.
23. Wu, D., LaRosa, G. J. & Simon, M. I. (1993) *Science* **261,** 101–103.
24. Inglese, J., Knoch, W. J., Caron, M. G. & Lefkowitz, R. J. (1992) *Nature (London)* **359,** 147–150.
25. Hawes, B. E., Touhara, K., Kurose, H., Lefkowitz, R. J. & Inglese, J. (1994) *J. Biol. Chem.* **269,** 29825–29830.
26. Xu, J., Wu, D., Slepak, V. Z. & Simon, M. I. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2086–2090.
27. Chen, J., DeVivo, M., Dingus, J., Harry, A., Sui, J., Carty, D., Blank, J., Exton, J. H., Stoffel, R. H., Inglese, J., Lefkowitz, R. J., Logothetis, D. E., Hildebrandt, J. D. & Iyengar, R. (1995) *Science* **268,** 1166–1169.