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Purification and characterization of recombinant $G_{16\alpha}$ from Sf9 cells: Activation of purified phospholipase C isozymes by G-protein α subunits

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ABSTRACT **A cDNA encoding $G_{16\alpha}$, the α subunit of a heterotrimeric guanine nucleotide-binding protein, was expressed in Sf9 cells using recombinant baculovirus. $G_{16\alpha}$ in membrane extracts of Sf9 cells activated phospholipase C-β1 (PLC-β1) in the presence of guanosine 5′-[γ-thio]triphosphate; the system could not be activated by Al^{3+}, Mg^{2+}, and F^-. The $G_{16\alpha}$ in the cytosolic fraction of Sf9 cells did not stimulate PLC-β1. Concurrent expression of the G-protein $\beta\gamma$ subunit complex increased the amount of $G_{16\alpha}$ in Sf9 cell membranes. The guanosine 5′-[γ-thio]triphosphate-activated form of $G_{16\alpha}$ was purified from cholate extracts of membranes from cells expressing $G_{16\alpha}$, and the G-protein β_2 and γ_2 subunits. $G_{16\alpha}$ activated PLC-β1, PLC-β2, and PLC-β3 in a manner essentially indistinguishable from that of $G_{q\alpha}$. $G_{16\alpha}$-mediated activation of PLC-β1 and PLC-β3 greatly exceeded that of PLC-β2. $G_{16\alpha}$ did not activate PLC-γ1 or PLC-δ1. Thus, two distantly related members of the $G_{q\alpha}$ family, $G_{q\alpha}$ and $G_{16\alpha}$, have the same ability to activate the known isoforms of PLC-β.**

The α subunits of heterotrimeric, signal-transducing guanine nucleotide-binding proteins (G proteins) can be classified into four major groups, based on amino acid sequence relationships and some major functional characteristics (1). The so-called $G_{q\alpha}$ class has four members—$G_{q\alpha}$, $G_{11\alpha}$, $G_{14\alpha}$, and $G_{15\alpha}/G_{16\alpha}$ (2–5). Within this group, $G_{q\alpha}$, $G_{11\alpha}$, and $G_{14\alpha}$ are very similar to one another. $G_{15\alpha}$ and $G_{16\alpha}$, which are now thought to be mouse and human homologs, are more distantly related to $G_{q\alpha}$ (58% and 57% amino acid identity, respectively); they are 85% identical to each other. All members of the $G_{q\alpha}$ class lack the cysteine residue near the carboxyl terminus that is the site of pertussis toxin-catalyzed ADP-ribosylation of members of the G_i class of α subunits. Thus, $G_{q\alpha}$ proteins are presumed to function in signaling pathways that are immune to disruption by the toxin.

Many hormones, neurotransmitters, and growth factors activate certain isozymes of phospholipase C (PLC) and thus stimulate the hydrolysis of the membrane lipid phosphatidylinositol 4,5-bisphosphate to yield two intracellular second messengers, inositol 1,4,5-trisphosphate ($Ins P_3$) and diacylglycerol (6). PLCs of the γ subtype are in general activated by receptors with intrinsic tyrosine kinase activity, whereas PLCs of the β subtype are regulated by G proteins (7). Mixtures of $G_{q\alpha}$ and $G_{11\alpha}$ isolated from bovine brain or bovine liver were first shown to activate PLC-β1 upon combination of the purified proteins (8, 9). We have further demonstrated that a mixture of brain $G_{q\alpha}/G_{11\alpha}$ and recombinant $G_{q\alpha}$ ($rG_{q\alpha}$) and $rG_{11\alpha}$, purified individually after baculovirus-directed expression in Sf9 insect cells, can activate purified PLC-β1, PLC-β2, and PLC-β3, but not PLC-γ1 or PLC-δ1 (10, 11).

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A cDNA that encodes $G_{16\alpha}$ was cloned from a human promyelocytic leukemia (HL-60) cell library (5). In contrast to $G_{q\alpha}$ and $G_{11\alpha}$, which are expressed ubiquitously, $G_{16\alpha}$ is synthesized predominantly in hematopoietic cells. We have purified the guanosine 5′-[γ-thio]triphosphate (GTP[γS])-activated form of $G_{16\alpha}$ after expression in Sf9 cells and characterized its interactions with various isozymes of PLC.

MATERIALS AND METHODS

Construction of Recombinant Baculovirus and Sf9 Cell Culture. A 1.25-kb DNA fragment containing the complete coding sequence for $G_{16\alpha}$ was excised from pKSG16 (5) by digestion with *Bal* I and *Xba* I and was subcloned into the *Sma* I and *Xba* I sites of the baculovirus transfer vector pVL1393. The resulting plasmid and linearized AcRP-lacZ viral DNA were transfected into Sf9 cells by lipofection. Recombinant virus was plaque-purified as described (12). Positive viral clones were identified by their capacity to direct the expression of $G_{16\alpha}$ protein in Sf9 cells, which was detected by immunoblotting with anti-$G_{16\alpha}$ antiserum B861. Sf9 cells were grown in suspension as described (11).

Measurement of PLC Activity. PLC activity was estimated as described (8, 11). In most cases PLC activity is expressed as pmol of $Ins P_3$ per min per ng of PLC. To quantify purification of $G_{16\alpha}$ (Table 1), PLC specific activity is expressed as nmol of $Ins P_3$ per min per mg of protein sample containing $G_{16\alpha}$. The amount of PLC used per assay was 2.9 ng for bovine brain PLC-β1, 1 ng for recombinant PLC-β1, 8 ng for recombinant PLC-β2, 0.2 ng for bovine brain PLC-β3, 2 ng for PLC-γ1, and 2 ng for PLC-δ1. Assays were performed for 5 min with PLC-β1 and for 10 min with the other phospholipases.

Purification of $G_{16\alpha}$ from Sf9 Cells. Sf9 cells (8-liter culture; 1.5×10^6 cells per ml) were infected with recombinant baculoviruses (2–5 plaque-forming units per cell) encoding $G_{16\alpha}$ and G-protein β_2 and γ_2 subunits (13). Cells were harvested after 48 hr by centrifugation at $1000 \times g$ for 10 min and were suspended in 800 ml of ice-cold lysis buffer [50 mM NaHepes, pH 7.2/1 mM EDTA/3 mM EGTA/5 mM $MgCl_2$/3 mM dithiothreitol/50 mM NaCl with the protease inhibitors [phenylmethanesulfonyl fluoride, 20 μg/ml; 7-amino-1-chloro-3-tosylamido-2-heptanone ("tosyllysine chloromethyl ketone"), 20 μg/ml; L-1-tosylamido-2-phenylethyl chloromethyl ketone, 30 μg/ml; and lima bean trypsin inhibitor, 30 μg/ml]. Cells were lysed by nitrogen cavitation (Parr bomb) at 500 psi (1 psi = 6.89 kPa) for 30 min at 4°C. Cell lysates were centrifuged at $750 \times g$ for 10 min to remove intact cells and nuclei, and the supernatant was further

Abbreviations: AMF, $Al^{3+}/Mg^{2+}/F^-$; GTP[γS], guanosine 5′-[γ-thio]triphosphate; $Ins P_3$, inositol 1,4,5-trisphosphate; PLC, phospholipase C; r (prefix), recombinant.

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Table 1. Purification of $rG_{16\alpha}$

Step	Volume, ml	Protein, mg	PLC activity*, units	Specific activity, units/mg
Cholate extract	700	1470	5700	3.8
Q-Sepharose	160	121	1200	10
Phenyl-Sepharose	110	4.5	ND	—
Mono-Q (octyl glucoside)	20	0.44	520	1200
Mono-Q (sodium cholate)	1	0.018	90	5000

ND, not done.
*One unit = 1 nmol of $InsP_3$ per minute per 2.9 ng of PLC-β1.

centrifuged at 100,000 × g for 40 min. Membrane pellets were suspended in 400 ml of lysis buffer with a Potter–Elvehjem homogenizer (10 strokes). The resulting suspension (about 5 mg of protein per ml) was frozen in liquid nitrogen and stored at −80°C.

For purification of $G_{16\alpha}$, membranes were thawed at 30°C. GTP[γS] was added (5 μM) and membranes were incubated with the nucleotide for 30 min at 30°C; all subsequent procedures were performed at 4°C. Membranes were extracted by addition of sodium cholate (1%, wt/vol) and stirring for 1 hr. This mixture was then centrifuged at 100,000 × g for 40 min, and the supernatant (membrane extract) was collected.

The membrane extract was applied to a 100-ml Q-Sepharose anion-exchange column (Pharmacia) which had been equilibrated with buffer A (50 mM NaHepes, pH 7.2/1 mM EDTA/3 mM EGTA/5 mM $MgCl_2$/3 mM dithiothreitol/1 μM GTP[γS]) containing 1% sodium cholate and 50 mM NaCl. The column was washed with 150 ml of the equilibration buffer, followed with a 500-ml linear gradient of NaCl (50–600 mM) in buffer A plus 1% sodium cholate. The column eluate (7-ml fractions) was assayed for capacity to activate bovine brain PLC-β1 and by immunoblotting using antiserum B861. Peak fractions (120 ml) were pooled and diluted 4-fold with buffer B [buffer A containing 35% (vol/vol) glycerol] containing 1.25 M $(NH_4)_2SO_4$. Any precipitate that formed at this stage was removed by centrifugation at 100,000 × g for 30 min. The supernatant was applied to a 30-ml column of phenyl-Sepharose CL-4B (Pharmacia), which had been equilibrated with buffer B containing 1 M $(NH_4)_2SO_4$ and 0.3% sodium cholate. The column was washed with 50 ml of equilibration buffer, followed with a 120-ml gradient containing linearly decreasing concentrations of $(NH_4)_2SO_4$ (1 M to 0) and linearly increasing concentrations of sodium cholate (0.3–1.5%) in buffer B. The column was finally washed with 120 ml of buffer B containing 1.5% sodium cholate. Fractions (3 ml) were collected into tubes containing 3 μl of 10 mM GTP[γS]. $G_{16\alpha}$ (immunoreactivity) was eluted broadly at the end of the gradient and during the final column wash. We could not assay the PLC-stimulating activity of $G_{16\alpha}$ in these fractions because of the inhibitory effect of $(NH_4)_2SO_4$. Fractions representing the peak of immunoreactivity were pooled and concentrated to 20 ml by ultrafiltration using an Amicon PM30 membrane. To change the detergent from sodium cholate to octyl glucoside, the sample was twice diluted 5-fold with buffer A containing 1% octyl glucoside and concentrated to 20 ml. The resulting sample was applied to a Mono-Q HR 5/5 anion-exchange column for FPLC (Pharmacia) that had been equilibrated with buffer A containing 1% octyl glucoside. The column was washed with 5 ml of equilibration buffer, followed by 40 ml of the same buffer containing a linear gradient of NaCl (0–500 mM). Most of the $G_{16\alpha}$ activity was detected in the flowthrough and wash. These fractions (20 ml) were collected, diluted 10-fold with buffer A containing 1% sodium cholate, and concentrated to 20 ml. The sample was then applied to a Mono-Q HR5/5 column equilibrated with buffer A containing 1% sodium cholate and 50 mM NaCl. The column was washed with 5 ml of equilibration buffer and a 30-ml gradient of NaCl (50–600 mM) in buffer A containing 1% sodium cholate. $G_{16\alpha}$ was eluted between 150 and 250 mM NaCl. Analysis of fractions by silver staining of SDS/polyacrylamide gels indicated that the fractions collected at the latter part of this peak were essentially pure $G_{16\alpha}$. These fractions were pooled and frozen at −80°C.

Antisera. Anti-$G_{16\alpha}$ antiserum B861 was made in rabbits against the synthetic 11-amino acid peptide corresponding to the carboxyl-terminal sequence of $G_{16\alpha}$. Antiserum W082 was raised in rabbits against a 19-amino acid peptide corresponding to an internal sequence of $G_{q\alpha}$ (amino acids 115–133) (14). Antiserum Z811 was raised in rabbits against a 15-amino acid peptide representing the carboxyl-terminal sequence shared by $G_{q\alpha}$ and $G_{11\alpha}$ (11, 14).

Miscellaneous Procedures. SDS/polyacrylamide gel electrophoresis of proteins was performed according to Laemmli (15). Protein concentrations were determined by staining with amido black, using bovine serum albumin as a standard (16). Immunoblotting was done with an ECL chemiluminescence system (Amersham) for detection. $rG_{q\alpha}$ (Sf9 cell-derived) and the $G_{q\alpha}$-like protein(s) endogenous to Sf9 cells were purified as described (11). rPLC-β1 and rPLC-β2 were purified from HeLa cells (17). PLC-β1, PLC-γ1, and PLC-δ1 were purified from bovine brain, while PLC-β3 was purified from rat brain (10, 18, 19). The following reagents were kindly provided by colleagues at the University of Texas Southwestern Medical Center: recombinant baculoviruses encoding G-protein β_2 and γ_2 subunits by Jorge Iñiguez-Lluhi; bovine brain $\beta\gamma$ by Jorge Iñiguez-Lluhi and Maurine Linder.

RESULTS

Recombinant $G_{16\alpha}$ in Sf9 Cells. We previously reported that active $rG_{q\alpha}$ and $rG_{11\alpha}$ could be synthesized in Sf9 cells and purified to homogeneity (11). Using the same methods, we attempted to purify $rG_{16\alpha}$, another member of the $G_{q\alpha}$ family. A recombinant baculovirus encoding $G_{16\alpha}$ was plaque-purified by monitoring immunoreactivity (Western blot) in Sf9 cells corresponding to a 44-kDa protein with antiserum B861 (Fig. 1). The immunoreactive band was not detected in uninfected cells or after reaction of the antiserum with the peptide used as immunogen.

Sf9 cells were infected with baculovirus encoding $G_{16\alpha}$, and membrane and cytosolic fractions were prepared after 48 hr. As shown in Fig. 1*A* (right two lanes), $rG_{16\alpha}$ was detected in both the particulate and soluble fractions in roughly equal amounts. When $rG_{16\alpha}$ was expressed together with G-protein β_2 and γ_2 subunits, most of the $rG_{16\alpha}$ immunoreactivity was found in membranes.

We next examined the capacity of $rG_{16\alpha}$ to activate PLC-β1. A sodium cholate extract of Sf9 cell membranes was assayed with PLC-β1 in the presence either of GTP[γS] or of AMF plus GDP. When GTP[γS] was the ligand, the extract containing $rG_{16\alpha}$ activated PLC-β1 to an extent similar to that observed with a cholate extract of membranes from cells expressing $rG_{q\alpha}$ (Fig. 1*B*). However, such activity was not detected when the assay was performed with AMF and GDP. In contrast, $rG_{q\alpha}$ activated PLC-β1 under either condition. Of interest, $rG_{16\alpha}$ in the cytosolic fraction was inactive under both conditions. Unlike $rG_{q\alpha}$ and $rG_{11\alpha}$, $rG_{16\alpha}$ in cholate extracts of Sf9 cell membranes did not aggregate, even when expressed without G-protein β or γ subunits (data not shown).

Unfortunately, the $G_{16\alpha}$ in detergent extracts of Sf9 cell membranes was unstable unless GTP[γS] was present. When $rG_{16\alpha}$ was extracted in the presence of AMF plus 200 μM

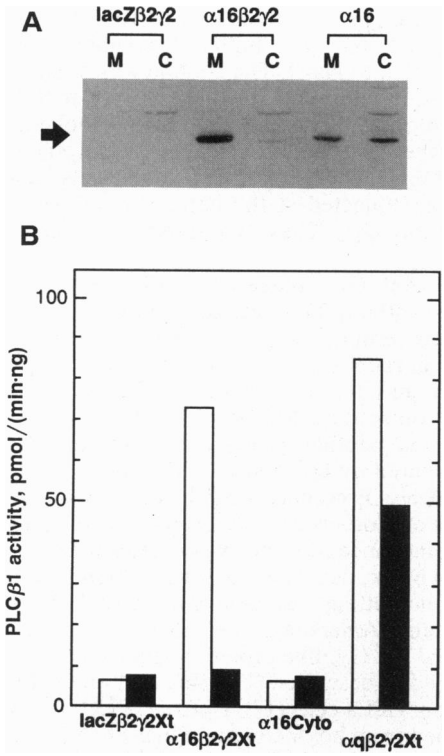

FIG. 1. Cellular location and activity of recombinant $G_{16\alpha}$ in Sf9 cells. Sf9 cells (50-ml culture) were infected with recombinant baculoviruses encoding β-galactosidase (*lacZ* gene product), β_2, and γ_2; $G_{16\alpha}$, β_2, and γ_2; $G_{q\alpha}$, β_2, and γ_2; or $G_{16\alpha}$. Membrane and cytosolic fractions were prepared as described under *Materials and Methods*. (*A*) Membrane (M) and cytosolic (C) fractions (10 μg of each) of lacZ$\beta_2\gamma_2$-, $G_{16\alpha}\beta_2\gamma_2$-, and $G_{16\alpha}$-expressing cells were subjected to SDS/polyacrylamide gel electrophoresis and analyzed by immunoblotting using anti-$G_{16\alpha}$ antiserum B861. Arrow indicates position of $rG_{16\alpha}$. (*B*) Membranes from cells expressing lacZ$\beta_2\gamma_2$, $G_{16\alpha}\beta_2\gamma_2$, and $G_{q\alpha}\beta_2\gamma_2$ were extracted (Xt) with 1% sodium cholate in the presence of 30 μM Al^{3+}/5 mM Mg^{2+}/10 mM F^- (AMF) plus 200 μM GDP. The cytosolic fraction (cyto) from $G_{16\alpha}$-expressing cells was similarly supplemented with sodium cholate, AMF, and GDP. Each fraction (5 μl) was assayed for capacity to stimulate bovine brain PLC-β1 (2.9 ng) in the presence of either 120 μM GTP[γS] (open bar) or AMF plus 200 μM GDP (filled bar). The fractions that were assayed with GTP[γS] were incubated with 1 mM GTP[γS] at 30°C for 30 min prior to assay.

GDP and held at 4°C, the capacity of the extract to activate PLC-β1 was lost after 24 hr (Fig. 2*A*). The activity of $rG_{q\alpha}$ was stable under the same conditions. In contrast, when we extracted $rG_{16\alpha}$ in the presence of 5 μM GTP[γS] and held the extract at 4°C for 24 hr, $G_{16\alpha}$ activity was undiminished (Fig. 2*B*); the activity of $rG_{16\alpha}$ was stable for at least 5 days under this condition. We attempted to stabilize nonactivated $rG_{16\alpha}$ in several ways, including addition of high concentrations of GDP (to 1 mM) and various concentrations of Mg^{2+} (to 50 mM). None was successful. We thus chose to purify GTP[γS]-activated $G_{16\alpha}$; the nucleotide was included in all buffers utilized for protein extraction and purification.

Purification of $rG_{16\alpha}$ from Sf9 Cells. We purified $rG_{16\alpha}$ from a membrane extract of 1.2×10^{10} Sf9 cells (8-liter culture) expressing $G_{16\alpha}\beta_2\gamma_2$ as described under *Materials and Methods*. Fig. 3*A* shows a silver-stained preparation of purified $G_{16\alpha}$ after SDS/polyacrylamide gel electrophoresis. The yield of $rG_{16\alpha}$ was 18 μg (Table 1).

The specificity of various antisera for $G_{16\alpha}$, Sf9 cell $G_{q\alpha}$-like protein(s), and $G_{q\alpha}$ is shown in Fig. 3*B*. Antiserum B861 recognizes $rG_{16\alpha}$ specifically and does not react with $rG_{q\alpha}$ or Sf9 cell $G_{q\alpha}$. Antiserum Z811 was raised against a 15-amino acid peptide representing the carboxyl-terminal sequence

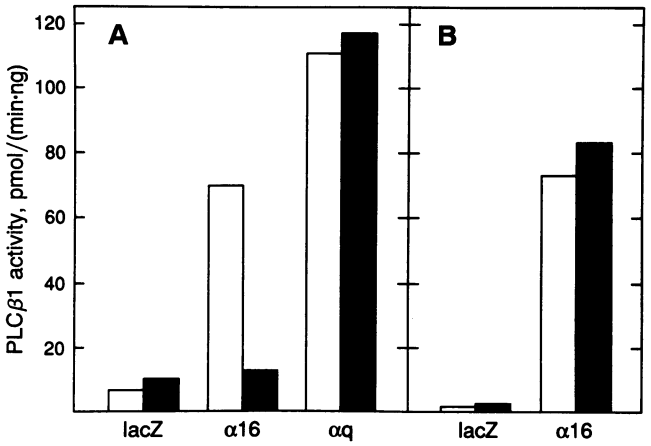

FIG. 2. Ability of AMF and GTP[γS] to stabilize recombinant $G_{16\alpha}$. (*A*) Membranes from cells expressing lacZ$\beta_2\gamma_2$ (lacZ), $G_{16\alpha}\beta_2\gamma_2$ (α_{16}), and $G_{q\alpha}\beta_2\gamma_2$ (α_q) were extracted with 1% sodium cholate in the presence of AMF plus 200 μM GDP. These extracts (5 μl) were assayed for their capacity to stimulate bovine brain PLC-β1 (2.9 ng) immediately after extraction (open bars) or after 24 hr at 4°C (filled bars). The extracts were incubated with 1 mM GTP[γS] at 30°C for 30 min prior to assay. The final concentration of GTP[γS] in the assay was 120 μM. (*B*) Membranes from cells expressing lacZ$\beta_2\gamma_2$ (lacZ) and $G_{16\alpha}\beta_2\gamma_2$ (α_{16}) were extracted with 1% sodium cholate in the presence of 5 μM GTP[γS]. These fractions (5 μl) were assayed for their capacity to stimulate bovine brain PLC-β1 (2.9 ng) immediately after extraction (open bars) or after 24 hr at 4°C (filled bars). The final concentration of GTP[γS] in the assay was 120 μM.

common to $G_{q\alpha}$ and $G_{11\alpha}$. $G_{16\alpha}$ has six identical amino acid residues within this peptide. Sf9 cell $G_{q\alpha}$ reacts with antiserum Z811, and $rG_{16\alpha}$ reacts weakly. $rG_{16\alpha}$ is not recognized by the $G_{q\alpha}$-specific antiserum W082.

Interactions of Purified $rG_{16\alpha}$ with PLC Isozymes. Both $rG_{q\alpha}$ and $rG_{11\alpha}$ stimulate all three isozymes of PLC-β (11). For comparison, $rG_{16\alpha}$ and $rG_{q\alpha}$ were reconstituted with rPLC-β1, rPLC-β2, and rat brain PLC-β3. $rG_{16\alpha}$ stimulated all three isozymes (Fig. 4), and the concentration–response curves for activation of PLC-β1 and PLC-β2 by $rG_{16\alpha}$ and $rG_{q\alpha}$ are superimposable. $rG_{16\alpha}$ appeared to be slightly less effective than $rG_{q\alpha}$ with PLC-β3. Similar to the situation described

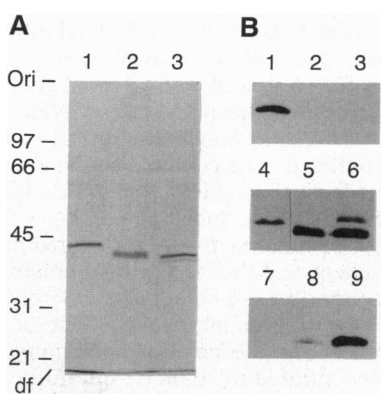

FIG. 3. SDS/polyacrylamide gel electrophoresis and immunoblot analysis of purified $G_{16\alpha}$, $G_{q\alpha}$, and endogenous Sf9 cell $G_{q\alpha}$-like protein(s). Purified $rG_{16\alpha}$, Sf9 cell $G_{q\alpha}$, and $rG_{q\alpha}$ (50 ng of each) were subjected to SDS/11% polyacrylamide gel electrophoresis. (*A*) Silver stain of the gel. Lane 1, $G_{16\alpha}$; lane 2, Sf9 $G_{q\alpha}$; lane 3, $G_{q\alpha}$. Scale at left shows the origin (Ori), positions of molecular weight markers ($M_r \times 10^{-3}$), and the dye front (df). (*B*) Immunoblots of $G_{16\alpha}$ (lanes 1, 4, and 7), Sf9 cell $G_{q\alpha}$ (lanes 2, 5, and 8), and $G_{q\alpha}$ (lanes 3, 6, and 9) with antisera B861 (lanes 1, 2, and 3), Z811 (lanes 4, 5, and 6), and W082 (lanes 7, 8, and 9). $rG_{q\alpha}$ is expressed as a doublet because of unexpectedly efficient reading of the altered polyhedron initiator codon upstream of the inserted $G_{q\alpha}$ sequence in pVL1393 (11).

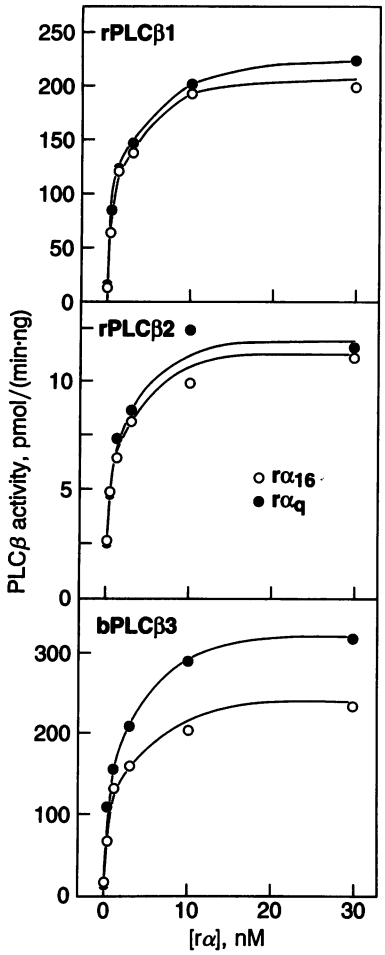

FIG. 4. Activation of PLC-β isoforms by $rG_{16\alpha}$ and $rG_{q\alpha}$. $rG_{16\alpha}$ (○) and $rG_{q\alpha}$ (●) were reconstituted with rPLC-β1 (1 ng) (*Top*), rPLC-β2 (8 ng) (*Middle*), or rat brain (b) PLC-β3 (0.2 ng) (*Bottom*). Reaction mixtures were incubated at 30°C for 5 min (PLC-β1) or 10 min (PLC-β2 and PLC-β3). $G_{q\alpha}$ was activated with 1 mM GTP[γS] for 1 hr at 30°C prior to assay. GTP[γS] was present in all assays at a final concentration of 120 μM.

previously with $rG_{q\alpha}$ and $rG_{11\alpha}$, $rG_{16\alpha}$ activated PLC-β1 and PLC-β3 to a much greater extent than was observed for PLC-β2. Under the assay conditions employed, the half-maximal concentrations of $rG_{16\alpha}$ required for activation of rPLC-β1, rPLC-β2, and PLC-β3 all appeared to be about 1 nM.

Several laboratories have demonstrated that the G-protein $\beta\gamma$ subunit complex can stimulate PLC-β isozyme activity (10, 11, 20–22). $rG_{16\alpha}$ did not change the sensitivity of PLC-β2 to stimulation by $\beta\gamma$ (Fig. 5); similar results were obtained with $rG_{q\alpha}$ and $rG_{11\alpha}$ (11).

$rG_{16\alpha}$ was also reconstituted with PLC-γ1 and PLC-δ1. $rG_{16\alpha}$ did not stimulate either of these phospholipases under the same conditions that were effective for PLC-β (Table 2). Both PLC-γ1 and PLC-δ1 were stimulated by high concentrations of Ca^{2+}. Similarly, $rG_{16\alpha}$ did not stimulate adenylylcyclase activity nor inhibit $G_{s\alpha}$-stimulated adenylylcyclase activity in S49 cyc^- cell membranes (data not shown).

DISCUSSION

$G_{16\alpha}$ protein was purified from a detergent extract of Sf9 cell membranes after infection of these cells with recombinant baculoviruses encoding $G_{16\alpha}$ and G-protein β_2 and γ_2 subunits. In contrast to the situation encountered with recombinant $G_{q\alpha}$ (11), $G_{16\alpha}$ did not aggregate, even when it was

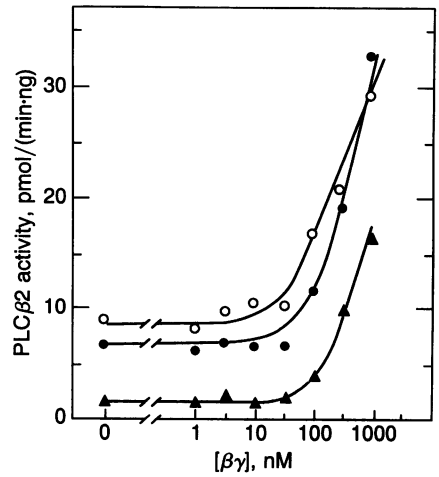

FIG. 5. Effect of $G_{16\alpha}$ and $G_{q\alpha}$ on activation of PLC-β2 by the G-protein $\beta\gamma$ subunit complex. Activation of rPLC-β2 (8 ng) by bovine brain $\beta\gamma$ was determined either in the absence (▲) or in the presence of 10 nM $G_{16\alpha}$ (○) or 10 nM $G_{q\alpha}$ (●). $G_{q\alpha}$ was activated with 1 mM GTP[γS] for 1 hr at 30°C prior to assay. GTP[γS] was present in all assays at a final concentration of 120 μM.

expressed without β and γ subunits. After extraction, membrane-bound recombinant $G_{16\alpha}$ was able to stimulate PLC-β1, whereas cytosolic $G_{16\alpha}$ was inactive. Thus, we expressed $G_{16\alpha}$ along with β_2 and γ_2 to increase the amount of $G_{16\alpha}$ in the membrane extract. Nevertheless, the yield was modest and only 18 μg of recombinant $G_{16\alpha}$ was purified from an 8-liter culture of Sf9 cells.

We do not understand the difference between the cytosolic and membrane-bound forms of $rG_{16\alpha}$. The cytosolic protein, unassociated with $\beta\gamma$, may denature quickly, perhaps because of rapid dissociation of GDP. Alternatively, cytosolic $G_{16\alpha}$ may lack some posttranslational modification necessary for activation of PLC-β. Concurrent expression of $\beta\gamma$ could be necessary for such modification, perhaps by facilitating translocation of $G_{16\alpha}$ to the membrane. The α subunits of most G proteins are now known to be palmitoylated (23). When expressed in Sf9 cells, membrane-bound forms of $G_{s\alpha}$, $G_{o\alpha}$, and $G_{q\alpha}$ are palmitoylated, but the cytosolic forms of these proteins are not. Membrane-bound $G_{16\alpha}$ is also labeled when Sf9 cells are incubated with [^{3}H]palmitate. It will be interesting to assess the effect of palmitoylation on the functions of $G_{16\alpha}$ and other G-protein α subunits.

We were not able to activate $G_{16\alpha}$ with AMF; this is unique among G-protein α subunits. Similarly, the combination of AMF and GDP failed to stabilize $G_{16\alpha}$ in detergent extracts of Sf9 cell membranes. AMF is thought to bind to the GDP-liganded form of G-protein α subunits, activating these proteins by mimicking the γ phosphate of GTP (24). The lack of effect of AMF on $G_{16\alpha}$ suggests that there may be a critical difference in the guanine nucleotide-binding domain of this protein. A highly conserved sequence, Gly-Ala-Gly-Glu-Ser-Gly-Lys-Ser, is found in the amino-terminal portion of the guanine nucleotide-binding domain of most G-protein α sub-

Table 2. Effect of $rG_{16\alpha}$ and $rG_{q\alpha}$ on PLC-γ1 and PLC-δ1

Addition	Activity, pmol/(min·ng)	
	PLC-γ1	PLC-δ1
None	9.9	2.2
30 nM $G_{16\alpha}$	9.2	1.6
30 nM $G_{q\alpha}$	9.0	3.1
30 nM Ca^{2+} (free)	4.2	2.3
100 μM Ca^{2+} (free)	12.3	53.3

units. The alanine residue in this sequence is replaced by threonine in members of the $G_{q\alpha}$ family other than $G_{15\alpha}/G_{16\alpha}$, and Ala-Gly-Glu is replaced by Thr-Ser-Asn in $G_{z\alpha}$. The guanine nucleotide-binding properties of $G_{q\alpha}$, $G_{11\alpha}$, and $G_{z\alpha}$ are known to differ substantially from those of other G-protein α subunits (11, 25). Perhaps the more drastic substitution of proline for alanine in $G_{16\alpha}$ is responsible for loss of activation of the protein by AMF. Purification of the nonactivated form of $G_{16\alpha}$ will be necessary to characterize the guanine nucleotide-binding properties of the protein.

$rG_{16\alpha}$ activated all three isoforms of PLC-β when the purified proteins were reconstituted. $G_{16\alpha}$ was not able to activate PLC-γ1, PLC-δ1, or adenylylcyclase. These data are generally consistent with previous experiments, wherein purified PLC isozymes were added to membranes from COS cells overexpressing $G_{16\alpha}$ (26, 27). However, we were unable to detect substantial differences between concentrations of $G_{16\alpha}$ and $G_{q\alpha}$ necessary to activate the PLC isoforms. Lee *et al.* (26) reported that $G_{16\alpha}$ was a more potent activator of PLC-β2 than was $G_{q\alpha}$ or $G_{11\alpha}$. This difference may be due to differences in assay conditions or to the method used to quantitate the amount of G_α protein.

There are many examples wherein different G-protein α subunits activate the same effector, including the capacity of $G_{q\alpha}$, $G_{11\alpha}$, and $G_{16\alpha}$, to activate PLC-β1, PLC-β2, and PLC-β3. The situation with $G_{16\alpha}$ appears unique, however, in that its amino acid sequence is substantially divergent from other members of the $G_{q\alpha}$ family. Of interest, $G_{15\alpha}$ and $G_{16\alpha}$ are now believed to represent mouse and human homologs of the same gene product (4), indicating that the sequence of this protein is much more variable among species than are the sequences of other G-protein α subunits. Perhaps there are other, unknown effectors that are regulated differentially by $G_{q\alpha}$, for example, and $G_{16\alpha}$. $G_{q\alpha}$ and $G_{16\alpha}$ may also interact with different sets of receptors. This possibility is probably enhanced by the fact that the distribution of $G_{16\alpha}$ is very restricted when compared with that of other members of the $G_{q\alpha}$ family.

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