Volume 313, number 3, 220–224 FEBS 11794 November 1992

Expression, characterization and purification of soluble G-protein $\beta\gamma$ dimers composed of defined subunits in baculovirus-infected insect cells

Alexander Dietrich[a], Michael Meister[a], Karsten Spicher[b], Günther Schultz[b], Montserrat Camps[a] and Peter Gierschik[a]

[a]*Abteilung Molekulare Pharmakologie, Deutsches Krebsforschungszentrum, Heidelberg, Germany,* and [b]*Institut für Pharmakologie der Freien Universität Berlin, Berlin, Germany*

Received 5 October 1992

Recombinant $\beta_1\gamma_2$ dimers of signal-transducing guanine nucleotide-binding proteins (G-proteins) carrying a mutation known to block isoprenylation of the γ_2 subunit were expressed as a soluble protein in baculovirus-infected insect cells. The soluble $\beta\gamma$ dimer was analyzed by sucrose density gradient centrifugation and purified to near homogeneity in the absence of detergents. The sedimentation velocity studies gave an $s_{20,w}$ value of 4.1 ± 0.4 S. The two subunits segregated as a dimer upon sucrose density gradient centrifugation and purification by sequential ion exchange and hydroxylapatite chromatography. The results show that baculovirus-infected insect cells can be employed for high level production of pure G-protein $\beta\gamma$ dimers suitable for functional and structural characterization.

G-protein; Signal transduction; Heterologous expression; Baculovirus; *Spodoptera frugiperda*; *Trichoplusia ni*

1. INTRODUCTION

Signal-transducing guanine nucleotide-binding proteins (G-proteins) couple an enormous variety of receptors to second messenger-generating effectors like adenylyl cyclase, ion channels or phospholipase C [1]. G-proteins are heterotrimeric proteins consisting of α, β, and γ subunits. All three subunits are members of large gene families [2]. The activated receptor interacts with the heterotrimeric G-protein and catalyzes the exchange of GTP for GDP bound to its α subunit. Activation of the GTP-bound form of the G-protein appears to coincide with its dissociation from the receptor as well as its own dissociation into a free α subunit and a tightly associated $\beta\gamma$ dimer. In certain cases, e.g. stimulation of various types of adenylyl cyclase or activation of the retinal cGMP phosphodiesterase, the α subunit alone is capable of effector regulation [3–5]. In other cases, however, free $\beta\gamma$ subunits may also be involved in controlling effector activity. Thus, $\beta\gamma$ dimers have been reported to stimulate phospholipase A_2 [6], open atrial muscarinic K^+ channels [7], inhibit type I adenylyl cyclase [4], facilitate the stimulation of type II and type IV adenylyl cyclase by activated α_s [4], regulate an unidentified effector moiety involved in the yeast pheromone response [8], and stimulate phospholipase C [9].

In order to study the regulation of effectors by, and to analyze the structure/function relationships, of $\beta\gamma$ dimers, it is highly desirable or even necessary to have access to sufficient quantities of $\beta\gamma$ dimers composed of defined subunits. In many cases, it is not possible to produce this material by conventional protein purification, because purified $\beta\gamma$ subunit preparations are usually complex mixtures composed of multiple β and/or γ subunits [10].

The baculovirus/insect cell expression system allows the large scale expression of properly folded and correctly processed mammalian proteins [11]. In addition, the simultaneous expression of multiple proteins or multisubunit polypeptides is feasible using this system [11]. These features prompted us to use baculovirus-infected insect cells to express recombinant $\beta\gamma$ dimers of defined composition. Here, we report the production and functional characterization of $\beta\gamma$ dimers composed of β_1 and of γ_2 subunits carrying a serine residue in place of the cysteine present in position −4 from the carboxyl terminus (γ_2C68S). This mutation was previously shown to preserve γ expression but block γ subunit isoprenylation and membrane attachment in cultured mammalian cells [12,13]. The recombinant $\beta_1\gamma_2$C68S subunit is soluble in aqueous buffers in the absence of detergents and is thus excellently suited to study the interaction of G-protein-regulated effectors with $\beta\gamma$ subunits.

Correspondence address: P. Gierschik, Molecular Pharmacology Division, German Cancer Research Center, Im Neuenheimer Feld 280/III, 6900 Heidelberg, Germany. Fax: (49) (6221) 423 352.

Abbreviations: G-protein, signal-transducing heterotrimeric guanine nucleotide-binding protein; Sf9 cells, *Spodoptera frugiperda* cells; AcNPV, *Autographa californica* nuclear polyhedrosis virus.

Published by Elsevier Science Publishers B.V.

2. MATERIALS AND METHODS

2.1. *Plasmid construction*

The cDNA of the γ_2 subunit was amplified from total RNA from bovine brain by reverse transcription and PCR using two synthetic oligonucleotides complementary to the 5′ and 3′ ends of the coding region and carrying a recognition site for *Bgl*II on either end. The downstream oligonucleotide encoded a serine residue instead of the cysteine present in position 68 of the wild-type γ_2 subunit [14]. The PCR product was ligated into pBluescript II SK− (Stratagene) as described [15] and sequenced. No difference between the expected and the actual DNA sequence was found. The cDNA was then cloned into the unique *Bgl*II site of the transplacement plasmid pAcUW51 (Pharmingen). The correct orientation of the insert to the p10 promoter was verified by DNA sequencing. The cDNA of the human β_1 subunit [16] was PCR amplified using two synthetic oligonucleotides complementary to the 5′ and 3′ ends of the coding region and carrying a recognition site for *Bam*HI on either end. The PCR product was ligated into pBluescript II SK− and sequenced. Except for a silent mutation in codon 232 (ATA to ATT), the DNA sequence of the PCR product was identical to the sequence published before [14] (see, however, [17]). The PCR product was ligated into the unique *Bam*HI site of the vector pAcUW51 already carrying the cDNA of γ_2C68S. The correct orientation of the insert to the polyhedrin promoter was verified by DNA sequencing.

2.2. *Production of recombinant baculovirus*

Recombinant baculoviruses were obtained by transfecting *Spodoptera frugiperda* cells (Sf9 cells, Invitrogen) with a 10:1 mixture of transfer vector and a modified baculovirus DNA (Baculogold, Pharmingen), which contains a lethal deletion and is rescued by the DNA of the transplacement plasmid. The recombinant baculovirus was detected by dot blot hybridization and amplified through 2 rounds of infection of 6×10^6 Sf9 cells, which resulted in a virus titer of 2×10^8/ml.

2.3. *Expression of recombinant $\beta\gamma$ subunits*

Trichoplusia ni 5B1-4 cells (High Five cells, Invitrogen) were maintained in Insect-Xpress protein-free medium (Whittaker) supplemented with 50 μg/ml of gentamycin (Sigma, G 1522) and 2.5 μg/ml amphotericin B (Fungizone, Gibco) at 27°C. For production of recombinant proteins, six 55 cm^2 culture dishes containing 6×10^6 cells each were incubated with recombinant baculovirus at a multiplicity of infection (MOI) of 5 in 2 ml of medium for 1 h at 27°C, followed by an incubation in 15 ml of fresh medium for 1–6 days. For production of larger quantities of recombinant proteins, Sf9 cells were infected with recombinant baculovirus at a MOI of 5 and maintained for 4 days at 27°C in suspension culture in TNM-FH medium (Sigma, T 1032) containing 10% fetal calf serum and the above supplements.

2.4. *Homogenization and fractionation of cells*

Cells were washed three times in ice-cold PBS (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.6 mM KCl, 136 mM NaCl, pH 7.2) scraped or suspended into lysis buffer (0.25 M sucrose, 20 mM Tris-HCl, pH 7.5, 1.5 mM $MgCl_2$, 1 mM ATP, 3 mM benzamidine, 1 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml soybean trypsin inhibitor) and lysed by nitrogen cavitation as described in [18], except that cells were pressurized with N_2 at 4 MPa for 30 min at 4°C. Removal of unbroken cells and nuclei and preparation of a crude membrane and a cytosolic fraction from the cavitate was performed as described [18,19]. Whole cell lysates were prepared by scraping the cells into ice-cold extraction buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 10 mM EGTA, 37 mM sodium cholate, 3 mM benzamidine, 43 mM 2-mercaptoethanol, and 100 μM phenylmethylsulfonyl fluoride), incubated for 1 h with gentle vortexing every 10 min, and then centrifuged at 15,000 × g for 20 min. The supernatant was snap-frozen in liquid N_2 and stored at −80°C.

2.5. *Sucrose density gradient centrifugation*

Linear gradients (4.8 ml) were prepared from 5 to 20% (w/w) sucrose in buffer A (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 3 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 2 μg/ml soybean trypsin inhibitor). 500 μl of the cytosolic fraction (30 mg protein) were combined with 200 μl buffer A containing a mixture of the calibrating proteins (catalase 11.3 S; lactate dehydrogenase 7.3 S; malate dehydrogenase 4.32 S; and cytochrome *c* 1.71 S [20]) and applied to the top of the gradient. Centrifugation was performed in a Beckman SW 50 rotor at 48,000 rpm for 16 h at 4°C. After centrifugation, the bottoms of the tubes were punctured and fractions of 6 drops each were collected. The calibrating proteins were assayed as described before [20].

2.6. *Purification of recombinant $\beta\gamma$ subunits*

Cytosolic extract (10 mg protein) was fractionated by anion-exchange chromatography using a Mono Q HR 5/5 column which had been equilibrated with 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol and 100 μM of phenylmethylsulfonyl fluoride (buffer A) at a flow rate of 0.5 ml/min. After application of the sample, the resin was washed with 5 ml of buffer B and eluted with a linear gradient (10 ml) of NaCl (0–500 mM) in buffer B followed by 5 ml of buffer B containing 500 mM NaCl. Fractions of 500 μl were collected and analyzed for $\beta\gamma$ subunits by SDS-PAGE and immunoblotting. A single peak containing recombinant $\beta\gamma$ subunits eluted at ≈135 mM NaCl. The peak fraction was applied to a column (0.5 × 4 cm) of hydroxylapatite (Calbiochem, HPLC grade) which had been equilibrated with buffer C (20 mM Tris-HCl, pH 7.5, 100 μM EDTA, 1 mM dithiothreitol and 100 μM of phenylmethylsulfonyl fluoride) at a flow rate of 0.2 ml/min. The resin was washed with 5 ml of buffer C and eluted with a linear gradient (10 ml) of K_2HPO_4/H_3PO_4, pH 7.5 (0–500 mM) in buffer C. Fractions of 250 μl were collected. A single peak containing recombinant $\beta\gamma$ subunits eluted at ≈15 mM K_2HPO_4/H_3PO_4.

2.7. *Miscellaneous*

SDS-PAGE and immunoblotting were performed as described [18], except that immunoreactive proteins were visualized using the Amersham ECL Western blotting detection system. In some cases, bipartite resolving gels were used containing 17% (w/v) and 12% (w/v) acrylamide in the lower and upper half, respectively, to improve the focussing of the γ_2 subunit [13]. A polyclonal antiserum reactive against the carboxyl terminus of β_1 (SW) [21] was obtained from Drs. William F. Simonds and Allen Spiegel. Antibody AS 292 was affinity purified from a serum raised in rabbits against a carboxyl terminal peptide of γ_2 (NH_2-SENPFREKKFFC-COOH) coupled to keyhole limpet hemocyanin. Protein was determined according to Bradford [22], using bovine IgG as standard.

3. RESULTS AND DISCUSSION

The strategy used in this study to express recombinant $\beta\gamma$ subunits was based on three considerations. First, we wanted to be sure that the two subunits are produced in the same cell at the same time. Second, we wished to produce recombinant $\beta\gamma$ subunits free of endogeneous $\beta\gamma$ subunits, which are reportedly present in non-infected insect cells [23]. Third, we wanted to generate $\beta\gamma$ subunits which are soluble in aqueous buffers to avoid potential artifacts which frequently arise when $\beta\gamma$ subunit regulation of effectors is analyzed using detergent-solubilized $\beta\gamma$ dimers purified from membraneous sources [24].

To achieve these objectives, we have replaced the cysteine present in position −4 from the carboxyl terminus of the γ_2 subunit with a serine by site-directed mutagenesis of the γ_2 cDNA, and have cloned both the

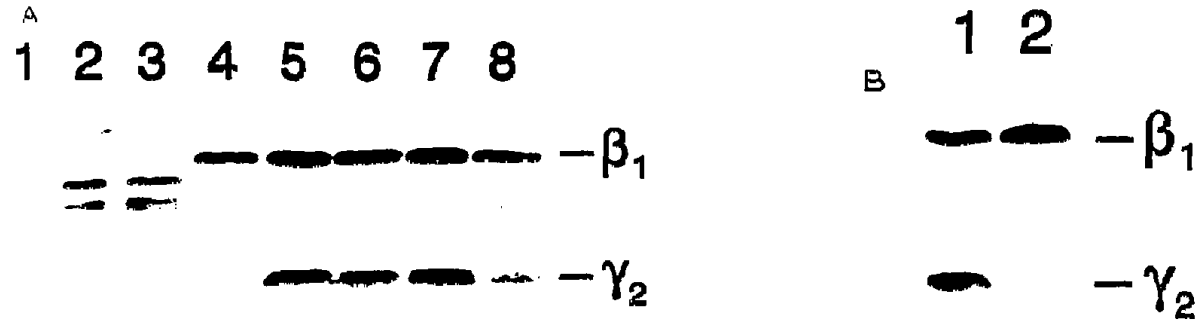

Fig. 1. Expression of recombinant β_1 and γ_2C68S in baculovirus-infected insect cells. (A) Time-course. High Five cells were infected with $\beta_1\gamma_2$C68S baculovirus and cell lysates were prepared after 1 (lane 3), 2 (lane 4), 3 (lane 5), 4 (lane 6), 5 (lane 7), and 6 days (lane 8) as described in section 2. Lysates of non-infected cells (lane 1) or cells infected with wild-type baculovirus for 4 days (lane 2) were analyzed for comparison. The lysates were subjected to SDS-PAGE (100 μg protein/lane) and immunoblotting was performed using an antiserum reactive against β_1 (upper panel) or affinity-purified antibodies reactive against γ_2 (lower panel). (B) Subcellular distribution. High Five cells were infected for 4 days with $\beta_1\gamma_2$C68S baculovirus, homogenized and fractionated into soluble (lane 1) and particulate (lane 2) fractions as described in section 2. Analysis of the samples by SDS-PAGE and immunoblotting was performed as described above. Only the ≈35 kDa and ≈6 kDa regions of the autoluminographs are shown.

mutated γ_2 cDNA and the β_1 cDNA into the baculovirus transfer vector pAcUW51. This vector is an AcNPV polyhedrin locus-based vector that contains a copy of the AcNPV p10 promoter and SV40 transcription termination signals inserted in tandem, upstream of the polyhedrin gene promoter, but in opposite orientation. The β_1 and γ_2C68S cDNAs were inserted into regions controlled by the polyhedrin and the p10 promoter, respectively. Both promoters are active during the very late phase of infection [11]. Thus, cells infected with recombinant baculoviruses carrying pAcUW51-borne foreign genes are expected to produce the corresponding polypeptides at the same time.

A time-course of the expression of he β_1 and the γ_2 subunit is shown in Fig. 1A. Neither the β nor the γ subunits were detected in detergent extracts of non-infected cells, in extracts of cells infected with wild-type baculovirus, or in extracts of cells infected with $\beta_1\gamma_2$C68S baculovirus on day 1 of the infection protocol. Synthesis of the β_1 and the γ_2C68S subunits was evident on day 2 and day 3, respectively, reached a plateau between day 3 and day 5, and decreased on day 6. Next, we determined the subcellular localization of the recombinant β_1 and γ_2C68S subunits in cells infected with $\beta_1\gamma_2$C68S baculovirus. The immunochemical analysis shown in Fig. 1B revealed that the particulate fraction contained only the β_1 subunit, whereas the soluble fraction contained both the β_1 and the γ_2C68S subunit. Neither of these subunits was detected by the antibodies used in this study in soluble or particulate fractions of non-infected cells or of cells infected with wild-type baculovirus (results not shown).

To investigate whether the co-expression of β_1 and γ_2C68S subunits led to an association of the two subunits, the soluble fraction of $\beta_1\gamma_2$C68S baculovirus-infected cells was subjected to sucrose density gradient centrifugation followed by immunochemical analysis of the fractions using antibodies reactive against β_1 and γ_2. Fig. 2A shows that β_1 and γ_2C68S were found in the same fractions of the gradient. The sedimentation coefficient $s_{20,w}$ of the two subunits was 4.1 ± 0.4 S (n = 3). Note that this value is considerably higher than the value obtained for γ_2C68S expressed in the absence of β_1 (≈0.7 S; Fig. 2B). Thus, the co-sedimentation of β_1 and γ_2C68S observed in Fig. 2A very likely reflects their dimerization and sedimentation as a tightly associated $\beta_1\gamma_2$C68S complex.

To examine the association of the β_1 and γ_2C68S subunits by more rigorous criteria, the recombinant subunits were purified from cytosolic fractions to near homogeneity by sequential ion exchange and hydroxylapatite chromatography. The immunochemical analysis of the column fractions obtained by the two chromatographic procedures showed that the β_1 and γ_2C68S subunits segregated as a dimer during purification (results not shown). Examination of the purified protein by SDS-PAGE revealed two major polypeptides with apparent molecular masses of approximately 35 and 6 kDa, which co-migrated with the native β_1 and γ_1 subunits, respectively, of retinal transducin (Fig. 3). Note that the two recombinant subunits stained with the same relative intensities as their native retinal counterparts, indicating that the subunit stoichiometry was the same (i.e. 1:1 [25]) for both $\beta\gamma$ dimers.

Our results demonstrate that a recombinant $\beta\gamma$ dimer carrying a mutation known to block isoprenylation of the γ subunit [12,13] can be expressed as a soluble protein in baculovirus-infected insect cells. The soluble $\beta\gamma$ dimer was analyzed by sucrose density gradient centrifugation and purified to near homogeneity in the absence of detergents. The sedimentation velocity studies gave an $s_{20,w}$ value which is similar to the values previously observed for $\beta\gamma_1$ in detergent-free gradients (3.9–4.15) [26]. Non-retinal native $\beta\gamma$ dimers are substantially

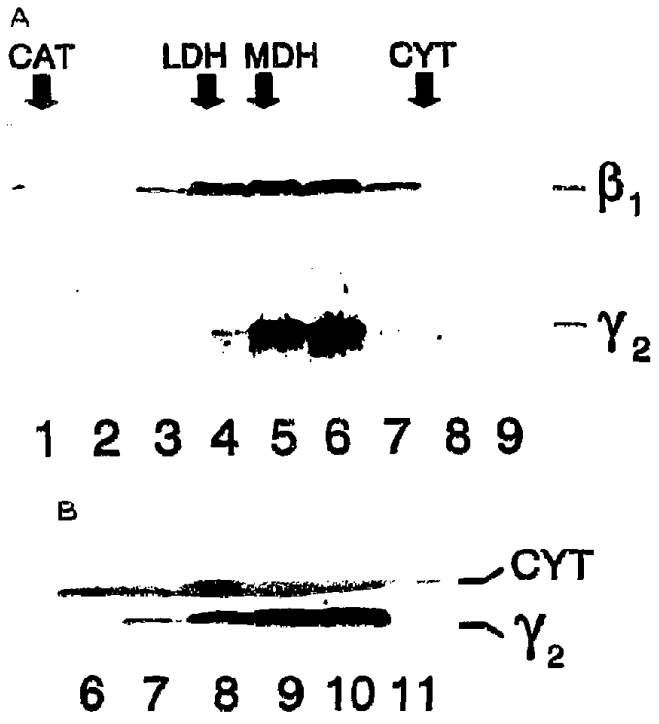

Fig. 2. Sucrose density gradient centrifugation of β_1 and γ_2C68S expressed in baculovirus-infected insect cells. Cytosolic preparations from High Five cells infected for 4 days with $\beta_1\gamma_2$C68S baculovirus (A) or γ_2C68S baculovirus (B) were subjected to sucrose density gradient centrifugation. Fractions were analyzed by SDS-PAGE and immunoblotting was performed using antibodies reactive against β_1 (A, upper panel) or γ_2 (A, lower panel, and B). The marker protein, cytochrome *c*, is visualized by non-specific staining in (B). The fraction numbers and the positions of the marker proteins catalase (CAT), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and cytochrome *c* (CYT) in the gradients are indicated.

more hydrophobic than $\beta\gamma_1$ and can be analyzed by sucrose density centrifugation only in the presence of detergents [27]. Some of these dimers bind large quantities of detergent [28]. It is thus not too surprising that their sedimentation behaviour is markedly dependent on the type of detergent used [29]. It is expected that production of soluble recombinant $\beta\gamma$ dimers will allow the precise determination of the hydrodynamic properties of this and other $\beta\gamma$ dimers without the influence of bound detergents.

The activity of several $\beta\gamma$ regulated effector moieties is highly sensitive to detergents [23,30,31]. In certain cases, the detergents present in non-retinal $\beta\gamma$ subunit preparations have been shown to severely compromise the functional analysis of the $\beta\gamma$–effector interaction (unpublished results and [23]). It is likely that the methodology outlined here will allow the production of sufficient quantities of purified, detergent-free $\beta\gamma$ subunits composed of various β and γ subunits to examine the specificity and efficacy of the $\beta\gamma$–effector interaction in considerable detail.

The availability of $\beta\gamma$ subunits lacking isoprenylation and, by inference, modification by carboxyl-terminal proteolysis and methylation, will also allow the examination of the role of these modifications in regulating the interaction of $\beta\gamma$ subunits with other transmembrane signaling components. Of interest, a retinal $\beta\gamma$ subunit lacking the carboxyl terminal isoprenylated cysteine residue has previously been shown to support the pertussis toxin-mediated ADP-ribosylation of the transducin α subunit only very poorly, indicating that the carboxyl terminal γ subunit modification is essential for the formation of the $\alpha\beta\gamma$ heterotrimer [32]. On the other hand, very recent results obtained by analyzing the interaction of rhodopsin kinase and β-adrenergic receptor kinase with the corresponding receptors are consistent with the notion that these receptors contain a docking site for carboxyl-terminally isoprenylated proteins [33,34]. This would indicate that γ subunit isoprenylation may be important for the receptor–$\beta\gamma$ subunit interaction.

While this work was in progress, Graber et al. [35] reported the expression of functional wild-type G-protein $\beta_1\gamma_1$ and $\beta_1\gamma_2$ subunits in baculovirus-infected insect cells. Detergent lysates of cells infected with the

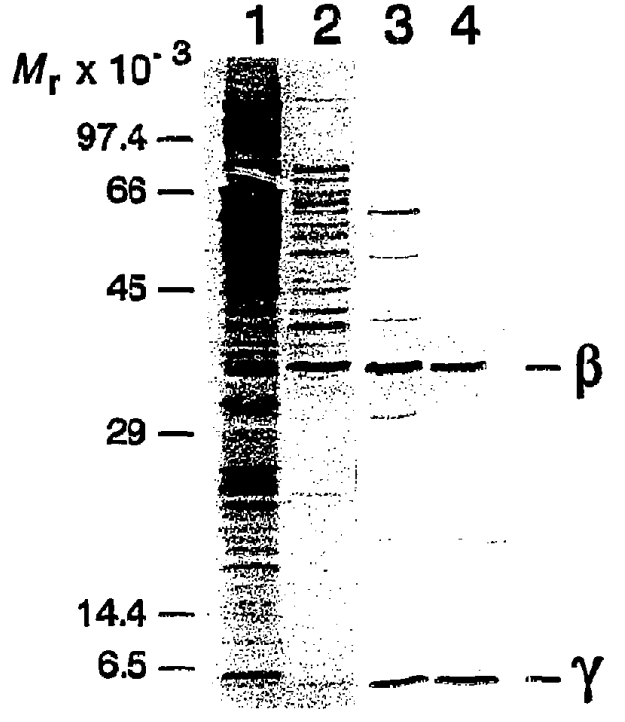

Fig. 3. Purification of $\beta_1\gamma_2$C68S. Sf9 cells were infected for 4 days with $\beta_1\gamma_2$C68S baculovirus. $\beta_1\gamma_2$C68S was purified from cytosolic fractions by sequential ion exchange and hydroxylapatite chromatography as described in section 2. Aliquots of the cytosolic fraction (lane 1), and the peak fractions obtained by chromatography on Mono Q (lane 2) and hydroxylapatite (lane 3) were subjected to SDS-PAGE. Purified $\beta\gamma_1$ was applied to the same gel for comparison (lane 4). Proteins were visualized by staining with Coomassie blue (lanes 1 and 2) or silver (lanes 3 and 4). The identities of β_1 and γ_2C68S in lane 3 were confirmed by immunoblotting (results not shown).

recombinant baculoviruses were capable of supporting the pertussis toxin-mediated ADP-ribosylation of exogeneous α_{i2}. However, no direct assessment of the $\beta\gamma$ dimer formation was provided and the $\beta\gamma$ subunits were not purified. Potential drawbacks of the approach used by these authors are the relatively high contamination (10–16%) of the recombinant $\beta\gamma$ dimers with endogeneous $\beta\gamma$ subunit activity and the fact that detergents were apparently required to solubilize functional $\beta\gamma$ subunits from the homogenate. Our observation that fractionation of soluble fractions from non- or mock-infected cells by Mono Q and hydroxylapatite chromatography did not yield $\beta\gamma$ subunits detectable by immunochemical means or by protein staining (not shown) strongly suggests that the purified preparation of $\beta_1\gamma_2$C68S characterized here is devoid of endogeneous $\beta\gamma$ dimers. Finally, using a single baculovirus encoding both the β_1 and γ_2 subunit (this study) rather than two separate viruses [36] is highly advantageous or even essential for large scale production of the $\beta\gamma$ dimer in cultured insect cells or reared insect larvae [11].

Acknowledgements: The expert technical assistance of Ulrike Schäfer is greatly appreciated. We wish to thank David Budiman for his cooperation, Drs. Juan Codina and Lutz Birnbaumer for providing the β_1 cDNA, and Drs. William F. Simonds and Allen Spiegel for their most generous gift of antiserum SW1. This work was supported by grants from the Deutsche Forschungsgemeinschaft.

REFERENCES

[1] Birnbaumer, L., Abramowitz, J. and Brown, A.M. (1990) Biochim. Biophys. Acta 1031, 163–224.
[2] Simon, M.I., Strathmann, M. and Gautam, N. (1991) Science 252, 802–808.
[3] Northup, J.K., Smigel, M.D., Sternweis, P.C. and Gilman, A.G. (1983) J. Biol. Chem. 258, 11369–11376.
[4] Tang, W.-J. and Gilman, A.G. (1991) Science 254, 1500–1503.
[5] Fung, B.K.-K., Hurley, J.B. and Stryer, L. (1981) Proc. Natl. Acad. Sci. USA 78, 152–156.
[6] Jelsema, C.L. and Axelrod, J. (1987) Proc. Natl. Acad. Sci. USA 84, 3623–3627.
[7] Logothetis, D.E., Kurachi, Y., Galper, J.B., Neer, E.J. and Clapham, D.E. (1987) Nature 325, 321–326.
[8] Kurjan, J. (1992) Annu. Rev. Biochem. 61, 1097–1129.
[9] Camps, M., Hou, C., Sidiropoulos, D., Stock, J.B., Jakobs, K.H. and Gierschik, P. (1992) Eur. J. Biochem. 206, 821–831.
[10] Tamir, H., Fawzi, A.B., Tamir, A., Evans, T. and Northup, J.K. (1991) Biochemistry 30, 3929–3936.
[11] O'Reilly, D.R., Miller, L.K. and Luckow, V.A. (1992) Baculovirus Expression Vectors: A Laboratory Manual, Freeman, New York.
[12] Simonds, W.F., Butrynski, J.E., Gautam, N., Unson, C.G. and Spiegel, A.M. (1991) J. Biol. Chem. 266, 5363–5366.
[13] Pronin, A.N. and Gautam, N. (1992) Proc. Natl. Acad. Sci. USA 89, 6220–6224.
[14] Gautam, N., Baetscher, M., Aebersold, R. and Simon, M.I. (1989) Science 244, 971–974.
[15] Marchuk, D., Drumm, M., Saulino, A. and Collins, F.S. (1991) Nucleic Acids Res. 19, 1154.
[16] Codina, J., Stengel, D., Woo, S.L.C. and Birnbaumer, L. (1986) FEBS Lett. 207, 187–192.
[17] Fong, H.K.W., Amatruda, T.T., Birren, B.W. and Simon, M.I. (1987) Proc. Natl. Acad. Sci. USA 84, 3792–3796.
[18] Gierschik, P., Sidiropoulos, D., Spiegel, A.M. and Jakobs, K.H. (1987) Eur. J. Biochem. 165, 185–1940.
[19] Gierschik, P., Steisslinger, M., Sidiropoulos, D., Herrmann, E. and Jakobs, K.H. (1989) Eur. J. Biochem. 183, 97–105.
[20] Haga, T., Haga, K. and Gilman, A.G. (1977) J. Biol. Chem. 252, 5776–5782.
[21] Murakami, T., Simonds, W.F. and Spiegel, A.M. (1992) Biochemistry 31, 2905–2911.
[22] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[23] Graber, S.G., Figler, R.A. and Garrison, J.C. (1992) J. Biol. Chem. 267, 1272–1278.
[24] Yatani, A., Okabe, K., Birnbaumer, L. and Brown, A.M. (1990) Am. J. Physiol. 258, H1507–H1514.
[25] Fung, B.K.-K. (1983) J. Biol. Chem. 258, 10495–10502.
[26] Wessling-Resnick, M. and Johnson, G.L. (1987) Biochemistry 26, 4316–4323.
[27] Sternweis, P.C. (1986) J. Biol. Chem. 261, 631–637.
[28] Neer, E.J. and Clapham, D.E. (1990) G Proteins (Iyengar, R. and Birnbaumer, L. eds.) pp. 41–61, Academic Press, San Diego.
[29] Neer, E.J., Pulsifer, L. and Wolf, L.G. (1988) J. Biol. Chem. 263, 8996–9000.
[30] Ross, E.M. (1981) J. Biol. Chem. 256, 1949–1953.
[31] Pind, S. and Kuksis, A. (1988) Biochim. Biophys. Acta 938, 211–221.
[32] Crooke, S.T. and Bennett, C.F. (1989) Cell Calcium 10, 309–323.
[33] Ohguro, H., Fukada, Y., Yoshizawa, T., Saito, T. and Akino, T. (1990) Biochem. Biophys. Res. Commun. 167, 1235–1241.
[34] Pitcher, J.A., Inglese, J., Higgins, J.B., Arriza, J.L., Casey, P.J., Kim C., Benovic, J.L., Kwatra, M.M., Caron, M.G. and Lefkowitz, R.J. (1992) Science 257, 1264–1267.
[35] Inglese, J., Koch, W.J., Caron, M.G. and Lefkowitz, R.J. (1992) Nature 359, 147–150.
[36] Graber, S.G., Figler, R.A., Kalman-Maltese, V.K., Robishaw, J.D. and Garrison, J.C. (1992) J. Biol. Chem. 267, 13123–13126.