G Protein–Effector Coupling: Binding of Rod Phosphodiesterase Inhibitory Subunit to Transducin†

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ABSTRACT: The cyclic GMP phosphodiesterase of retinal rods is composed of three distinct polypeptides: α (90 kDa), β (86 kDa), and γ (10 kDa). In this multimeric form, the enzyme is inhibited. Its activity is stimulated by the interaction with the GTP-bound form of the T_α subunit of transducin and reversed upon the recombination of the inhibitory γ subunit with the catalytic $\alpha\beta$ subunit. We show here by a novel coimmunoprecipitation technique that the γ subunit, but not the $\alpha\beta$ subunit, forms a 1:1 complex with T_α. The binding of γ to T_α is nucleotide-dependent and is facilitated by GTPγS or Gpp(NH)p. This study provides convincing evidence that the T_α–GTP subunit of transducin stimulates phosphodiesterase activity by binding to γ and physically carrying it away from $\alpha\beta$.

The regulation of effector enzymes by receptor-coupled G proteins is of major interest in understanding the mechanism of signal transduction. One of the best studied biological systems regulated by G proteins is the phototransduction process in vertebrate retinal rods, where the direct activation of a cGMP-specific phosphodiesterase (PDE)[1] by transducin (retinal G protein) has been conclusively demonstrated. In this system, photolyzed rhodopsin catalyzes the exchange of GTP for GDP bound to transducin, which, in turn, stimulates the phosphodiesterase activity in ROS. The reduction of the intracellular level of cGMP then leads to the closure of many cGMP-regulated cation channels in the plasma membrane, resulting in a decrease in Na^+ conductance and the hyperpolarization of the rod (Fung, 1986; Stryer, 1986; Hurley, 1987; Liebman et al., 1987).

The rod PDE is a peripheral membrane protein consisting of α (M_r = 90 000), β (M_r = 86 000), and γ (M_r = 10 000) polypeptides (Baehr et al., 1979). In this multimeric form, PDE is inhibited (Hurley & Stryer, 1982). The inhibition can be relieved by limited digestion, which selectively destroys the γ subunit (Hurley & Stryer, 1982), or alternatively, by the addition of an excess amount of the GTP-bound form of the T_α subunit of transducin (Fung et al., 1981; Wensel & Stryer, 1986). These findings strongly suggest that the $\alpha\beta$ subunit contains the catalytic domain and that the T_α–GTP complex activates PDE by releasing the inhibitory constraint exerted by γ. Whether or not T_α–GTP directly removes γ or interacts with $\alpha\beta$ to block the action of γ under physiological conditions, however, is not entirely clear. Sitaramayya et al. (1986) have reported a large difference in the K_m for cGMP hydrolysis between the trypsin-activated PDE and the transducin-activated PDE. They interpreted this finding as evidence that γ remains interacting with $\alpha\beta$ of activated PDE. We have also found that PDE can be coimmunoprecipitated together with T_α–GTPγS, which seems to show that transducin can interact with the holoenzyme (Navon & Fung, 1987). On the other hand, Yamazaki et al. (1983) have reported the elution of an inhibitory factor from frog ROS with Gpp(NH)p. Similarly, Deterre et al. (1986) have detected by ion-exchange chromatography a small population of the γ–T_α–GTPγS complex in a protein preparation extracted from the ROS membranes at low ionic strength. In the present paper, we resolve this controversy by studying in a reconstituted system the interaction between T_α–GTPγS and the purified PDE subunits. We show here that purified γ subunit can be readily coimmunoprecipitated with T_α–GTPγS using a monoclonal antibody directed against T_α. Our results provide conclusive evidence that transducin stimulates the phosphodiesterase activity by binding to the γ inhibitory subunit of PDE.

Experimental Procedures

Materials. Frozen bovine retinas were purchased from American Stores Packing Co., Lincoln, NE; Pansorbin was a product of Calbiochem; immobilized TPCK–trypsin was obtained from Pierce. ^{125}I protein A was prepared by iodination with chloramine T. The isolation of bovine ROS membranes and the preparation of the reconstituted membrane vesicles containing purified rhodopsin were performed according to the procedure of Hong and Hubbell (1973). Transducin was eluted from photolyzed ROS membranes with GTP and purified by column chromatography as previously described (Fung et al., 1981). Activated transducin was prepared by incubating the purified protein with GTPγS in the presence of a catalytic amount of photolyzed rhodopsin in reconstituted membrane vesicles (Fung, 1983). The properties of mAb TF16 were reported earlier (Navon & Fung, 1987, 1988). The compositions of the buffered solutions were as follows: buffer A (10 mM morpholinopropanesulfonic acid, pH 7.5, 2 mM $MgCl_2$, 1 mM DTT, 200 mM NaCl) and buffer B (20 mM Tris, pH 7.5, 0.5 M NaCl, 0.05% Tween 20).

Preparation of Phosphodiesterase Subunits. Rod PDE was extracted from photolyzed ROS membranes and purified by column chromatography according to procedures described previously (Baehr et al., 1979; Fung & Nash, 1983). To prepare trypsin-activated PDE containing only the $\alpha\beta$ polypeptides, purified PDE (2 mg/mL) in buffer A was allowed to pass slowly through a small column (0.15 mL) of immo-

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[1] Abbreviations: GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); Gpp(NH)p, guanosine 5′-(β,γ-imidotriphosphate); mAb, monoclonal antibody; ROS, rod outer segment; PDE, retinal rod cGMP phosphodiesterase; SDS, sodium dodecyl sulfate; TPCK, L-1-(tosylamino)-2-phenylethyl chloromethyl ketone; T_α and $T_{\beta\gamma}$, the α and $\beta\gamma$ subunits of transducin; α, β, and γ, subunits of phosphodiesterase.

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bilized TPCK–trypsin. By adjustment of the flow rate at which the PDE passed through the column, γ was selectively destroyed and removed (Hurley & Stryer, 1982). Those fractions containing the phosphodiesterase activity were pooled and stored frozen at −20 °C.

The inhibitory γ subunit was isolated according to a modified procedure of Hurley and Stryer (1982). Briefly, 15 mg of PDE isolated by DEAE-Sephadex column chromatography (Baehr et al., 1979) was concentrated by ultrafiltration (Amicon type YM-100) to 2 mL. This step removed proteins of mass less than 30 kDa from the PDE extract. The protein concentrate was then diluted to 2 mg/mL with buffer A and added dropwise to an equal volume of 0.2 M formic acid solution. The mixture was then heated at 70 °C for 15 min, allowed to cool to room temperature, and centrifuged at 13000*g* for 20 min to remove a small amount of protein aggregate. The clear supernatant, which contained approximately 85% PDE, was again concentrated to about 25 mg/mL by using a Centricon 10 microconcentrator (Amicon). To separate γ from $\alpha\beta$, the protein concentrate was chromatographed on a Bio-Gel P-100 column (0.6 × 75 cm) and eluted with 0.2 M formic acid at a flow rate of 2 mL/h. The peak fractions (0.4 mL) containing γ were pooled and stored in formic acid at 4 °C. γ remained active for several weeks under these conditions.

Two methods were used to calibrate the concentration of purified γ in a standard preparation. First, four samples of γ of known volume were subjected to quantitative amino acid analysis. Thirteen different sets of γ concentrations were obtained by dividing the amount of each amino acid determined from the amino acid analysis with the known number of residues in γ (Ovchinnikov et al., 1986). An average molar concentration of γ was then calculated. Second, the amount of cysteine in four γ samples of known volume was determined. Since γ contains only one cysteine residue, this analysis provides an independent and accurate measure of the molar concentration of γ. Essentially the same concentration of γ was obtained with these two methods in three independent analyses. The variations were less than 10% between the two methods and less than 15% between different analyses.

Preparation of Rabbit Antisera against PDE and γ. Peptide (Arg-Lys-Gly-Pro-Pro-Lys-Phe-Lys-Gln-Arg-Gln-Thr-Arg-Gln-Phe-Lys-Ser-Lys) with a sequence corresponding to residues 24–41 of γ (Ovchinnikov et al., 1986) was synthesized by the Merrifield solid-phase method at the Peptide Synthesis Facility of UCLA. The peptide was purified by gel filtration and then conjugated to keyhole limpet hemocyanin with glutaraldehyde. To produce antisera, rabbits were injected subcutaneously at multiple points with 0.5 mg of the peptide conjugate in complete Freund's adjuvant every 4 weeks. Antisera directed against PDE were produced by the same procedure. Approximately 0.5 mg of purified PDE was used per injection. The specificity of the antisera toward PDE was confirmed on immunoblot with resolved subunits.

Measurement of γ *Binding to* T_α *by Coimmunoprecipitation.* The coimmunoprecipitation of γ with T_α by mAb TF16 was performed according to a modified procedure of Navon and Fung (1987). As a precautionary step to avoid the formation of γ aggregates during prolonged incubation, an immunocomplex containing T_α attached to mAb TF16–Pansorbin was first prepared by the following procedure. Transducin or its GTPγS-bound form (0.25 mg/mL) was incubated with mAb TF16 (0.15 mg/mL) in 20 μL of buffer A for 30 min. Then 10 μL of Pansorbin was added, and the reaction was allowed to continue for an additional 1 h. The mixture was diluted with 1 mL of buffer B, pelleted by centrifugation, and redispersed in 10 μL of buffer B. To assay for binding, purified γ in 0.2 M formic acid was first brought to pH 7.5 with 0.2 M NaOH in buffer B. Immediately after the pH neutralization and centrifugation to remove a small amount of protein aggregates, 10 μL of γ diluted to various concentrations with buffer B was added to each aliquot of the T_α–TF16–Pansorbin suspension. After 5 min of incubation, the mixture was diluted with 1 mL of buffer B and the protein complex was pelleted by centrifugation. Bound proteins were then eluted from the pellet with 20 μL of electrophoresis sample buffer (Laemmli, 1970) by heating to 70 °C for 15 min. After centrifugation to remove the Pansorbin, the supernatant containing the extracted proteins was analyzed by SDS–polyacrylamide gel electrophoresis. To detect the presence of γ, the proteins were blotted onto nitrocellulose membranes, treated with antipeptide antisera, and detected with ^{125}I protein A.

Determination of Amount of Bound T_α *and* γ *in the Immunoprecipitates.* Quantitative immunoblot analysis was used to determine the amount of γ that was coimmunoprecipitated. Samples containing coimmunoprecipitated γ and known amounts of purified γ standards were processed on the same immunoblot and detected with ^{125}I protein A. The γ bands of the standards were excised and counted to generate a calibration curve for γ. The amount of protein present in each immunoprecipitate was then determined by comparing the radoactivity of the γ band with those in the calibration curve. This method was found to be accurate in the range of 0–400 ng of γ.

The amount of T_α bound to mAb TF16 was determined from the radioactivity in the immunoprecipitate using transducin–[^{35}S]GTPγS in the reaction mixture. In a typical experiment in which this information was required, four additional immunoprecipitates containing the bound T_α–[^{35}S]-GTPγS complex were prepared along with the samples. The amount of [^{35}S]GTPγS in each pellet was then determined by scintillation counting. The average radioactivity was then used to calculate the amount of T_α in the immunoprecipitates.

Analytical Methods. Phosphodiesterase activity was determined by measuring proton release due to cyclic GMP hydrolysis (Yee & Liebman, 1978). The activity of γ was measured by its ability to inhibit trypsin-activated PDE (Hurley & Stryer, 1982). Protein concentrations were routinely determined by the method of Coomassie blue binding (Bradford, 1976) with γ-globulin from Bio-Rad Laboratories as the standard. SDS–polyacrylamide gel (13%) electrophoresis was performed by the method of Laemmli (1970). Lysozyme (14 400), soybean trypsin inhibitor (21 500), carbonic anhydrase (31 000), ovalbumin (45 000), bovine serum albumin (68 000), and phosphorylase B (92 500) were used as molecular weight standards. Immunoblot analysis was carried out as described previously (Navon et al., 1986). Amino acid analysis was performed by the Protein Sequencing Facility at UCLA.

Results

Transducin Interacts with PDE through Binding to the γ *Subunit.* Although we have recently reported the coimmunoprecipitation of PDE with T_α–GTPγS (Navon & Fung, 1987), it is not clear from this result which PDE subunit is actually involved in binding to T_α–GTPγS. To answer this question, we repeated the experiment using either purified γ or $\alpha\beta$ generated by controlled proteolysis with trypsin. The SDS–polyacrylamide gel patterns of the proteins used in these experiments are shown in the left panel of Figure 1. To enhance the detection of γ, the protein bands were also visualized with a mixture of anti-PDE and anti-γ antisera (right

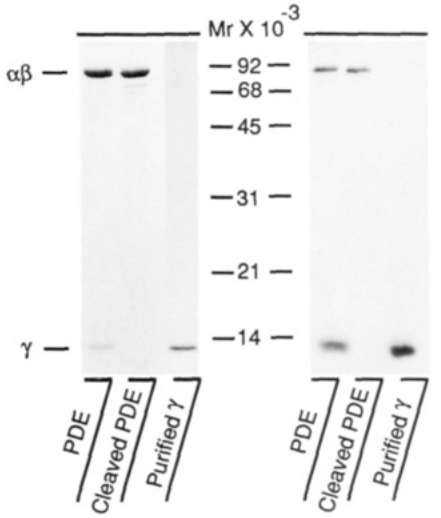

FIGURE 1: SDS–polyacrylamide gel electrophoresis analysis (left panel) and Western blot analysis (right panel) of PDE, trypsin-activated PDE, and purified γ. Proteins were detected by staining with Coomassie Blue. Immunoreactivity was identified with a mixture of anti-PDE and anti-γ antisera to enhance the detection of γ and visualized with ^{125}I protein A.

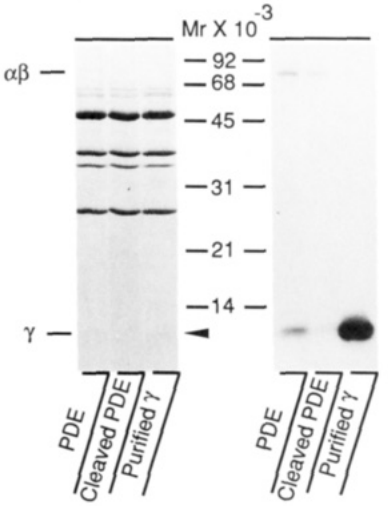

FIGURE 2: Coimmunoprecipitation of PDE, trypsin-activated PDE, and purified γ with T_α–GTPγS. PDE (32 μg), trypsin-activated PDE (32 μg), or γ (4.5 μg) was coimmunoprecipitated with T_α–GTPγS (8 μg) bound to the TF16–Pansorbin complex, washed, and eluted with 250 μL of sample buffer as described under Experimental Procedures. The eluents (80 μL) were analyzed by SDS–polyacrylamide gel electrophoresis and detected with Coomassie Blue staining (left panel). The same samples (40 μL) were blotted onto nitrocellulose paper and detected with a mixture of anti-PDE and anti-γ antisera. The arrow denotes the precipitated γ.

panel). As was reported previously (Hurley & Stryer, 1982), the αβ subunit of the trypsin-cleaved PDE remained intact, whereas γ was almost completely degraded. The trypsin-activated PDE generated by this procedure retained approximately 95% of the maximal phosphodiesterase activity, which is consistent with the exclusive degradation of the inhibitory γ subunit (data not shown). The purity of the γ subunit isolated in 0.2 M formic acid by gel filtration chromatography was approximately 95–98% (Figure 1). Purified γ can recombine with trypsin-activated PDE and totally inhibit its activity (data not shown).

Figure 2 shows the SDS–polyacrylamide gel and the Western blot of proteins eluted from the immunoprecipitates derived from incubating purified PDE, trypsin-activated PDE, and γ with the T_α–GTPγS–TF16–Pansorbin complex. As has

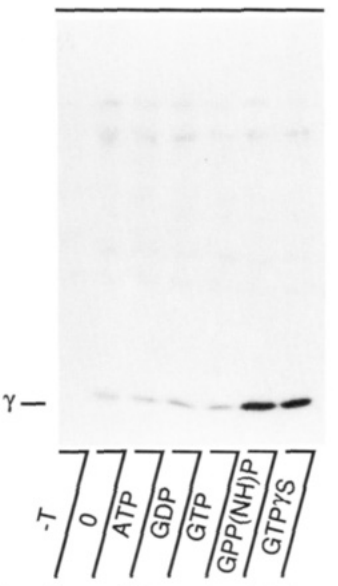

FIGURE 3: Nucleotide specificity of T_α binding to γ. Purified transducin (1 mg/mL) in buffer A containing 0.2 mg/mL photolyzed rhodopsin in the reconstituted membrane vesicle was preincubated with 0.25 mM amounts of various nucleotides for 3 h. After the membranes were removed by centrifugation, the supernatants were adjusted to 0.5 mg/mL and used to precipitate γ by the method described under Experimental Procedures. γ extracted from the immunoprecipitates were analyzed on Western blots with anti-γ antisera. Lane –T, γ immunoprecipitated with TF16–Pansorbin only.

been reported previously (Navon & Fung, 1987), a small amount of PDE can be brought down by the T_α–GTPγS–TF16 complex. The coimmunoprecipitated PDE was found to contain bound γ, indicating that it is the inhibited holoenzyme that interacts with T_α–GTPγS. We estimated from the PDE standards processed in the same blot that only 0.5 pmol of PDE was coimmunoprecipitated. This amount represented approximately 2% of the total PDE in the reaction mixture and 40-fold less than the amount of T_α–GTPγS in the immunoprecipitate. Hence, the binding of PDE to the T_α–GTPγS–TF16 complex must be very weak. After the γ subunit was removed by trypsin digestion, the amount of αβ coimmunoprecipitated with T_α–GTPγS under the same conditions further declined by aproximately 2.5-fold. Moreover, the densitometric scan of the autoradiogram indicated that at least 60% of the immunoprecipitated αβ contained bound γ. The most likely explanation of this result is that the T_α–GTPγS–TF16 complex did not bind αβ but selectively precipitated the 5% intact PDE still remaining in our trypsin-activated PDE preparation. In contrast, when purified γ was used in the same experiment, the molar amount of coimmunoprecipitated γ was 17 times higher than that of the PDE preparation. These results demonstrate that γ, but not αβ, binds to the T_α–GTPγS complex and strongly suggest that T_α–GTPγS interacts with PDE through binding to the γ subunit.

Nonhydrolyzable GTP Analogues Promote the Binding of γ to T_α. Previous studies have demonstrated that bound GTP or its nonhydrolyzable analogues facilitate the stimulation of the phosphodiesterase activity by tranducin (Fung et al., 1981). If the observed γ binding to T_α is a part of the PDE activation process, the formation of the T_α–γ complex should exhibit the same requirement for guanine nucleotides. Figure 3 shows the result of such an experiment in which transducin was preincubated with various nucleotides in the presence of photolyzed rhodopsin prior to the immunoprecipitation step. As shown in the Western blot of Figure 3, γ was not detected

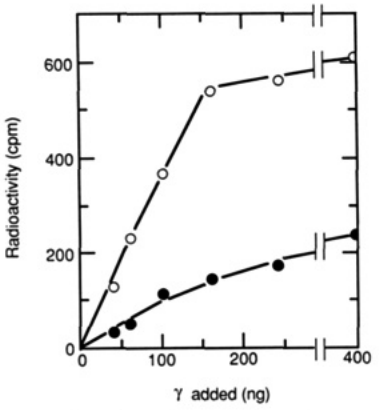

FIGURE 4: Concentration-dependent binding of γ to T_α–GTPγS (○) or T_α (●). Bound γ were quantified from the radioactivity of ^{125}I protein A of the immunoblot according to the method given under Experimental Procedures. The amounts of γ in the immunoprecipitates were determined from a standard curve. The radioactivity of 550 cpm at saturation corresponded to 15.5 pmol of bound γ in the immunoprecipitate. The amount of T_α–GTPγS in the immunoprecipitate, determined from the radioactivity of bound [^{35}S]GTPγS, was 14 pmol.

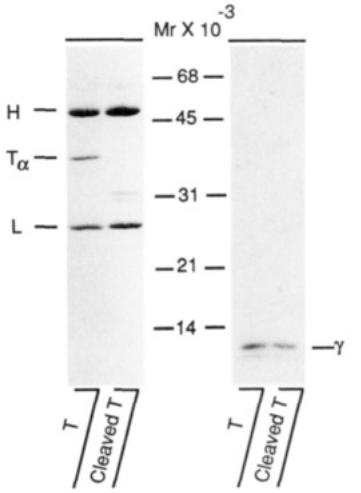

FIGURE 5: Coimmunoprecipitation of γ with the trypsin-cleaved T_α–GTPγS. Transducin–GTPγS (0.8 mg/mL) in buffer A was digested with TPCK–trypsin (0.02 mg/mL) at 22 °C for 3 h. After the addition of a 25-fold molar excess of trypsin inhibitor, the mixture was used in precipitating γ as described under Experimental Procedures. Proteins extracted from the immunoprecipitates were analyzed by SDS–polyacrylamide gel electrophoresis and detected with Coomassie Blue staining (left panel). The same samples were blotted onto nitrocellulose paper and detected with anti-γ antisera (right panel).

in the immunoprecipitate in the absence of T_α. A small amount of γ, however, was found to coimmunoprecipitate with T_α or T_α preincubated with ATP, GDP, and GTP. Since the bound GTP was hydrolyzed by the intrinsic GTPase activity associated with T_α during the period of immunoprecipitation, it is not surprising to find very little difference between the amounts of precipitated γ in the T_α–GTP and the T_α–GDP samples. In the presence of bound Gpp(NH)p or GTPγS, however, there was a 4-fold increase in the amount of coimmunoprecipitated γ. This result indicates that the bound nonhydrolyzable analogues of GTP, and most likely bound GTP, markedly enhance the T_α–γ interaction.

We have also tested the effect of a number of chemical reagents, ions, and synthetic peptides on the binding of γ to T_α–GTPγS. The binding was found not to be affected by the addition of EDTA (5 mM), $CaCl_2$ (5 mM), cGMP (2 mM), LiCl (10 mM), or synthetic peptides (0.5 mg/mL) with amino acid sequences corresponding to residues 2–16, 24–41, 44–54, and 73–87 of γ.

T_α–GTPγS Binds a Stoichiometric Amount of γ. Figure 4 shows the binding of γ to T_α–GTPγS and T_α as a function of the amount of added γ. The amount of γ in the immunoprecipitates was determined from the radioactivity of ^{125}I protein A by quantitative Western blot analysis. The radioactivity associated with the binding of γ to T_α–GTPγS was 3–4-fold higher than to T_α at all levels of added γ, in agreement with the result shown in Figure 3. Furthermore, the amount of γ bound to T_α–GTPγS increased almost linearly with increasing amounts of added γ and saturated at approximately 150 ng. The stoichiometry of γ to T_α–GTPγS at saturation can be obtained from the amounts of γ and T_α in the immunoprecipitates determined from a standard curve of γ and from the amount of bound GTPγS, respectively (see Experimental Procedures). At a saturated amount of added γ, we estimated there were 15.5 pmol of bound γ and 14 pmol of T_α in the immunoprecipitate, which gave a molar ratio of γ:T_α of 1:1.1. This result indicates that each T_α–GTPγS complex can bind one molecule of γ.

Cleaved T_α–GTPγS Retains Its Ability to Bind γ. To further define the γ binding domain of T_α, the GTPγS-bound form of transducin was subjected to limited proteolysis with trypsin prior to assaying for γ binding. We (Fung & Nash, 1983) and others (Hurley et al., 1984) have previously shown that prolonged proteolysis of transducin–Gpp(NH)p with trypsin produces a mixture of 32- and 31-kDa fragments, which retains the bound Gpp(NH)p (Fung & Nash, 1983). These fragments are immunoreactive to mAb TF16 (Navon & Fung, 1988). Consistent with this finding, the SDS–polyacrylamide gel pattern of the trypsin-digested transducin in the immunoprecipitate shows a mixture of 32- and 31-kDa fragments (left panel of Figure 5). When the corresponding immunoblot was analyzed with anti-γ antibody, almost an equal amount of γ was found to be coimmunoprecipitated with either the cleaved T_α or the intact T_α (right panel of Figure 5). This result indicates that trypsin-cleaved T_α–GTPγS is capable of interacting with γ.

Discussion

PDE in the dark-adapted ROS is inhibited, but can be activated by the addition of the T_α–Gpp(NH)p complex (Fung et al., 1981). These findings, together with the observed inhibitory effect of γ, have led to the initial proposal that transducin activates PDE by relieving an inhibitory constraint exerted by γ (Hurley & Stryer, 1982). Recently Wensel and Stryer (1986) have demonstrated that γ also reverses the activating effect of transducin and thus provides an additional support for a central role for γ in the regulation of phosphodiesterase activity. Exactly how T_α–GTP stimulates the phosphodiesterase activity under physiological conditions, however, is not entirely clear. Three possible mechanisms of activation can be envisaged. First, T_α–GTP might interact with $\alpha\beta$ to release the inhibitory γ subunit. Since T_α–GTPγS does not bind to $\alpha\beta$ (Figure 2), this possibility can be eliminated. Second, T_α–GTP might activate PDE by binding to the holoenzyme, altering the interaction between γ and $\alpha\beta$. At first sight, the result of the coimmunoprecipitation of PDE with T_α–GTPγS (Navon & Fung, 1987) appears to be compatible with this mechanism. However, in view of our present finding that T_α–GTPγS binds tightly to γ, the coimmunoprecipitation of the weakly bound PDE by T_α–GTPγS is most likely a result of its association through γ. This interpretation is further supported by the detection of a stoichiometric

amount of γ associated with $\alpha\beta$ in the PDE immunoprecipitate, indicating that the immunoprecipitated PDE is most probably inhibited. Consistent with our result is the report that γ reverses the stimulatory effect of T_α–GTPγS, which argues against the activation of PDE by a simple alteration of the interaction between γ and $\alpha\beta$ (Wensel & Stryer, 1986). Third, another possible mechanism is that T_α–GTPγS binds to and removes the γ subunit from the holoenzyme, leaving an active $\alpha\beta$ subunit. This is the mechanism that we favor and is also most consistent with our finding that γ and T_α–GTPγS form a tight complex. This mechanism is also in agreement with the results of many laboratories. For example, Deterre et al. (1986) have detected by ion-exchange chromatography a small population of the γ–T_α–GTPγS complex in a protein preparation extracted from ROS at low ionic strength. Yamazaki et al. (1983) have also reported the elution of an inhibitory factor from frog ROS into the supernatant with Gpp(NH)p. Similar results were obtained by Wensel and Stryer (1986) using bovine ROS membranes, except that γ is found to be associated with the membranes. The combined results of these studies provide a convincing argument for the formation and release of the γ–T_α–GTPγS complex from $\alpha\beta$ as a most probable mechanism of PDE activation.

We would like to emphasize that the release of γ with T_α–GTP from PDE is unlikely to be the only step involved in the activation of PDE in ROS under physiological conditions. Other factors might be involved for the following reasons. In a reconstituted system consisting of purified PDE and urea-stripped ROS membranes, the activation of PDE requires micromolar concentrations of transducin (Fung et al., 1981) but is inhibited with nanomolar concentrations of γ (Wensel & Stryer, 1986). Hence, the equilibrium must highly favor the formation of inhibited PDE. This finding is in agreement with a very low dissociation constant in the range of 0.005–0.13 nM for γ binding to trypsin-activated PDE (Wensel & Stryer, 1986; Hurley & Stryer, 1982). We have also observed that the phosphodiesterase activity of trypsin-activated PDE was rapidly quenched by the addition of the preformed γ–T_α–GTPγS complex, indicating that γ was readily released from T_α–GTPγS to recombine with $\alpha\beta$ (unpublished observation). If the affinity of γ for $\alpha\beta$ is so much higher than for T_α–GTP, how does transducin activate PDE in the rod? One possible explanation, as proposed by Wensel and Stryer (1986), is the existence of two populations of transducin molecules, with one form of transducin having a higher affinity for γ. Another possibility is the presence of an unknown factor in ROS membranes that can either interact with the activated $\alpha\beta$ catalytic subunit to lower its affinity for γ or, alternatively, stabilize the γ–T_α–GTP complex by increasing the affinity of γ for T_α–GTP. In either case, the addition of this factor would shift the equilibrium more in favor of the formation of the γ–T_α–GTPγS complex. Circumstantial evidence has suggested that one such factor is in the membranes. As we have shown previously (Fung & Nash, 1983), PDE in solution is activated only to a limited extent by T_α–Gpp(NH)p but can become fully activated with the addition of reconstituted membrane vesicles containing rhodopsin. One possible explanation of this interesting observation would be the formation of a stable γ–T_α–GTP complex at the charged membrane surface. This hypothesis is consistent with the work of Wensel and Stryer (1986), which shows that the activation of PDE by T_α–GTPγS does not release γ from the membranes. It also explains why a much higher concentration of γ is required to fully inhibit light-activated PDE than trypsin-activated PDE (Sitaramayya et al., 1986), since most of the γ added to the photolyzed ROS membranes will be stably bound to T_α–GTP and will be unavailable for inhibiting the activated PDE. Along the same line of thinking, the absence of membranes may lower the affinity of γ for T_α–GTP and hinder the release of the γ–T_α–GTP complex from the activated PDE. This would explain why in solution T_α–GTPγS is much less effective in activating PDE and forms a weakly associated complex with PDE while binding to γ. Additional experiments in the future, however, will be needed to establish firmly the existence of this factor in the membranes and to shed more light on its role in stabilizing the activated PDE.

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