Size Changes of Phosphodiesterase in Bovine Rod Outer Segments on Illumination†

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ABSTRACT: Light activates a 3′,5′-cyclic GMP phosphodiesterase (PDE) in bovine retinal rod outer segments. The light is absorbed by rhodopsin situated in the disk membranes. PDE is a three-subunit peripheral protein on the disks and appears to be activated via a guanine nucleotide binding protein (G) in the presence of activated rhodopsin and GTP. Does the activation occur by collision coupling of G and PDE? We have studied the protein–protein interactions of PDE in situ in disk membranes by radiation inactivation. Irradiation of a protein with high-energy electrons leads to loss of activity in proportion to radiation dose and the molecular weight of the protein. We see no change in the size of PDE upon activation by light and 100 μM guanosine 5′-(β,γ-imidotriphosphate) (Gpp[NH]p) compared with PDE in dark with 260 μM GTP. Application of statistics to our data shows that a 27 000 change in molecular weight would be significant at the 95% level but that smaller changes would go undetected. The apparent molecular weight is 176 000 ± 27 000 (mean ± 95% confidence limit), in agreement with the size determined by polyacrylamide gel electrophoresis. Thus there appears to be either (i) no permanent change in PDE size on activation or (ii) a small change, undetectable by the technique, or (iii) an exchange of subunits such that no net change in molecular weight is seen.

Retinal rod cells are responsible for black and white vision and are sensitive over 8 orders of magnitude of light intensity (Dowling & Ripps, 1972). The primary action of light upon rod outer segments (ROS)[1] is to bleach the protein rhodopsin in the disk membrane (Ostroy, 1977). This triggers an amplification pathway which eventually causes a decrease in the Na^+ permeability (Tomita, 1970; Hagins, 1972; Yau et al., 1981) of the spatially distinct (Cohen, 1968) plasma membrane [for brief reviews, see Bownds (1981) and Stryer et al. (1981)]. The exact nature of this exquisitely sensitive and adaptable second messenger system is not known. An increase in cytoplasmic free calcium (Hagins & Yoshikami, 1974) or a decrease in cyclic GMP (Woodruff et al., 1977; Liebman & Pugh, 1979) has been proposed as the primary light transducing signal. However, it appears that interactions of several second messengers are needed to expain all the phenomena (Hermolin et al., 1982). A cyclic GMP phosphodiesterase (PDE) on the disk membrane can be activated by bleached rhodopsin (R*) (Miki et al., 1975; Yee & Liebman, 1978; Pober & Bitensky, 1979). This causes a drop in cytoplasmic cyclic GMP, and this in turn could shut Na^+ channels (Miller & Nicol, 1979).

Many groups have demonstrated that the activation of PDE by bleached rhodopsin requires the presence of GTP (Yee & Liebman, 1978). A guanine nucleotide binding protein (G) is required for PDE activation, and a number of studies (Fung & Stryer, 1980; Godchaux & Zimmerman, 1979) have shown that one direct effect of R* is to cause exchange of GTP for bound GDP on the G-protein. This activated G·GTP complex has been proposed to activate PDE through collision coupling (Liebman & Pugh, 1980; Fung et al., 1981), but this has not been experimentally tested in situ. Hydrolysis of GTP leads to inactivation of PDE (Liebman & Pugh, 1980) which can be prevented by use of the nonhydrolyzable GTP analogue guanosine 5′-(β,γ-imidotriphosphate) (Gpp[NH]p) (Wheeler & Bitensky, 1977).

Does G·GTP bind directly to PDE to activate it and its release lead to inactivation? We decided to investigate this possibility by using radiation inactivation of PDE in situ on the disk membrane to examine its protein–protein interactions under various conditions.

Radiation inactivation (Kempner & Schlegel, 1979) assumes that the inactivation of a multisubunit protein by a dose of high-energy electrons will be proportional to the size of that protein complex. One incident electron on any part of the complex will inactivate the whole complex. Thus, if the complex size of a protein changes (e.g., G binds to PDE), the sensitivity to radiation will change in proportion.

This technique has been used in many systems to determine molecular weights (Kempner & Schlegel, 1979; Martin et al., 1979; Levinson & Ellory, 1974; Kepner & Macey, 1968), and in the majority of cases these have corresponded to those found by other methods. However, care must be taken in assigning absolute molecular weights and the technique is best suited to examining changes in size of complexes of protein subunits.

The G-protein has three subunits of M_r 40 000, 37 000, and 6000 (Godchaux & Zimmerman, 1979; Wheeler et al., 1977; Kuhn, 1980; Baehr et al., 1982), the largest carrying the guanine nucleotide binding site (Fung et al., 1981). The PDE may also have three subunits, of M_r 88 000, 85 000, and 11 000 (Baehr et al., 1979; Fung et al., 1981; Kohnken et al., 1981), in bovine ROS although other authors obtain different figures for frog ROS (Miki et al., 1975). Thus, in a whole cell a large number of interactions are, in principle, possible. The simplest interaction is binding of G to PDE upon light activation which should increase the PDE complex size from 184 000 to 267 000.

However, Fung et al. (1981) have shown that the M_r 40 000 subunit of G, when irreversibly activated with Gpp[NH]p, will activate PDE in the absence of other G subunits. So other interactions by binding of stimulatory or dissociation of in-

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[1] Abbreviations: ROS, rod outer segments; PDE, 3′,5′-cyclic GMP phosphodiesterase; R, rhodopsin; G, guanine nucleotide binding protein; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; Gpp[NH]p, guanosine 5′-(β,γ-imidotriphosphate); GDPβS, guanosine 5′-(β-thiodiphosphate); $NaDodSO_4$, sodium dodecyl sulfate.

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hibitory subunits from PDE are possible.

We report that activation of PDE by light and Gpp[NH]p or light and GTP does not change the complex size of PDE compared with dark and GTP or dark and GDPβS within the accuracy of the technique. The PDE size is measured as 176000 ± 27000 (mean ± 95% confidence limit).

Experimental Procedures

Materials. ^{3}H-Labeled cyclic GMP was from Amersham, GDPβS was a gift from Aviva Tolkovsky, and all other chemicals were from Sigma or Aldrich Chemical Co. and of analytical grade. Fresh bovine eyes were obtained from Garnham's, Ltd., Cambridge, England.

Rod Outer Segment Preparation. Rod outer segments were prepared by a modified method of Schnetkamp et al. (1979). Retinas dissected from dark-adapted bovine eyes under dim red light (Kodak Wratten Series 1 filter) were shaken in ice-cold 45% w/v sucrose, 10 mM glucose, 1 mM $CaCl_2$, 1 mM dithiothreitol, and 20 mM Hepes, pH 7.4, (buffer 1). Fragments of retinas were removed with a 0.5-mm mesh sieve. The suspension was centrifuged for 5 min at 3000*g*, and the supernatant was diluted 3-fold with buffer 1 without sucrose. This suspension was spun again at 3000*g* for 5 min, and the pellet formed was resuspended in ice-cold 15% w/v sucrose, 5% Ficoll 400, 1 mM dithiothreitol, 10 mM glucose, and 20 mM Hepes, pH 7.4 (buffer 2), and applied to a linear density gradient of 15 mL of buffer 2 and 15 mL of 20% w/v sucrose, 16% Ficoll 400, and 1 mM dithiothreitol at about 20 retinas per gradient. These gradients were spun at 4 °C in a Beckman SW27 rotor at 25000 rpm (40000–100000*g*) for 1 h. The main rhodopsin containing band was collected, diluted 2-fold in buffer 1 without sucrose, and spun for 8 min at 9000*g*. The resulting pellet was resuspended in buffer 1 with 15% w/v sucrose and stored at 0–4 °C for not more than 24 h before use. ROS were examined by light and electron microscopy and found to consist of fragments of cells containing many disks.

Rhodopsin Spectral Assays. Rhodopsin in the preparations was assayed in 0.1% cetyltrimethylammonium bromide, 12% w/v sucrose, 8 mM glucose, 0.8 mM $CaCl_2$, and 16 mM Hepes, pH 7.4, with 10 μL of ROS suspension/mL by using a Perkin-Elmer 557 double-beam spectrophotometer. $A_{280/500}$ was routinely 2.0–2.5, and rhodopsin stock containing 1–3 nmol of rhodopsin/retina was calculated by using a molar extinction coefficient of 40600.

Radiation Inactivation. Radiation inactivation is a physical technique for examining the size of multisubunit proteins in intact membranes. A sample is irradiated with high-energy electrons from a linear accelerator. Any protein which is hit by an electron loses its biological activity due to the large (66 eV) energy transfer. When this loss of activity is measured at different radiation doses, a simple, empirically determined relationship gives the apparent molecular weight of the functional unit. The method is particularly good for looking at changes in the functional interactions of proteins in situ in membranes under different experimental conditions.

The probability of a protein being hit by an electron from the accelerator is proportional to the size of the protein and the radiation dose. Kepner & Macey (1968) determined an empirical relationship between dose and molecular weight. This relationship relies on four reasonable assumptions: (a) electrons give discrete, highly localized ionizations; (b) one hit inactivates a whole protein complex through energy transfer between closely approximated subunits; (c) primary events (hits) are randomly distributed with respect to the electron density of the sample; (d) there are no effects due to the diffusion of free radicals formed by the radiation.

These considerations lead to the expression that

$$M_r = 6.55 \times 10^{11}/D_{37}$$

where D_{37} is the dose in rads required to leave 37% of the activity found in the unirradiated samples.

The strategy of experiments using this technique is that ROS are preincubated under the desired test conditions, rapidly frozen, freeze-dried, irradiated, resuspended, and assayed for remaining PDE activity. A detailed description is given sequentially in the following three sections.

(*1*) *Preparation of Samples for Irradiation.* ROS were preincubated with conditions and treatments as indicated in figure legends. Small samples (3 nmol of rhodopsin) were rapidly frozen in liquid nitrogen and freeze-dried in the dark at 0.03 millibar for 12–18 h. Complete freeze-drying is essential for accurate results.

Freeze-dried samples were sealed under nitrogen or argon in order to minimize the effects of free radicals and kept in the dark throughout irradiation. Human red blood cell membranes were freeze-dried in parallel, gassed, and placed in dark containers close to ROS samples during irradiation to act as calibration as described below.

(*2*) *Irradiation Procedure.* Samples were placed in the beam of 15-MeV electrons from a Phillips MEL SL75-20 linear accelerator at the Department of Radiotherapeutics, New Addenbrooke's Hospital, Cambridge, England. Precooled sample tubes were kept cool during irradiation by a continuous stream of dry ice cooled air. To avoid heating effects no more than 5 Mrad was given to any sample without a cooling period. In practice the technique is best suited to the study of large proteins and to large changes in complex size owing to the difficulty of precise calibration of the various doses. In order to calibrate dose as accurately as possible three procedures were used. (a) The beam was calibrated periodically by using the Perspex optical density method of Berry & Marshall (1969). (b) The dose rate was 2 Mrad/min and the dose was determined by an integrated current flow to earth from the aluminum sample support block. (c) The calibration of radiation dose as measured in (b) was checked on each day's experiment by using freeze-dried human red blood cell membranes. Irradiation of the acetylcholinesterase in these membranes has been shown to give an apparent molecular weight of 75000 (Levinson & Ellory, 1974). Our values had a mean of 74400. If the size determined by any individual experiment differed from 75000 by more than 15%, the experiment was discarded. This occurred once in seven experiments. Some inaccuracies of dose calibration involving inhomogeneities of the irradiating field or of sample freeze-drying have been overcome in a recent modification of the technique by Lo et al. (1982).

(*3*) *Preparation of Samples for PDE Assay.* After irradiation, samples were resuspended thoroughly in deionized water to the same volume as was present before freeze-drying and then assayed for remaining PDE activity. It is crucial to activate fully both light and dark preincubated samples so that all the PDE remaining in each is available to the assay. This was done by having 260 μM GTP and fluorescent light present throughout all the assays.

Other proteins are involved in the activation of PDE (R and G), and so inactivation of these components by radiation could lead to artifacts. In our system this is unlikely for two reasons. First, the G-protein is present in at least 6-fold excess over PDE (Kuhn, 1980) and R in about 100-fold excess (Baehr et al., 1979) so that even a large percentage of inactivation of these proteins would still leave sufficient present for full PDE

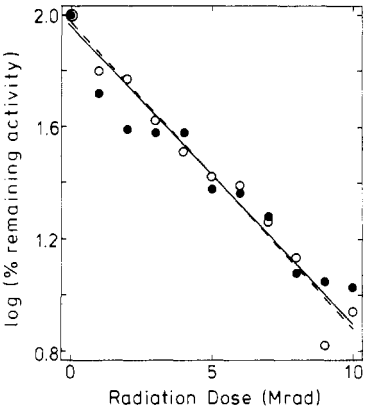

FIGURE 1: Radiation inactivation of PDE in light and dark. Samples preincubated with 260 μM GTP in the dark (closed symbols) or with 100 μM Gpp[NH]p after a 5–10% rhodopsin bleach (open symbols) were lyophilized, irradiated, and assayed for remaining PDE activity as described under Experimental Procedures. Preincubation was in 150 mM Tris-HCl, pH 8.0, at a rhodopsin concentration of 5–50 μM. No effect of varying rhodopsin concentration was observed in the results. After irradiation, resuspended ROS were added at an amount equivalent to 0.15–0.87 nmol of unirradiated rhodopsin to 0.4 mL of assay medium containing 2.0–2.5 mM cyclic GMP.

activation. Second, the PDE, as measured by $NaDodSO_4$–polyacrylamide gel electrophoresis (Baehr et al., 1979; Kohnken et al., 1981), is larger than either G or R (180000:90000:37000, respectively) and so would be expected to be inactivated more rapidly than them if all were free in the membrane.

Phosphodiesterase Assay. This was by a modified method of Thompson & Appleman (1971) in which [^{3}H]GMP formed by PDE is converted to guanosine and separated from unhydrolyzed cyclic GMP by ion-exchange chromatography. Samples were incubated in 150 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, [^{3}H]guanosine cyclic 3′,5′-monophosphate (150000 cpm) at the concentration specified in the figure legends, 250 μM GTP, and 0.03% v/v β-mercaptoethanol at 30 °C for 2 min under the conditions in the figure legends. Sample addition was used to start the assay in a 0.4-mL final volume in glass tubes to facilitate rapid temperature equilibration. Tubes were transferred to a boiling water bath for 1 min followed by freezing saline for 1 min. After addition of 0.1 mL of 1 mg/mL *Ophiophagus hannah* venom (5′-nucleotidase) samples were incubated for 15 min at 30 °C. Methanol (1 mL) was added to each tube on ice, and the whole sample was transferred to 0.6 × 1.5 cm Dowex 1-X8-400 columns and washed with a further 1 mL of methanol into 10 mL of scintillant containing 4 g of 2,5-diphenyloxazole, 10 mg of 1,4-bis(5-phenyloxazol-2-yl)benzene, 0.33 L of Triton X-100, and 0.67 L of toluene per L for liquid scintillation counting.

Treatment of Data. The data in Figure 1 are from four separate experiments. Each estimate was calculated relative to the mean zero dose PDE activity for that experiment and condition, and then all estimates at any individual dose were averaged to give the points shown. Not all points have the same number of estimates averaged into them. The lines drawn are linear regression fits to all the data.

Results

Figure 1 shows the results of irradiation of freeze-dried ROS PDE after preincubation under two conditions designed to compare the size of PDE in the dark, inactive state and in a fully activated light state. The dark state had GTP present to simulate the situation in vivo, while in the light Gpp[NH]p, a nonhydrolyzable GTP analogue, was used to ensure full activation of the PDE (Wheeler & Bitensky, 1977).

The plot of log (percent remaining activity of PDE) against dose yields, in both light and dark, a straight line of negative gradient passing close to 100% at zero dose as predicted by theory. The lines correspond to a PDE complex size of 177000 in the dark and 175000 in the light. The average 100% PDE activity (i.e., that in the unirradiated samples) was 0.30 μmol of cyclic GMP min^{-1} (nmol of rhodopsin)$^{-1}$ for the light condition and 0.26 μmol of cyclic GMP min^{-1} (nmol of rhodopsin)$^{-1}$ for the dark condition. The same experiment was performed with the nonphosphorylatable GDP analogue GDPβS in order to fix the PDE in the most inactive state obtainable. GDPβS has been shown by Kuhn et al. (1981) to compete with GTP for the G-protein and thus reduce the possibility of G activating PDE. No difference in complex size was seen compared to that of the light with Gpp[NH]p condition. We have also irradiated in the absence of any added guanine nucleotides and again find no significant change in the complex size (results not shown).

Controls were performed on each freeze-dried preparation to show that the assay system responded linearly to the amount of PDE added at the cyclic GMP concentrataion used.

A statistical treatment of the data which are incorporated in Figure 1 showed that a 27000 change in complex size could be expected to be seen at a 95% confidence level. This value was calculated by using a test of similarity of gradient of pairs of lines from individual days and then an F test of significance of difference in the mean slopes under the two conditions using days as blocks over all experiments.

Controls must be performed to check that after freeze-drying and resuspension the PDE activity and properties do not alter. Figure 2A shows that neither in the light nor in the dark state is total activity of the PDE decreased by this treatment. Three conclusions can be drawn from this figure: (i) the activities in both freeze-dried ROS after resuspension and those stored frozen are closely parallel, indicating that freeze-drying does not inactivate PDE, (ii) the assay system gives a linear response to the amount of PDE added and hence is suitable for measuring the amount of PDE remaining after irradiation, and (iii) the actual activity of the light and Gpp[NH]p treated PDE in these samples was 0.76 μmol of cyclic GMP min^{-1} (nmol of rhodopsin)$^{-1}$. In the dark the PDE activity was 0.15 μmol of cyclic GMP min^{-1} (nmol of rhodopsin)$^{-1}$. Thus, in this experiment a 5-fold activation of PDE by light and Gpp[NH]p is seen. The average activation obtained was 6.8-fold on the preparations used in Figure 1.

Figure 2B shows an experiment to demonstrate that the light-sensitive properties of PDE have not changed after freeze-drying. Over the range of PDE activities and rhodopsin concentrations used in these experiments, it can be seen that (i) the light-preincubated samples have the same activity whether assayed in the light or dark, indicating that PDE is probably in its active form when irradiated, and (ii) the dark samples are still fully activatable by light present during the assay so the freeze-drying has not selectively damaged the PDE in the dark state. When assayed in light the PDE given a dark preincubation is slightly less active than the light-preincubated PDE. This may be due to the presence of Gpp[NH]p in the latter which will prevent GTP hydrolysis and so maintain 100% active PDE. This phenomenon is also seen in the PDE activity of the unirradiated samples in Figure 1 in which the average zero dose PDE activity is 15% greater with the light than the dark preincubation (see above).

We estimate that the *maximum* temperature the samples could reach in our cold-air cooling system is 60 °C over the

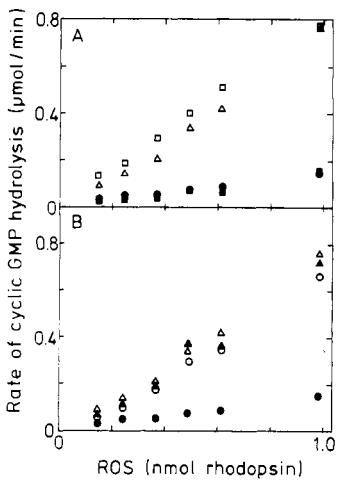

FIGURE 2: No effect of freeze-drying on PDE. In (A) ROS were preincubated in 150 mM Tris-HCl, pH 8.0, and 100 μM Gpp[NH]p for 2 min after a flash sufficient to bleach 5–10% rhodopsin (open symbols) or in 260 μM GTP in the same buffer in the dark for 2 min (closed symbols). Each suspension was divided into two parts and frozen in liquid nitrogen. One part was lyophilized and resuspended in the dark and then assayed for PDE activity at various rhodopsin concentrations (open triangles and closed circles). The other part was treated in the same way except that it was not lyophilized but stored in the dark at −20 °C before thawing and assaying (squares). The light preincubated samples were assayed in room light while the dark preincubated ones were assayed under dim red light as described under Experimental Procedures in the presence of 5 mM cyclic GMP. In (B) ROS were preincubated with 260 μM GTP in the dark (circles) or with 100 μM Gpp[NH]p after a 5–10% bleach (triangles) in 150 mM Tris-HCl, pH 8.0, and lyophilized as described under Experimental Procedures in the presence of 5 mM cyclic GMP. After resuspension samples were assayed for PDE activity at various rhodopsin concentrations in either light (open symbols) or dark (closed symbols). The open triangles and closed circles are the same data as in (A) replotted for comparative purposes. Points are means of duplicates from a single experiment.

2.5-min maximum irradiation period. However, the actual temperature is more likely to be around 30 °C. We have checked that heating of freeze-dried samples to 70 °C for 3 min had no effect on PDE activity (results not shown) and thus the inactivation of PDE in our cooled system is very unlikely to be due to heat inactivation.

Discussion

The results shown here indicate that there is no observable change in the size of the cyclic GMP PDE complex of bovine retinal rod outer segments whether in the dark or light-activated state. The apparent molecular weight of the complex is around 176 000 under both conditions. This agrees well with the size as measured by other groups (Baehr et al., 1979; Fung et al., 1981; Kohnken et al., 1981) using $NaDodSO_4$–polyacrylamide gel electrophoresis.

We suggest six possible interpretations of this result. (a) There is in fact no change in the interaction of the PDE protein complex with other proteins during its activation. (b) A transient interaction of PDE with other proteins (probably G-protein) serves to activate it, but this interaction rapidly reverses without GTP hydrolysis, leaving active PDE. (c) One set of subunits exchanges with another, leading to an undetectable net size change. (d) A small size change does occur in the PDE, but this is below the resolution limit of the technique, i.e., the binding or removal of a small polypeptide chain (M_r less than 27 000). (e) The rapid freezing process causes a reversal of an activating interaction of G·GTP with PDE, leading to an artifactual appearance of no change. (f) This system is one of the minority of cases in which, as discussed by Kempner & Schlegel (1979), the technique does not pick up an interaction which is occurring due to poor energy transfer between subunits of a complex.

How do these possibilities fit in with the observations of others? Miki et al. (1975) have shown that a diffusible transmitter of the light activation can cause activation of PDE on disks in which no rhodopsin has been bleached. This transmitter has been confirmed as the G·GTP complex which is known to be formed by the action of R* on G·GDP (Fung & Stryer, 1980). Fung et al. (1981) have since shown that the isolated 40 000 subunit of G, when bound to Gpp[NH]p, can activate PDE on dark-adapted disks. Whether this is a direct effect of the 40 000 subunit alone or whether it acts by recombining with inactive G-protein on the dark-adapted disks is not clear. A model has been proposed by Fung et al. (1981) and Liebman & Pugh (1981) in which the activated G·GTP complex binds to PDE, causing its activation. This is followed by GTP hydrolysis on this complex, leading to G·GDP·PDE separation and concomitant loss of PDE activity. This model would lead to a large increase in molecular weight on light activation of PDE. This is not consistent with our observations, assuming possibilities e and f are not interfering.

A problem with our work is the possibility e that during freezing of samples prior to freeze-drying the low temperature causes reversible separation of a G·GTP·PDE interaction which does in fact occur. This phenomenon has not been observed with other membrane proteins [e.g., adenylate cyclase (Martin et al., 1979)] but would be increased in likelihood if G·GTP·PDE interactions were weak. Such a weak interaction would mean that all the G-protein would be required for full PDE activation. Our data preclude a weak binding equilibrium between PDE and the 6–10-fold excess of G·GTP leading to formation of an active G·GTP·PDE complex. This would show up as an increased sensitivity to radiation due to inactivation of G-protein (the concentration of which would now be critical in the final assay). However, we see a PDE size remarkably close to that estimated by $NaDodSO_4$–polyacrylamide gel electrophoresis and hence inconsistent with a very weak interaction.

In addition Kohnken et al. (1981) have recently isolated PDE in a complex with G-protein after a series of hypo- and hypertonic washes, indicating that under some conditions PDE·G interactions can be strong. Indeed, interpretation c may be correct as this would fit with the observation of Kohnken et al. (1981) that both a PDE·G complex and PDE alone have similar sizes as measured by nondenaturing techniques.

Work by Hurley et al. (1981) and Yamazaki et al. (1982) has indicated that a small heat-stable polypeptide can act to inhibit the PDE. Recent data of Hurley & Stryer (1982) show that removal of a small (11 000) subunit from PDE can activate the remaining two subunits.

Bitensky et al. (1981) have proposed a model of PDE activation in which G·GTP binding to PDE causes release of the inhibitory polypeptide, giving active PDE. G·GTP *may* then dissociate, leaving PDE in the active form, until the free inhibitory polypeptide rebinds to inactivate it. Our interpretation b is consistent with this possibility so long as the major form of active PDE is free and not bound to G·GTP.

Hurley & Stryer (1982) also propose that G·GTP alters the interaction of the inhibitory polypeptide with PDE. Whether G·GTP simply causes an alteration of the interaction of the inhibitory subunit with the PDE or whether G·GTP, binding to the inhibitory subunit, removes it completely from the PDE is not known. However, our data suggest that it is not even

binding of the 40 000 subunit of G to the PDE. Rather, the statistical treatment shows that if there is any change it must be under 27 000. This suggests that G·GTP or the 40 000 subunit–GTP complex could cause removal of the inhibitory 11 000 subunit from the rest of the PDE (as proposed in interpretation d above) and thereby promote activation of cyclic GMP hydrolysis. So, if this interpretation is correct, the important question now is whether the inhibitory polypeptide is simply released free or whether it is removed from PDE by binding directly to G·GTP.

Thus, although not resolving the mechanism of activation of PDE by R*, the results presented here preclude a number of suggested mechanisms and indicate where efforts should be directed in future. Clearly, the radiation inactivation technique could be extended to examine the G-protein and its interactions with both R* and PDE under different light and GTP conditions.

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