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Light- and Nucleotide-Dependent Binding of Phosphodiesterase to Rod Disk Membranes: Correlation with Light-Scattering Changes and Vesicle Aggregation†

Antonio Caretta‡,§ and Peter J. Stein*,‡

Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut 06510, and Istituto di Fisiologia Umana, Universita di Parma, Parma, Italy

Received October 11, 1985; Revised Manuscript Received December 17, 1985

ABSTRACT: Under conditions in which large guanosine cyclic 3′,5′-phosphate (cGMP)- and phosphodiesterase (PDE)-dependent changes in near-infrared transmission and vesicle aggregation and disaggregation occur, we have observed a striking change in the binding of PDE to rod disk membranes. The change in PDE binding is nucleotide and light dependent as are the light-scattering changes. The cGMP- and PDE-dependent light-scattering signal can be produced by a 500-nm light flash which bleaches $1/(1 \times 10^7)$ rhodopsin molecules. Mg ions are an essential cofactor for the nucleotide-dependent PDE binding and light-scattering changes. 3-Isobutyl-1-methylxanthine and other competitive inhibitors of PDE hydrolytic activity support increased PDE binding to the disk membrane, vesicle aggregation, and the light-scattering signal. However, treatments which block GTP-dependent activation of PDE hydrolytic activity (colchicine, GDP, or ethylenediaminetetraacetic acid) also block these phenomena. Thus, GTP-dependent activation of PDE rather than its hydrolytic activity appears to be correlated with the light-scattering signal.

Many near-infrared light-scattering signals have been reported to occur in rod outer segment membrane preparations in vitro (Hofmann et al., 1976; Uhl et al., 1978, 1979a,b; Bignetti et al., 1980; Kuhn et al., 1981; Vuong et al., 1984; Borys et al., 1983; Thacher, 1983; Lewis et al., 1983; Caretta & Stein, 1985; Kamps et al., 1985). Bignetti et al. reported a light-dependent decrease in turbidity of rod outer segment membrane suspensions in the presence of extrinsic membrane proteins and GTP. The binding and dissociation signals reported by Kuhn et al. (1981) and the P signal reported by Hofmann et al. (1976) were proposed to be related to the binding and guanine nucleotide dependent release of the G protein[1] from disk membranes. A larger infrared scattering signal (G+) also related to the presence of GTP and extrinsic membrane proteins has been reported by Lewis et al. (1983).

In a previous paper (Caretta & Stein, 1985), we described changes in near-infrared light scattering of rod outer segment (ROS) membranes dependent on bleached rhodopsin, G protein, PDE, GTP, and cGMP. Under the same conditions, disk vesicle–disk vesicle aggregation–disaggregation can be observed. We have continued our investigation of the cGMP- and PDE-dependent light-scattering change in order to identify the molecular events responsible for the light-scattering changes and the aggregation–disaggregation phenomenon. We have examined the partitioning of extrinsic membrane proteins between the membrane and supernatant fraction during light-scattering experiments. PDE binding to the membrane increases during the membrane aggregation (decreased infrared light transmission) phase, and PDE binding is reduced during the membrane disaggregation phase (increased infrared light transmission). Our results indicate that PDE undergoes a light- and nucleotide-dependent binding to the membranes which appears to correlate with both the aggregation dynamics of disk vesicles and the concurrent light-scattering changes.

Materials and Methods

Membranes were prepared as previously described (Caretta & Stein, 1985). Fresh calf eyes were dissected under dim red light (Kodak wratten filter 1), and the retinas were shaken

† This work was supported by National Institutes of Health Grant EY 05074-01A1 (P.J.S.), by AFOSR Grant 84-NL-024 (P.J.S.), by Research to Prevent Blindness, Inc., Grant EY00085, and by the Connecticut Lions.

‡ Yale University School of Medicine.
§ Universita di Parma.

[1] Abbreviations: G protein, three polypeptide subunits of M_r 39 000, 37 000, and 6000 which comprise the GTPase; PDE, three polypeptide subunits of 94, 92, and 13 kilodaltons which comprise phosphodiesterase; cGMP, guanosine cyclic 3′,5′-phosphate; 8Br-cGMP, 8-bromoguanosine cyclic 3′,5′-phosphate; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); DTT, dithiothreitol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; IBMX, 3-isobutyl-1-methylxanthine; ROS, rod outer segment(s); SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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in 40% sucrose containing 100 mM Tris-HCl, pH 7.7, 1 mM $MgCl_2$, and 5 mM DTT (2 mL/retina) at 4 °C and then centrifuged for 1 h at 100000*g*. The rod disks floated and were collected from the sucrose–buffer interface with a syringe. The membranes were washed 2 times with 100 mM Tris-HCl, pH 7.7 (250 μL/retina), 1 mM $MgCl_2$, and 5 mM DTT. The G protein and PDE were extracted according to the procedures described by Kuhn (1980). No further purification of PDE was made. The G protein was dialyzed free of GTP and concentrated on an Amicon CF-25 filter to a concentration of 10–15 μM. Enzymes and membranes were stored at 0 °C in 5 mM Tris-HCl (ph 7.9) and 5 mM DTT and used within 72 h.

Light-scattering measurements (transmission changes) were made as previously described (Caretta & Stein, 1985) using a Shimadzu UV-3000 recording spectrophotometer. Measuring light was at 710 nm (unless otherwise indicated); the phototube was at 0° (±5.5°) transmission angle. The optical path length was 1 cm. The apparent optical density of the sample was 0.4–0.5 ODU.

To determine the light sensitivity of the scattering signal and PDE binding, we employed ROS membranes from retinas of *Bufo marinus* dark adapted for 12–18 h before sacrifice. The membranes were prepared and manipulated with an infrared image converter, as previously described (Stein et al., 1985). In these experiments, light-scattering measurements were made at 870 nm.

Reconstitution experiments were made by mixing PDE and G protein with membranes washed free of peripheral membrane proteins. Tris-HCl (pH 7.7) and $MgCl_2$ were added to final concentrations of 100 and 1 mM, respectively. The membrane suspension was stirred with a magnetic stirrer in a 3-mL cuvette. Phosphodiesterase activity was measured according to the method of Bignetti et al. (1978). Light microscopic observations were made with a phase-contrast microscope, as previously described. In the experiments in which GTP was used, the samples were photographed within 2 min from the collection from the cuvette.

To analyze protein partitioning between membranes and supernatant fraction, we removed aliquots of the membrane suspension [400 μL, in 100 mM Tris-HCl (pH 7.8) and 1 mM $MgCl_2$] from the cuvette during light-scattering experiments. The samples were centrifuged at 39000*g* for 4 min. The supernatant solution (moderate ionic strength wash) was collected, and the membrane pellets were resuspended in 400 μL of distilled water, passed 30 times through a 25-gauge syringe needle, and centrifuged at 39000*g* for 20 min. This low ionic strength wash was collected. Under these conditions, the moderate ionic strength wash contains the soluble (nonmembrane-bound) proteins. At the rhodopsin concentrations employed for these experiments (2.5–4 μM rhodopsin concentrations), the low ionic strength wash extracts more than 90% of the membrane-bound PDE (data not shown). The amount of PDE released by the low ionic strength wash was considered to be the amount of PDE that was membrane bound. Both the moderate and low ionic strength washes were diluted in SDS Laemmli sample buffer to a final SDS concentration of 3% and analyzed by 11% PAGE. Under these conditions, PDE runs as a single band with a molecular weight of approximately 92 000.

Results

Figure 1A shows the effect of addition of GTPγS and 8Br-cGMP on light scattering of a suspension of bleached membranes reconstituted with G protein and PDE. As previously described (Caretta & Stein, 1985), a decrease in

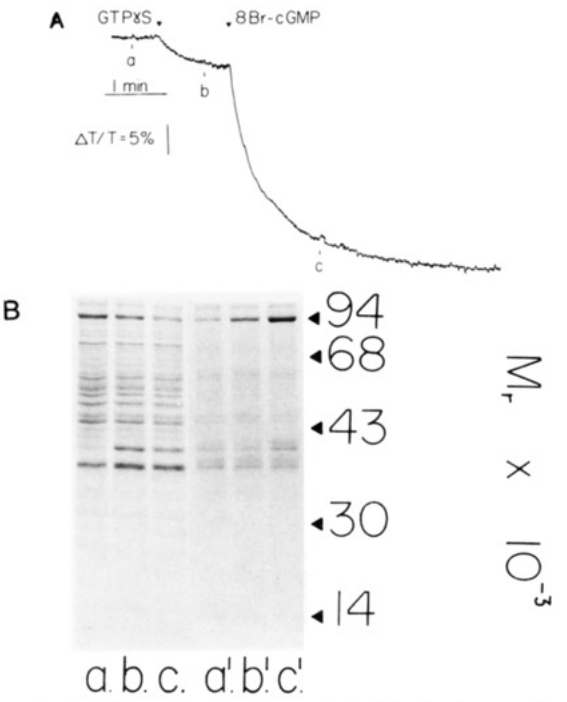

FIGURE 1: Addition of nucleotides to fully bleached reconstituted membranes: effect on near-infrared light scattering at 710 nm. (A) [Rhodopsin] = 2.5 μM, [G protein] = 250 nM, and [PDE] = 100 nM. After the addition, [GTPγS] = 3 μM and [8Br-cGMP] = 500 μM. At the times indicated by a–c, samples were collected from the cuvette and spun at 39000*g* for 4 min. (B) SDS gel showing protein partitioning between moderate and low ionic strength supernatants. Lanes a, b, and c show the moderate ionic strength washes of the samples collected at times a, b, and c, respectively, as indicated in panel A. Lanes a′, b′, and c′ show the proteins extracted by low ionic strength washing of the pellets of the same samples.

near-infrared light transmission occurs after addition of GTPγS. This decrease is further enhanced by addition of 8Br-cGMP. Analysis of protein partitioning (Figure 1B) during this experiment shows that addition of GTPγS decreases the amount of PDE in the moderate ionic strength wash (lane b compared with lane a) and increases the amount of PDE in the low ionic strength wash (lane b′ compared with lane a′). Thus, the amount of PDE associated with the membrane (as indicated by the amount released by low ionic strength washing) has increased. Upon addition of 8Br-cGMP, a further increase in PDE binding to the membrane occurs (Figure 1B, lane c′ compared with lane b′).

Addition of GTP to bleached disk membranes, reconstituted with PDE, G protein, and 8Br-cGMP, results in a large reversible decrease in near-infrared light transmission (light-scattering increase) (Figure 2A). The light-scattering change is reversible as the signal returns to its base-line value (Figure 2A). The return of the signal to the base-line level occurs when GTP hydrolysis is completed (Caretta & Stein, 1985). Subsequent addition of GTPγS produces an irreversible transmission decrease. In other experiments (data not shown), up to four additions of GTP were capable of eliciting transmission decreases of similar amplitude. Figure 2B shows the distribution of proteins between moderate and low ionic strength fractions obtained from aliquots of the membrane suspensions taken from the cuvette during the scattering experiments. Before the addition of GTP (Figure 2B), a small amount of PDE is present in the low ionic strength wash (lane a′), while most PDE is in the moderate ionic strength wash (lane a). After addition of GTP (Figure 2B), most PDE is present in the low ionic strength wash (lane b′), and only a small amount is in the moderate ionic strength fraction (lane b). When the

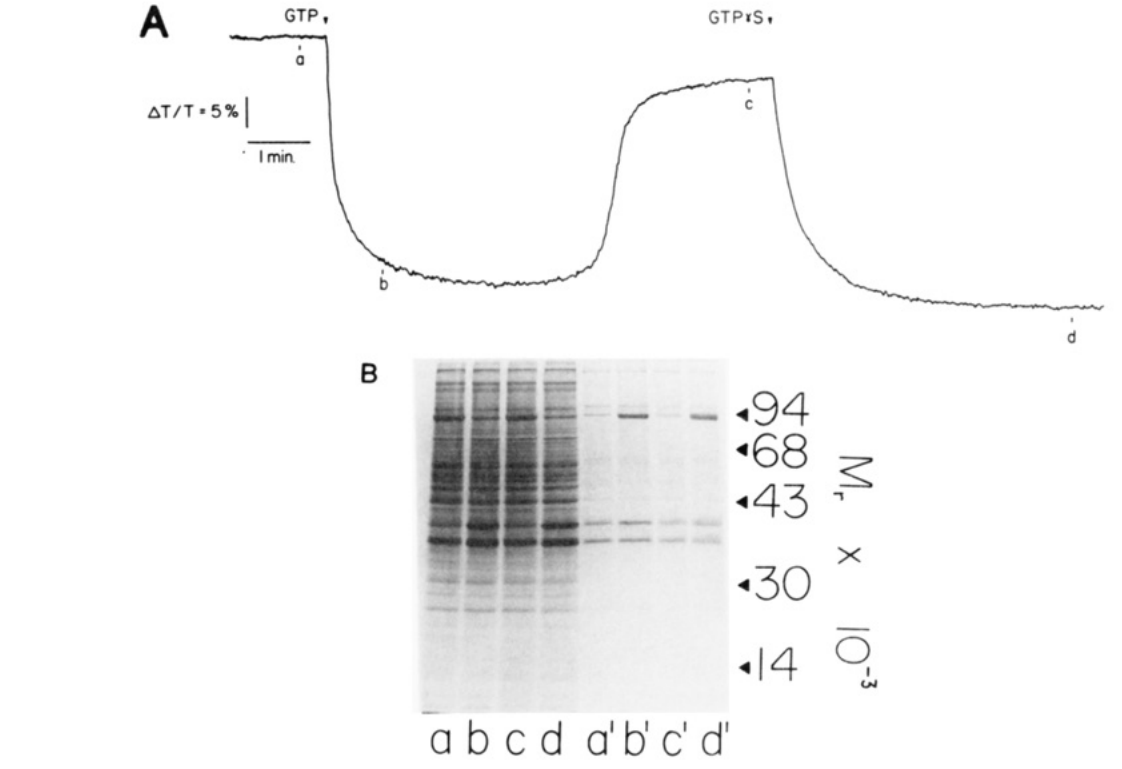

FIGURE 2: Addition of GTP (5 μM) and GTPγS (3 μM) to fully bleached reconstituted membranes: effect on near-infrared light scattering at 710 nm. (A) [Rhodopsin] = 3 μM, [G protein] = 300 nM, [PDE] = 120 nM, and [8Br-cGMP] = 500 μM. At the times indicated by a–d, samples were collected from the cuvette. (B) Protein partitioning between moderate and low ionic strength supernatants. Lanes a, b, c, and d show the moderate ionic strength supernatant of the samples collected at times a, b, c, and d, respectively, as indicated in panel A. Lanes a′, b′, c′, and d′ show the proteins extracted by low ionic strength washing of the pellet of the same samples. (C) Phase-contrast light micrographs of the samples collected at times a–d. Bar = 5 μM for all the samples.

scattering signal is again at its base-line value, the original distribution of PDE is restored (Figure 2B, lanes c′ and c). After addition of GTPγS (Figure 2B), the amount of PDE increased in the low ionic strength fraction (lane d′), and a reduced amount is present in the moderate ionic strength supernatant (lane d).

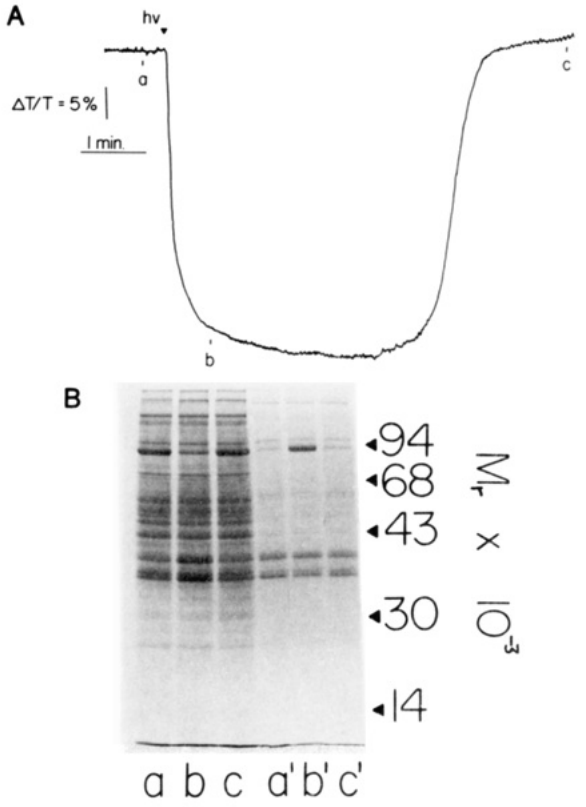

FIGURE 3: Near-infrared light-scattering changes of a suspension of unbleached reconstituted disk membranes. (A) [Rhodopsin] = 3 μM, [G protein] = 200 nM, [PDE] = 130 nM, [8Br-cGMP] = 500 μM, and [GTP] = 5 μM. The flash (indicated by the arrowhead) bleached 2.5% rhodopsin. At the times indicated by a–c, samples were collected from the cuvette. (B) SDS gel showing protein partitioning during the light-scattering changes. Lanes a, b, and c show the moderate ionic strength supernatants of the samples collected at times a, b, and c, respectively, as indicated in panel A. Lanes a′, b′, and c′ show the proteins extracted at low ionic strength from the same samples.

Figure 2C shows the state of vesicle aggregation under identical experimental conditions. It is clear that when the vesicles are in the aggregated state, an increasing population of PDE molecules is bound. When the vesicles disaggregate, the PDE molecules reappear in the moderate ionic strength fraction. Thus, it appears likely that PDE binding and release from the membranes may be related to vesicle aggregation and to the nucleotide- and enzyme-dependent light-scattering changes in these membrane suspensions.

Both PDE binding and the infrared light-scattering changes can be shown to be light dependent. Flash bleaching of dark-adapted membranes in the presence of GTP and 8Br-cGMP triggers a reversible increase in light scattering, as previously described (Caretta & Stein, 1985). Under these same conditions, we have analyzed protein partitioning. We observe that photobleaching, which triggers the light-scattering signal (Figure 3A), also triggers the change in PDE binding (Figure 3B). Figure 4A shows the light-scattering signal recorded with 500-nm light flashes of different intensities. The kinetics of the signal vary enormously over the intensity range illustrated. The data show that a signal can be elicited by bleaching $1/(1 \times 10^7)$ rhodopsins. This sensitivity is close to the value reported for PDE activation by Yee and Liebman (1978). Figure 4B shows the relationship between light-scattering changes and rhodopsin bleaching. It can be noted that it does not resemble the intensity response function for rod excitation. We also observe that PDE binding increases as the extent of bleaching increases. Figure 4C shows that this effect can be produced by bleaching $1/(1 \times 10^6)$ rhodopsins (the lowest intensity we have tested). These preliminary experiments demonstrate that PDE binding is light dependent and related to light intensity.

Figure 5 shows the light-scattering change elicited by addition of GTPγS to a suspension of bleached membranes reconstituted with G protein and PDE in the presence and absence of IBMX, a competitive inhibitor of PDE hydrolytic activity. The data show that IBMX can substitute for cGMP or 8Br-cGMP. Other competitive inhibitors of PDE hydrolytic activity can also substitute for cGMP. The apparent K_m's for the light-scattering change (measured as $dT/T\ s^{-1}$) are IBMX = 85 μM, theophylline = 330 μM, aminophylline = 400 μM, and caffeine = 1.5 mM. We believe that occupation of the cGMP binding site by the competitive inhibitor is sufficient to support the light-scattering change. These data further support our previous suggestion that cGMP binding, rather than hydrolysis, is essential for the cGMP- and PDE-dependent light-scattering change. Under these conditions, we also observe a correlation between PDE binding and the decrease in light transmission similar to that described in Figure 1A,B.

A number of treatments reverse the nucleotide- and enzyme-dependent light-scattering change. Figure 6A shows that following initiation of the transmission decrease by GTP, addition of EDTA (1 mM) results in a rapid increase in transmission which stabilizes at the base-line level. Subsequent addition of 3 mM $MgCl_2$ reactivates the light transmission decrease. Analysis of PDE partitioning between the supernatant and membrane fraction (Figure 6B) illustrates that PDE binding roughly correlates with the light transmission decrease and PDE release correlates with the transmission increase. Figure 7 (upper trace) shows that addition of GTP in the presence of EDTA has no effect on light scattering but subsequent addition of $MgCl_2$ elicits a large light-scattering change. Figure 7 (lower trace) shows that addition of $CaCl_2$ or $MgCl_2$ in the presence of EDTA but in the absence of GTP has no effect on light scattering. Subsequent addition of GTP elicits the usual light-scattering change. In all these conditions, the presence of EGTA (a chelating substance selective for Ca over Mg) has no effect on light-scattering changes (data not shown). From these data, it appears that the cGMP- and PDE-dependent light-scattering change does not result from a charge effect of divalent cations but rather requires the presence of Mg (not Ca) as a nucleotide cofactor.

GDP, at millimolar concentration, can reverse the GTP-dependent activation of the infrared light-scattering change (Figure 8, upper trace). GDP, if added before GTP, can prevent the GTP-dependent light transmission decrease. ADP at similar concentration is ineffective. It is interesting to note that GDP cannot reverse the activation by GTPγS, suggesting that GTPγS is more effective than GTP in activating the light-scattering change.

Colchicine, at high concentration (25 mM), is also capable of reversing the GTP-dependent decrease in light transmission (Figure 9). If colchicine is added before GTP, the effect of GTP addition is blocked. Under these conditions, the binding and release of PDE correlate with the infrared light transmission decrease and subsequent increase (data not shown). Lumicolchicine at high concentration is similarly effective.[2]

[2] The effect of colchicine in these experiments appears not to be related to tubulin or microtubules since the concentrations necessary are orders of magnitude above those routinely employed for microtubule disruption. In addition, lumicolchicine appears to be as effective as colchicine. Effects of this drug at high concentration, not related to tubulin or microtubules, have been reported both in photoreceptors (O'Connor & Burnside, 1981) and in other neural tissues (Landowne et al., 1983).

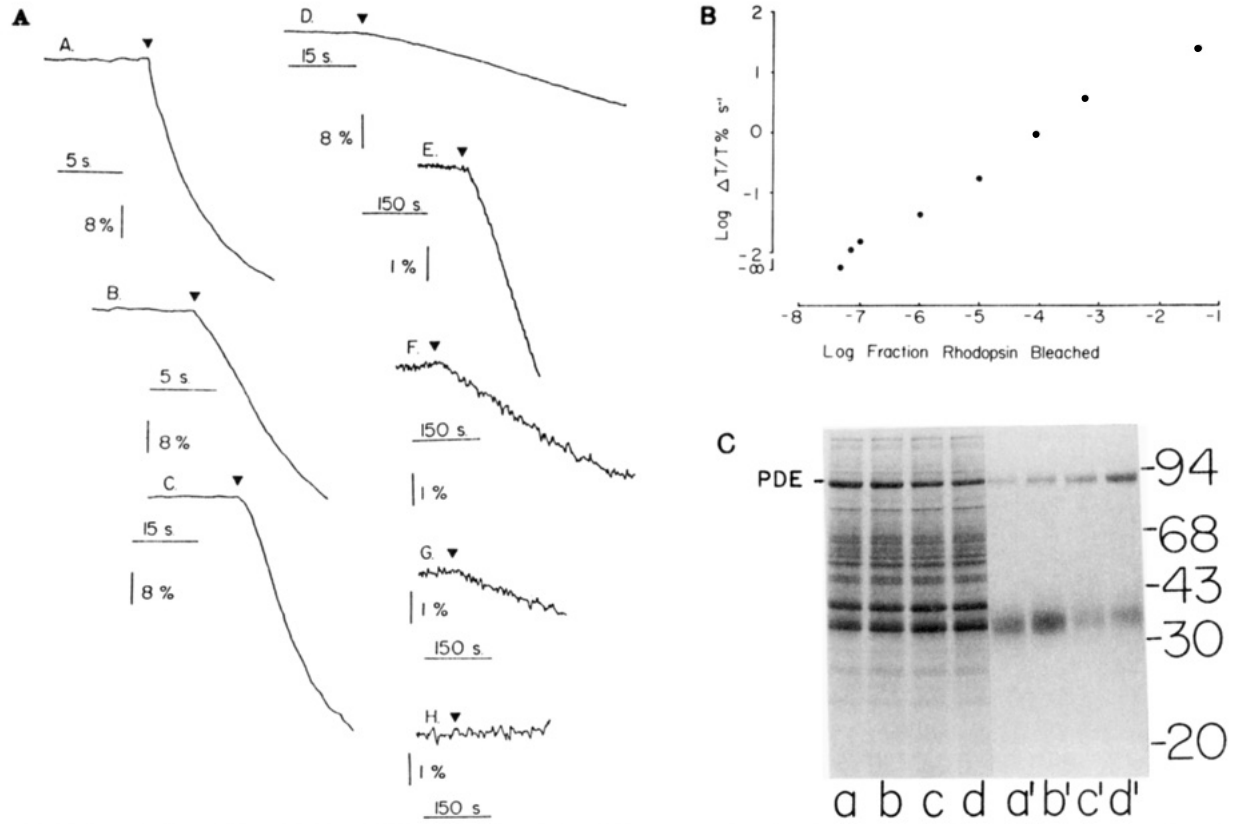

FIGURE 4: Light sensitivity of the cGMP- and PDE-dependent infrared light-scattering signal. (A) Low ionic strength washed toad membranes prepared under infrared light were reconstituted with bovine G protein and PDE. Final concentrations were rhodopsin = 5 μM, G protein = 900 nM, PDE = 100 nM, GTPγS = 5 μM, and 8Br-cGMP = 500 μM. Measurement of the scattering signal was performed at 870 nm. Note the change in time and transmission scales in various panels. Trace A, $4/(1 \times 10^2)$ rhodopsins bleached; trace B, $1/(1 \times 10^3)$ rhodopsins bleached; trace C, $1/(1 \times 10^4)$ rhodopsins bleached; trace D, $1/(1 \times 10^5)$ rhodopsins bleached; trace E, $1/(1 \times 10^6)$ rhodopsins bleached; trace F, $1/(1 \times 10^7)$ rhodopsins bleached; trace G, $5/(1 \times 10^8)$ rhodopsins bleached; trace H, $2/(1 \times 10^8)$ rhodopsins bleached. (B) Plot of the initial speed of $dT/T\%\ s^{-1}$ against fractional bleaching of rhodopsin. Data from panel A. (C) SDS gel showing protein partitioning as a function of bleaching intensity in the experiment in panel A. Lanes a and a′ are the moderate and low ionic strength washes of dark membranes, respectively. Lanes b, c, and d and b′, c′, and d′ are the moderate and low ionic strength supernatants of samples collected at the completion of the experiments illustrated in panel A, traces F, D, and B, respectively.

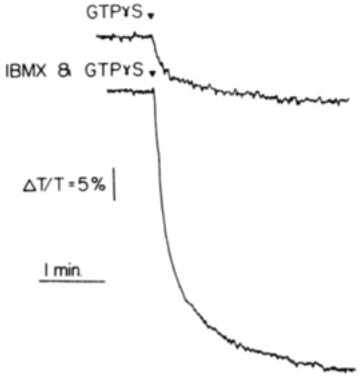

FIGURE 5: Near-infrared light-scattering changes (710 nm) of a suspension of fully bleached reconstituted disk membranes. Effect of GTPγS (3 μM) addition in the absence (upper trace) and in the presence (lower trace) of 500 μM IBMX. [Rhodopsin] = 3 μM, [G protein] = 300 nM, and [PDE] = 120 nM.

We have measured PDE hydrolytic activity under the same experimental conditions as those described above for EDTA, GDP, and colchicine. In the presence of these agents, GTP-dependent activation of PDE is completely blocked. In the presence of IBMX (a competitive inhibitor of PDE hydrolytic activity), GTP-dependent activation of cGMP hydrolysis by PDE is still present (Figure 10).

Discussion

The data presented show that light and nucleotide affect PDE binding to ROS disk membranes in reconstitution experiments.[3] Under conditions which enhance PDE binding to the membranes, G protein is released from the membrane. It appears under these experimental conditions that only G protein and PDE are changing their association with the membrane (albeit, in opposite directions). Thus, increased PDE binding is not merely a consequence of the membrane aggregation process which occurs under the same conditions. A simple explanation of these phenomena would be that PDE release from the membranes is diminished by aspects of vesicle aggregation, e.g., decrease in exposed surface area or mechanical trapping of proteins etc. However, careful examination of the gels shows that while there are many other proteins in our partially purified extracts, only PDE and G protein undergo nucleotide-dependent binding and release. It is also possible that there is a competition between G protein and PDE for a binding site on the disk membrane. As G protein binding is decreased (by GTP addition), PDE binding could increase. However, increasing the ratio of G protein to PDE in the reconstitution does not decrease PDE binding as would be expected if there were competition for a common binding site. Furthermore, decreasing the ratio of G protein to PDE decreases PDE binding (data not shown). Since ad-

[3] We reported previously that the minimum stimulus necessary to produce the cGMP- and PDE-dependent light-scattering signal bleached $2/(1 \times 10^5)$ rhodopsins. In the present work, we used toad ROS membranes prepared under infrared light, 870-nm measuring beam (instead of 740 nm), with a hydrolysis-resistant guanine nucleotide analogue (instead of GTP), 0.5-mm slit (instead of 5.0 mm).

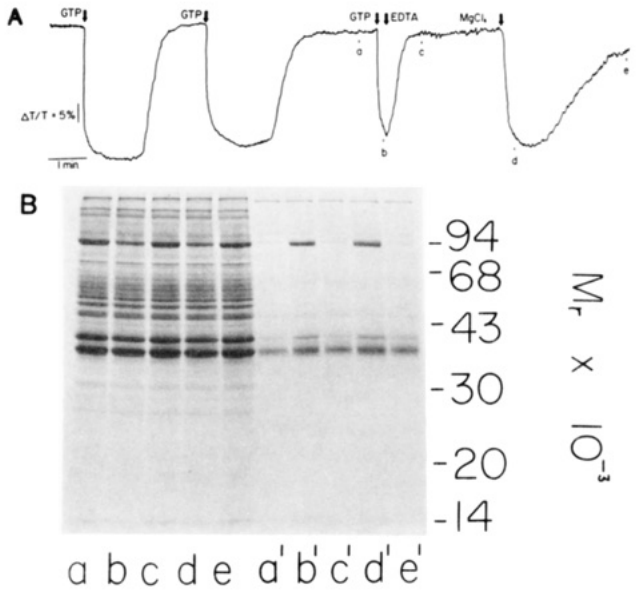

FIGURE 6: Effect of EDTA and $MgCl_2$ on the cGMP- and PDE-dependent near-infrared light-scattering change of a suspension of reconstituted fully bleached disk membranes. (A) [Rhodopsin] = 3 μM, [G protein] = 300 nM, [PDE] = 120 nM, [Br-cGMP] = 500 μM, and [$MgCl_2$] = 200 μM, present from the beginning. Effect of subsequent addition of GTP (3 μM), EDTA (1 mM), and $MgCl_2$ (3 mM). At the times indicated by a–e, samples were collected from the cuvette. (B) SDS gel showing protein partitioning between moderate and low ionic strength washes during the experiments presented in panel A. Lanes a, b, c, d, and e show the moderate ionic strength washes of the samples collected at times a, b, c, d, and e, respectively. Lanes a′, b′, c′, d′, and e′ show the proteins extracted from the pellets of the same samples at low ionic strength.

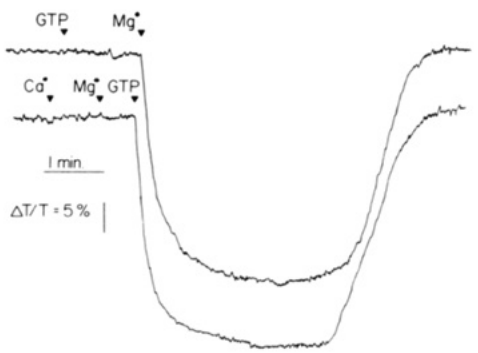

FIGURE 7: Effect of chelating agents and divalent cation on the cGMP- and PDE-dependent light-scattering changes. [Rhodopsin] = 3 μM, [G protein] = 300 nM, [PDE] = 120 nM, and [8Br-cGMP] = 500 μM, present from the beginning. (Upper trace) [EDTA] = 200 μM, present from the beginning. Effect of subsequent addition of GTP (3 μM) and $MgCl_2$ (1 mM). (Lower trace) [EDTA] = 200 μM, present from the beginning. Effect of subsequent addition of $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), and GTP (3 μM).

dition of GTP (or its hydrolysis-resistant analogues) in the presence of G protein activates PDE, it seems possible that this activation may alter the conformation of PDE so that its affinity for the membrane is increased. While this seems to be the most promising interpretation of the data, we cannot yet define the mechanism(s) responsible for the increase in PDE affinity for the membranes in these experiments.

GDP, EDTA, and colchicine can reverse the nucleotide- and PDE-dependent near-infrared light-scattering signal, vesicle aggregation, and PDE binding to disk membranes. Each of these agents also blocks GTP-dependent activation of PDE hydrolysis. Thus, when activation of PDE hydrolytic activity by G protein and GTP is blocked, PDE binding, vesicle aggregation, and the infrared light-scattering change are also

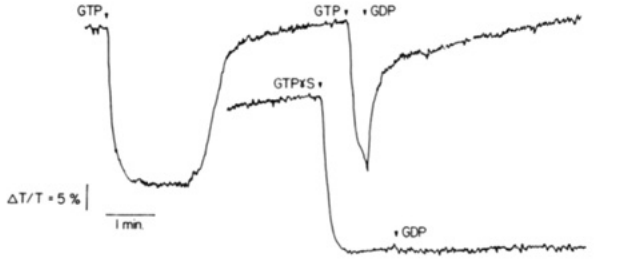

FIGURE 8: Effect of GDP addition on light-scattering changes (710 nm) of a suspension of fully bleached reconstituted membranes. [$MgCl_2$] = 5 mM, [rhodopsin] = 3 μM, [G protein] = 300 nM, [PDE] = 120 nM, and [8Br-cGMP] = 500 μM, present from the beginning. (Upper trace) After addition, [GTP] = 6 μM and [GDP] = 1.5 mM. (Lower trace) After addition, [GTPγS] = 3 μM and [GDP] = 1.5 mM.

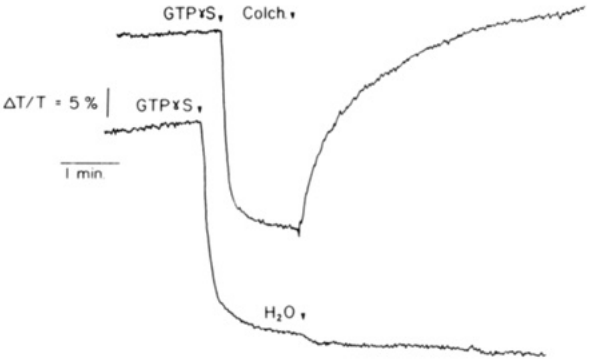

FIGURE 9: Effect of colchicine on the cGMP- and PDE-dependent light-scattering changes of a suspension of fully bleached reconstituted disk membranes. [Rhodopsin] = 3 μM, [G protein] = 300 nM, [PDE] = 120 nM, and [8Br-cGMP] = 500 μM, present from the beginning. After additions, [GTPγS] = 5 μM and [colchicine] = 25 mM.

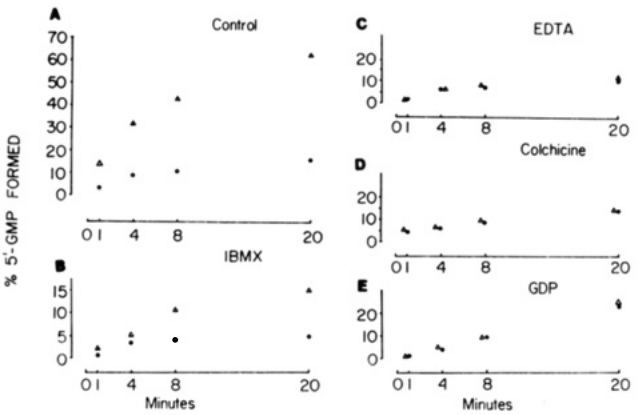

FIGURE 10: PDE hydrolytic activity in the presence (▲) and in the absence (●) of GTP. [Rhodopsin] = 1 μM, [G protein] = 100 nM, [PDE] = 40 μM, [GTP] = 10 μM, and [cGMP] = 1.5 mM. GMP formation is plotted against time. (A) Control; (B) [IBMX] = 500 μM; (C) [EDTA] = 3 mM; (D) [colchicine] = 25 mM; (E) [GDP] = 2 mM.

blocked. By contrast, IBMX, which inhibits PDE hydrolytic activity by a competitive mechanism (not by blocking GTP-dependent activation), fully supports PDE binding, vesicle aggregation, and the near-infrared light transmission decrease. Similarly, in the presence of GTPγS (or GTP) and 8Br-cGMP or IBMX (without cGMP), the "active" conformation of PDE can be induced by G protein, while cGMP hydrolysis is greatly reduced or cannot occur. However, increased PDE binding, vesicle aggregation, and the light-scattering signal can be readily observed. Thus, these three phenomena appear to be related to the state of activation (not hydrolytic activity) of the enzyme.

We have not been able to observe light- and nucleotide-dependent PDE binding in disrupted, unwashed, ROS membranes without increasing the PDE concentration. The light- and nucleotide-dependent light-scattering signal is present in native membranes, but it is extremely small compared to the signal in the reconstituted system (Caretta & Stein, 1985). If in native membranes there is a high ratio of PDE binding sites to PDE, then most PDE will be bound and the change in PDE binding will be difficult to detect. We believe increasing the PDE concentration makes it possible to observe the PDE binding/release phenomenon which is present but not normally detectable in native membranes or intact ROS.

The physical basis of the PDE- and nucleotide-dependent infrared light-scattering signal reported both here and previously (Caretta & Stein, 1985) appears to be disk vesicle aggregation/disaggregation. The data suggest that GTP-dependent activation of PDE results in enhanced PDE binding to the disk membrane under our experimental conditions and PDE inactivation by GTP hydrolysis may release PDE from the membrane. We believe that the changes in PDE binding/release are related to vesicle aggregation/disaggregation. Therefore, we propose that the changes in near-infrared light-scattering observed in these experiments reflect PDE activation/inactivation. A similar relationship between PDE activation and near-infrared light-scattering changes has recently been proposed by Kamps et al. (1985) on the basis of studies of permeabilized ROS.

ACKNOWLEDGMENTS

We thank Drs. James W. Clack and Robert T. Sorbi for their critical reading of the manuscript and helpful comments. We thank Dr. Gary D. Bernard for valuable discussions during the course of the work.

Registry No. PDE, 9068-52-4; GTPγS, 37589-80-3; 8Br-cGMP, 31356-94-2; GTP, 86-01-1; IBMX, 28822-58-4; cGMP, 7665-99-8; GDP, 146-91-8; Mg, 7439-95-4; theophylline, 58-55-9; aminophylline, 317-34-0; caffeine, 58-08-2; cholchicine, 64-86-8.

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