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cGMP- and Phosphodiesterase-Dependent Light-Scattering Changes in Rod Disk Membrane Vesicles: Relationship to Disk Vesicle–Disk Vesicle Aggregation†

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ABSTRACT: Visible light activates a large guanosine cyclic 3′,5′-phosphate (cGMP)- and phosphodiesterase (PDE)-dependent infrared light-scattering change in suspensions of photoreceptor disk membranes. Reconstitution experiments show that this signal requires bleached rhodopsin, G protein (three polypeptide subunits of M_r 39 000, 37 000, and 6000 which comprise the GTPase), phosphodiesterase, cGMP, and GTP. The lowest light intensity which elicits the light-scattering signal bleaches 0.002% rhodopsin. cGMP and GTP hydrolysis occurs more slowly than the initial phase of the scattering signal, and the kinetics of nucleotide hydrolysis do not correlate with any phase of the signal. Hydrolysis-resistant analogues of cGMP and GTP support the initial decreasing phase of the signal. Thus, the signal apparently depends upon nucleotide binding rather than hydrolysis. Microscopic observations made under the same conditions as light-scattering experiments show that vesicle–vesicle aggregation and disaggregation occur. The data suggest that light and nucleotide activations of the cyclic nucleotide cascade enzymes are responsible for the vesicle aggregation process and nucleotide hydrolysis for vesicle disaggregation. The vesicle aggregation–disaggregation phenomenon appears likely to be the physical basis of the cGMP- and PDE-dependent changes in infrared transmission.

A variety of infrared light-scattering signals have been reported to occur in suspensions of broken rod outer segment (ROS)[1] membranes after a flash of visible light. Small changes in transmittance, which occur in the absence of extrinsic membrane proteins, have been correlated with rhodopsin bleaching (Hofmann et al., 1976; Uhl et al., 1978). Similarly, large changes in transmittance dependent on ATP hydrolysis have been correlated with ion movements through disk membranes (Uhl et al., 1979a,b; Borys et al., 1983). An increase in transmittance dependent on GTP and extrinsic membrane proteins (Bignetti et al., 1980), attributed to light activation of the enzymes, has been demonstrated to depend on rapid interaction of rhodopsin, G protein, and GTP (Kuhn et al., 1981; Emeis et al., 1982). A slower and larger light-scattering change dependent on GTP and extrinsic membrane proteins also has recently been reported (Lewis et al., 1983).

In this paper, we describe a large infrared light-scattering signal. In contrast to those reported previously, the infrared transmission changes observed depend upon both GTP and cGMP. In addition, the signal requires the presence of G protein, PDE, and bleached disk vesicles. Reconstitution experiments demonstrate that the scattering signal can be triggered either by light, in dark-adapted membranes, or by nu-

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[1] Abbreviations: ROS, rod outer segment(s); G protein, three polypeptide subunits of M_r 39 000, 37 000, and 6000 which comprise the GTPase; PDE, phosphodiesterase; cGMP, guanosine cyclic 3′,5′-phosphate; 8Br-cGMP, 8-bromoguanosine cyclic 3′,5′-phosphate; cAMP, adenosine cyclic 3′,5′-phosphate; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); DTT, dithiothreitol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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cleotide addition to fully bleached membranes. We describe the temperature, light intensity, and nucleotide dependence of various phases of the signal.

Microscopic observations document that disk vesicle–disk vesicle aggregation occurs and that the extent of aggregation correlates roughly with the scattering signal. These data suggest that changes in the extent of disk vesicle–disk vesicle aggregation are responsible for the large cGMP- and PDE-dependent changes in light scattering in the membrane suspensions.

Materials and Methods

Fresh bovine (calf) eyes were obtained from a local slaughterhouse. The eyes were transported in light-tight containers at ambient temperature (45–60 min) before the retinas were dissected. The retinas were shaken in 40% (w/w) sucrose containing 100 mM Tris-HCl (pH 7.7), 1 mM $MgCl_2$, and 5 mM DTT (2 mL per retina) at 4 °C and then centrifuged for 1 h at 100000*g*. The rod disks floated and were collected with a syringe and disrupted by passage 3 times through a 25-gauge syringe needle. These membranes were used for experiments without reconstitution. For reconstitution experiments, the membranes were washed 4 times with 100 mM Tris-HCl (250 μL per retina), 1 mM $MgCl_2$, and 5 mM DTT. G protein and PDE were extracted according to Kuhn (1980). The G protein was washed free of GTP and concentrated with an Amicon CF-25 filter cone to a concentration of 10–15 μM. No further purification of PDE was made. The enzymes and membranes were stored in 5 mM Tris-HCl (pH 7.9) and 5 mM DTT at 0 °C. For experiments with bleached rhodopsin, disk vesicles were totally bleached with multiple flashes from a photographic strobe and stored on ice in the light for 1–3 h. The experiments were performed within 72 h of the death of the animals. No decay of enzyme activity was observed within this time.

Light-scattering changes were measured as optical transmission changes at 710 nm (or as indicated) in a Shimadzu UV-3000 recording spectrophotometer in the sample compartment furthest from the phototube at a 0° (±5.5°) transmittance angle. The phototube window was shielded from the exciting light with a 660-nm cutoff filter. Photoexcitation was performed with a photographic flash at a 90° angle from the measuring beam through a 498-nm (10-nm bandwidth) interference filter. An unattenuated flash bleached 2% of the rhodopsin at 5 μM concentration. Neutral density filters were employed, when necessary, to attenuate the flash. The optical path length was 1 cm. The apparent optical densities of the samples were 0.4–0.5 ODU (710 nm). All scattering experiments were performed at 21 °C, unless otherwise indicated.

Reconstitution experiments were performed by mixing PDE and G protein with membranes washed free of peripheral proteins using a solution containing 5 mM Tris-HCl (pH 7.7) and 5 mM DTT (no $MgCl_2$). Tris-HCl (pH 7.7) and $MgCl_2$ were added to final concentrations of 100 and 1 mM, respectively. The experiments were routinely performed in this buffer. The samples were flash bleached after a 3-min incubation in the dark, in the presence of the nucleotides. In experiments where nucleotides were added to bleached membranes, the suspensions were stirred at 60 rpm in a 3-mL cuvette by using a magnetic stirrer.

For measurement of PDE and GTPase activity, samples were collected from the cuvette while light-scattering changes were recorded. Hydrolysis of [^{32}P]GTP was measured according to the method of Yamazaki et al. (1983), and hydrolysis of [^{3}H]cGMP was determined as previously described (Goridis & Virmaux, 1974). Protein concentrations were determined according to the method of Bradford (1976).

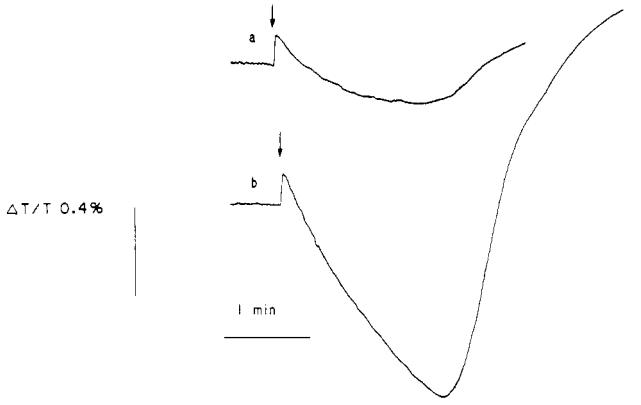

FIGURE 1: Near-infrared light-scattering changes of a suspension of disrupted disk membranes. Stacked disks were disrupted by passing them through a 25-gauge syringe needle as described under Materials and Methods. [Rhodopsin] = 2 μM. The flash (indicated by the arrow) bleached 0.2% rhodopsin. Trace a, [GTP] = 2 μM, no cGMP; trace b, [GTP] = 2 μM, [cGMP] = 1 mM.

Negative staining of disk membranes was made with grids coated with carbon film. The disks were stained for 1 min with a solution containing 20 μg/mL bacitracin in 2% phosphotungstic acid, adjusted to pH 7.4 with NaOH.

Light microscopic observations of disk vesicle aggregation were made with a phase-contrast microscope. Aliquots of disk membranes, treated as described in the legend of Figure 9, were removed from the cuvette while light-scattering changes were recorded. The aliquots were applied to a glass slide, and a coverslip was placed on top. Excess solution was removed with a tissue. With the use of a 40× phase objective, the membrane aggregation–disaggregation phenomenon was observed and photographed.

Results

Flash bleaches of native disk membranes in the presence of GTP elicited a fast increase in transmission followed by a slower and larger transmission decrease. After this infrared transmission decrease, there was a slow transmission increase (Figure 1, trace a). Addition of cGMP to these membranes caused a large change in the signal elicited by a light flash. In this condition, the speed and amplitude of the transmission decrease, and the subsequent increase is greatly enhanced (Figure 1, trace b).

In order to understand the molecular basis for these complex transmission changes, we employed reconstitution experiments. When low ionic strength washed membranes are reconstituted with purified G protein, a flash of light elicits a fast and persistent decrease in transmittance (Figure 2A, trace a), as previously described (Kuhn et al., 1981). If GTP is added to this reconstituted system, light elicits a rapid increase in transmittance which slowly returns to the base line as GTP is hydrolyzed (Figure 2A, trace b). These signals have been called the binding and dissociation signals, respectively (Kuhn et al., 1981). Under these conditions, no further transmission changes are detected.

Addition of PDE to disk membranes, in the presence of G protein and GTP, results in the light-induced dissociation signal being followed by a transmittance decrease (as observed in nonreconstituted membranes; Figure 1, traces a and b). If traces b and c in Figure 2A are compared, this difference is clear. Furthermore, Figure 2B shows that addition of increasing amounts of PDE increases the speed and amplitude of the transmission decrease. This decrease in transmittance is followed by a late and slow increase in transmittance (Figure

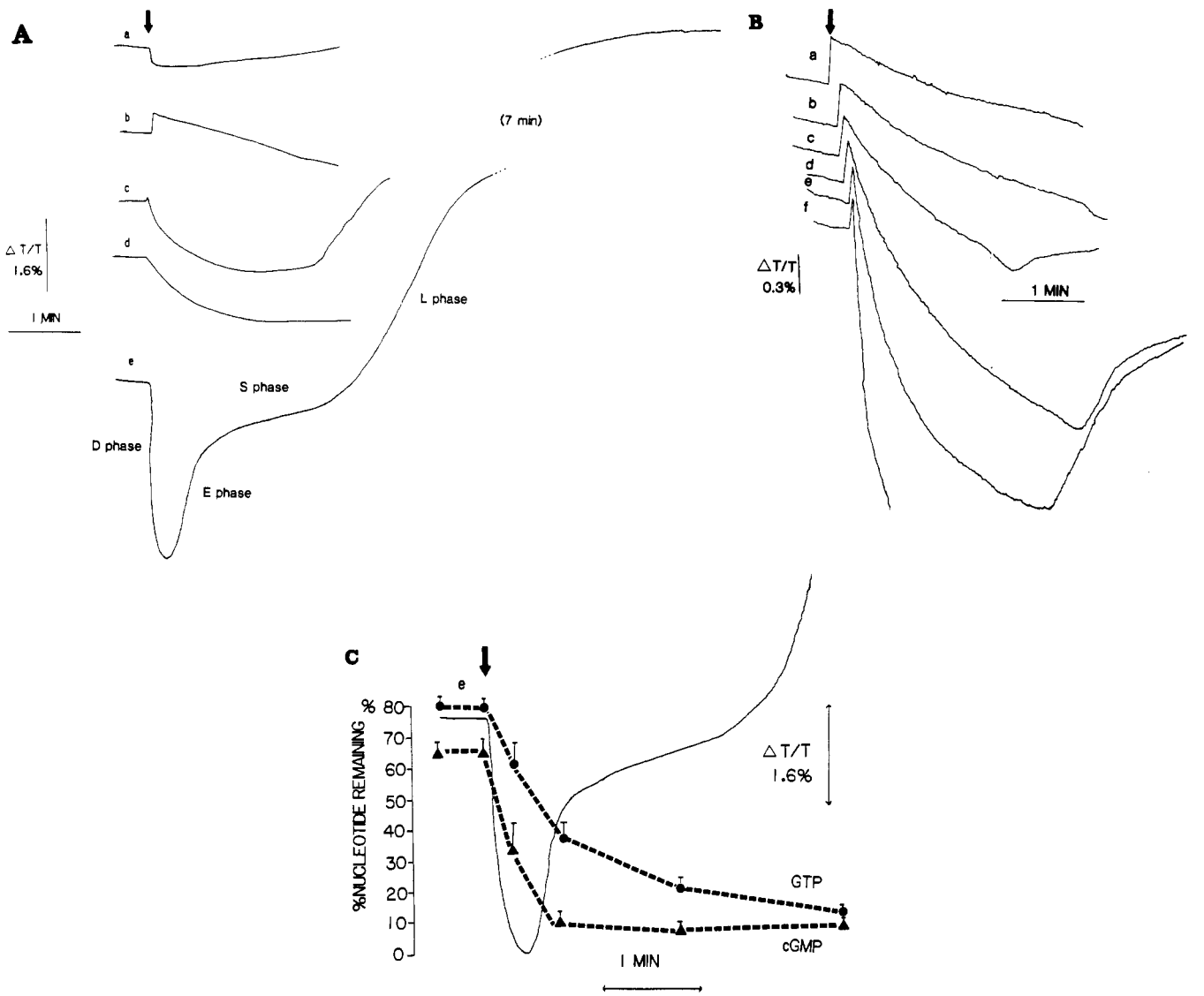

FIGURE 2: (A) Near-infrared (710 nm) light-scattering changes of disk membranes reconstituted with G protein and/or PDE and with different nucleotide composition. [Rhodopsin] = 2 μM. The flash (indicated by the arrow) bleached 2% rhodopsin. Trace a, [G protein] = 200 nM, [PDE] = 60 nM, no GTP. The presence or absence of 1 mM cGMP makes no difference (binding signal; Kuhn et al., 1981). Trace b, [G protein] = 200 nM, no PDE, [GTP] = 3.5 μM, no cGMP (dissociation signal; Kuhn et al., 1981). Trace c, [G protein] = 200 nM, [PDE] = 60 nM, [GTP] = 3.5 μM, no cGMP. Trace d, no added G protein, [PDE] = 60 nM, [GTP] = 3.5 μM, [cGMP] = 1 mM. Trace e, [G protein] = 200 nM, [PDE] = 60 nM, [GTP] = 3.5 μM, [cGMP] = 1 mM. (B) Near-infrared (710 nm) light-scattering changes of disk membranes reconstituted with G protein, GTP, and increasing amounts of PDE. No cGMP present. The flash bleached 0.2% rhodopsin. [Rhodopsin] = 2 μM, [G protein] = 600 nM, and [GTP] = 2.5 μM. Trace a, no PDE; trace b, [PDE] = 20 nM; trace c, [PDE] = 30 nM; trace d, [PDE] = 40 nM; trace e, [PDE] = 60 nM; trace f, [PDE] = 120 nM. (C) Same conditions as (A), trace e; 0.2 μCi of [^{3}H]cGMP and [^{32}P]GTP was present in order to measure PDE and GTPase activities. Every point, mean ± SD of three experiments.

2A, trace c). Under these conditions, the signal resembles that reported by Lewis et al. (1983).

Addition of cGMP to this reconstituted system greatly alters the kinetics and wave form of the scattering signal; the initial transmittance decrease (D phase) becomes larger and more rapid. With cGMP present, the previously observed dissociation signal (Figure 2A, trace b) can no longer be detected because it is masked by this large and rapid transmittance decrease (Figure 2A, trace e). In the presence of cGMP, the D phase is followed by a small increase in transmittance (early phase, E phase) whose amplitude is smaller than the D phase (Figure 2A, trace e). After this partial recovery (E phase), the transmittance becomes relatively stable (S phase) before a second transmittance increase (late phase, L phase) occurs which usually exceeds the starting transmission value (Figure 2A, trace e).

The traces in Figure 2 show that this multiphasic response requires the presence of G protein, PDE, GTP, cGMP, and disk membranes. When any one of the components is missing, the complete signal is no longer observed. The manner in which the signal is altered depends upon which component is missing from the reconstitution. In particular, cGMP has no effect at all if G protein, GTP, or PDE is absent. The slow decrease detected in Figure 2A, trace d, is likely to depend upon slight contamination of the PDE fraction with G protein (for comparison, see Figure 2A, trace e).

We have examined the relationship of the kinetics of the scattering signal to the enzyme activities of GTPase and PDE present in the reconstituted system. The D phase persists until cGMP hydrolysis is almost completed (less than 10% left, Figure 2C). This figure shows that the kinetics of the D phase appear to be more rapid than the hydrolysis of either cGMP or GTP. The L phase does not begin until GTP hydrolysis is almost complete (Figure 2C). The L phase eventually reaches a stable value of transmittance usually somewhat greater than the value before the flash (Figure 2A, trace e,

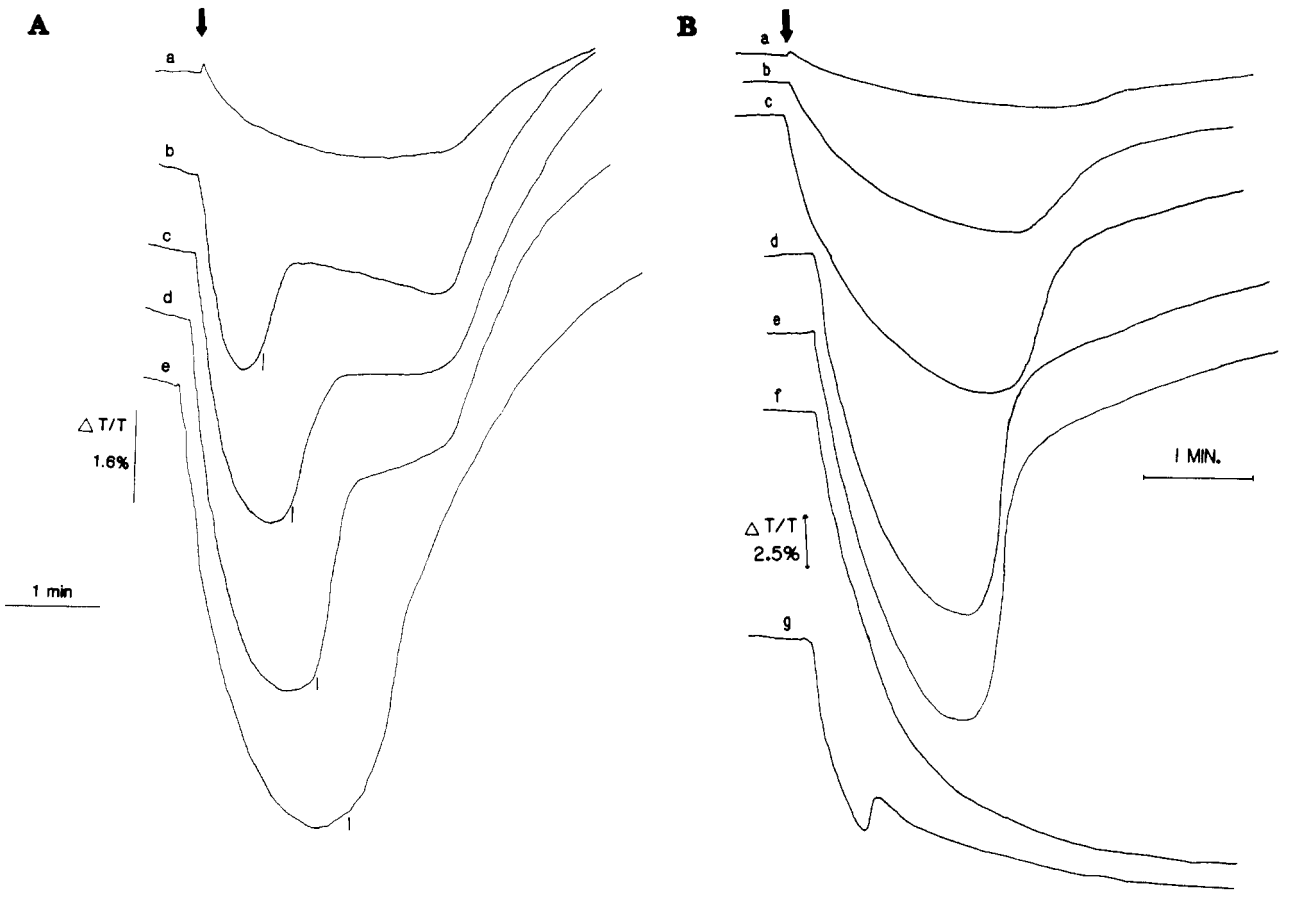

FIGURE 3: (A) Effect of increasing concentrations of cGMP on near-infrared (710 nm) light-scattering changes of disk membranes reconstituted with G protein and PDE. [Rhodopsin] = 2 μM, [G protein] = 600 nM, [PDE] = 60 nM, and [GTP] = 2.5 μM. The flash (indicated by the arrowhead) bleached 0.2% rhodopsin. Trace a, no cGMP; trace b, [cGMP] = 0.5 mM; trace c, [cGMP] = 1 mM; trace d, [cGMP] = 1.5 mM; trace e, [cGMP] = 2 mM. The vertical bars near the traces indicate the time of complete cGMP hydrolysis. (B) Membrane and protein concentrations identical with those in (A). The flash (indicated by the arrowhead) bleached 0.2% rhodopsin. Trace a, [GTP] = 2.5 μM, no cGMP; trace b, [GTP] = 2.5 μM, [8Br-cGMP] = 100 μM; trace c, [GTP] = 2.5 μM, [8Br-cGMP] = 250 μM; trace d, [GTP] = 2.5 μM, [8Br-cGMP] = 500 μM; trace e, [GTP] = 2.5 μM, [8Br-cGMP] = 1 mM; trace f, [GTPγS] = 2.5 μM, [8Br-cGMP] = 1 mM; trace g, [GTPγS] = 2.5 μM, [cGMP] = 1 mM.

and Figure 2C). The reason for the discrepancy between the initial and final transmission values is unclear.

In the presence of GTP, the amplitude, speed, and length of the D phase depend upon cGMP concentration (Figure 3A). It is not possible to determine a precise value of K_m for the cGMP effect on the D phase because of its continuous and rapid hydrolysis by PDE. If 8Br-cGMP (a hydrolysis-resistant analogue) is used instead of cGMP, the shape of the signal is altered (Figure 3B, traces b–e). The D phase is prolonged and persists until GTP hydrolysis is almost completed. At this time, an increase in transmittance occurs whose amplitude is smaller than the initial decrease (Figure 3B, traces a–e). From its time course, it appears that this transmission increase may be the L phase which no longer exceeds the starting transmission value. The apparent K_m for the 8Br-cGMP effect on the D phase is 125 μM (calculated by measuring dT/T s^{-1}).

GTP concentration above the micromolar range is essential for observing the scattering signal. If GTPγS (a hydrolysis-resistant GTP analogue) is used rather than GTP, the shape of the signal is modified: in the presence of 8Br-cGMP and GTPγS, the D phase occurs, but both the E and L phases are absent (Figure 3B, trace f). In the presence of GTPγS and cGMP, light still elicits a large D phase followed by a small E phase. The L phase is absent (Figure 3B, trace g). The apparent K_m for GTPγS is 0.4 μM (calculated by measuring dT/T s^{-1}). Both the D phase and the E phase require at least micromolar GTP concentration. When GTP concentration is increased above the micromolar level, the S phase is lengthened (Figure 4), so the onset of the L phase is delayed. The D phase and the E phase are unchanged. Of the other nucleotides tested, ATP does not substitute for GTP, guanosine and GMP are ineffective, and cAMP is about 50 times less effective than cGMP (5 mM cAMP = 100 μM cGMP) in supporting the light-scattering changes (data not shown).

Temperature affects both the speed and wave form of the scattering signal (Figure 5). The component phases of the signal are differentially affected by temperature. At low temperature, the D phase becomes slower and larger, presumably because of the slower hydrolysis of cGMP. Similarly, the onset of the L phase is delayed, presumably because of the reduced rate of GTP hydrolysis. The amplitude of the E phase appears to be greatly reduced. However, because of the complexity of the signal wave-form, measurements of the Q_{10} are difficult to make and interpret.

The relationship of the scattering signal to light intensity is illustrated in Figure 6A. At low light intensities, the wave form appears less complex and slower than at higher intensities. The lowest light intensity at which we observed the scattering signal bleached 0.002% rhodopsin. The relationship between rhodopsin bleaching and the amplitude of the transmission decrease is shown in Figure 6B.

The scattering signal reported above requires the presence of both extrinsic membrane proteins, disk membranes, and nucleotides. Reconstitution experiments indicate that different phases of the transmittance change have different relationships to both enzyme and nucleotide concentrations. The rela-

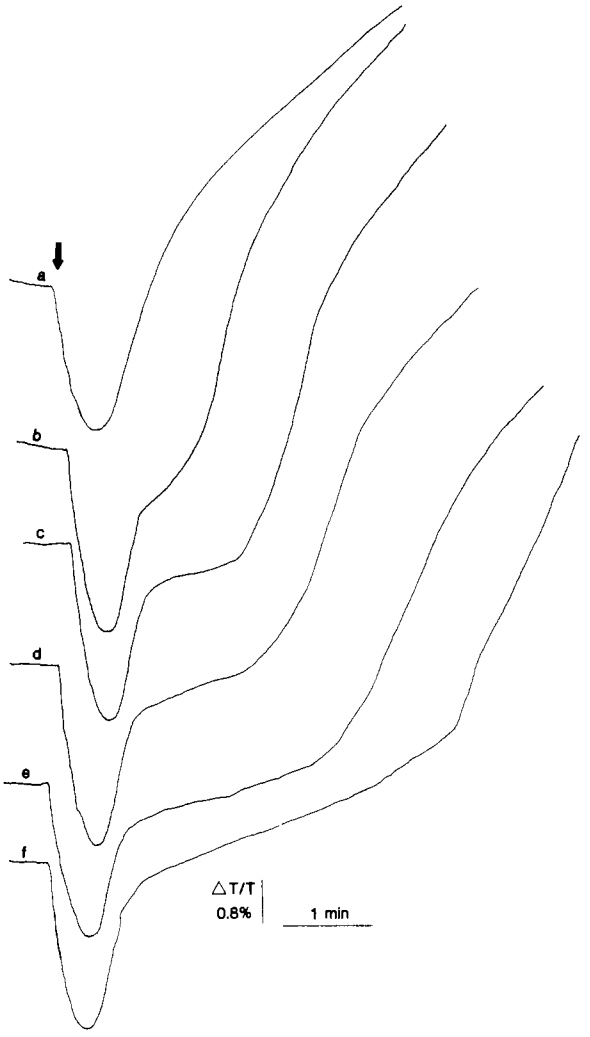

FIGURE 4: Effect of increasing concentrations of GTP on near-infrared (710 nm) light-scattering changes of disk membranes reconstituted with G protein and PDE. [Rhodopsin] = 2 μM, [G protein] = 200 nM, [PDE] = 60 nM, and [cGMP] = 1 mM. The flash (indicated by the arrowhead) bleached 0.2% rhodopsin. Trace a, [GTP] = 1 μM; trace b, [GTP] = 1.8 μM; trace c, [GTP] = 2.5 μM; trace d, [GTP] = 3 μM; trace e, [GTP] = 4 μM; trace f, [GTP] = 5 μM.

tionship between rhodopsin concentration and the speed of the D phase is a linear one (Figure 7A). The speed of the D phase is a quadratic function of the concentration of the extrinsic proteins (G protein and PDE) present (Figure 7B). The G protein concentration, while not affecting the amplitude of the D phase, increases the speed of this phase (Figure 7C). In reconstitution experiments, the complete scattering signal is observed with urea-washed membranes, as well as frozen–thawed and sonicated membranes. Urea-washed membranes are extremely leaky to ions (A. Caretta, unpublished results); thus, it appears unlikely that permeability changes are involved in the scattering signal. Urea treatment and freeze–thawing abolish the scattering signal if applied to G protein and PDE.

In an attempt to understand the physical basis of the scattering signal, we made both light and electron microscopic observations. To accomplish these experiments, we took advantage of the fact that addition of GTPγS and 8Br-cGMP to a fully bleached, reconstituted system results in a scattering signal almost identical with that elicited by flash-bleaching dark membranes in the presence of these nucleotides (Figure 8A; for comparison, see Figure 3B, trace f). When GTP is used instead of GTPγS, the transmission decrease is followed by a transmission increase which returns close to the starting

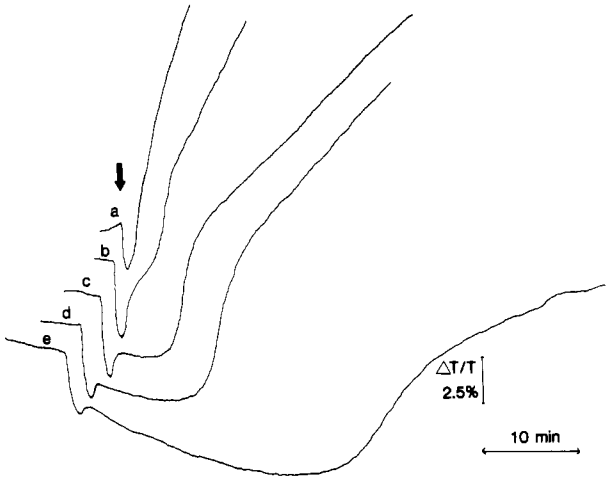

FIGURE 5: Near-infrared (710 nm) light-scattering changes as a function of temperature of disk membranes reconstituted with G protein and PDE. [Rhodopsin] = 2 μM, [G protein] = 200 nM, [PDE] = 60 nM, [GTP] = 2.5 μM, and [cGMP] = 1 mM. The flash bleached 0.2% rhodopsin. Trace a, T = 27.5 °C; trace b, T = 21.5 °C; trace c, T = 14.5 °C; trace d, T = 9.5 °C; trace e, T = 4 °C.

transmission level (compare Figure 8B and Figure 3B, trace e). The return occurs following completion of GTP hydrolysis (data not shown). Addition of a second aliquot of GTP elicits a second transmission change (Figure 8B). Nucleotide addition to dark-adapted membranes has no effect.

Light microscopic observations on unstained material (Figure 9) show that addition of GTPγS and 8Br-cGMP to bleached membranes, reconstituted with G protein and PDE, triggers the formation of large, irregular structures of 2–10 μm in length. Electron microscopic observations of negatively stained membranes, similarly reconstituted, demonstrate that these large structures are aggregates of disk vesicles (Figure 10). Microscopic observations (data not shown) made when GTP is added rather than GTPγS indicate that there is increasing disk vesicle–disk vesicle aggregation as the transmission decreases. As the transmission increase occurs, the vesicles disaggregate. The time course of these changes under these conditions is too rapid for us to photograph with our present technique. No aggregation phenomenon was observed when any one of the components (G protein, PDE, GTP, or cGMP) was absent from the reconstitution.

Discussion

The cGMP- and PDE-dependent infrared transmission changes reported in the present paper appear to be different from those previously reported. The binding and dissociation signals (Kuhn et al., 1981) are much smaller in amplitude and do not require PDE and cGMP. The infrared light-scattering changes reported by Uhl et al. (1979a,b) and Borys et al. (1983) are affected by cGMP, as is our signal, but require ATP rather than GTP. Their signal is also affected by nucleotides in dark-adapted membranes, while this does not occur in our system. Finally, Lewis et al. (1983) report a GTP-dependent infrared scattering signal. The initial transmission decrease (D phase) and the late recovery (L phase) observed in our experiments are similar to those observed in their experiments. In both systems, the latency before the L phase appears to be related to GTP concentration. However, in our experiments, both phases appear to be greatly enhanced by cGMP. The E phase and S phase observed in our experiments were not reported by Lewis et al. (1983). Reconstitution experiments indicate that G protein and PDE are essential for the expression of the complete signal. The ratio of these proteins is important in determining the amplitude and kinetics

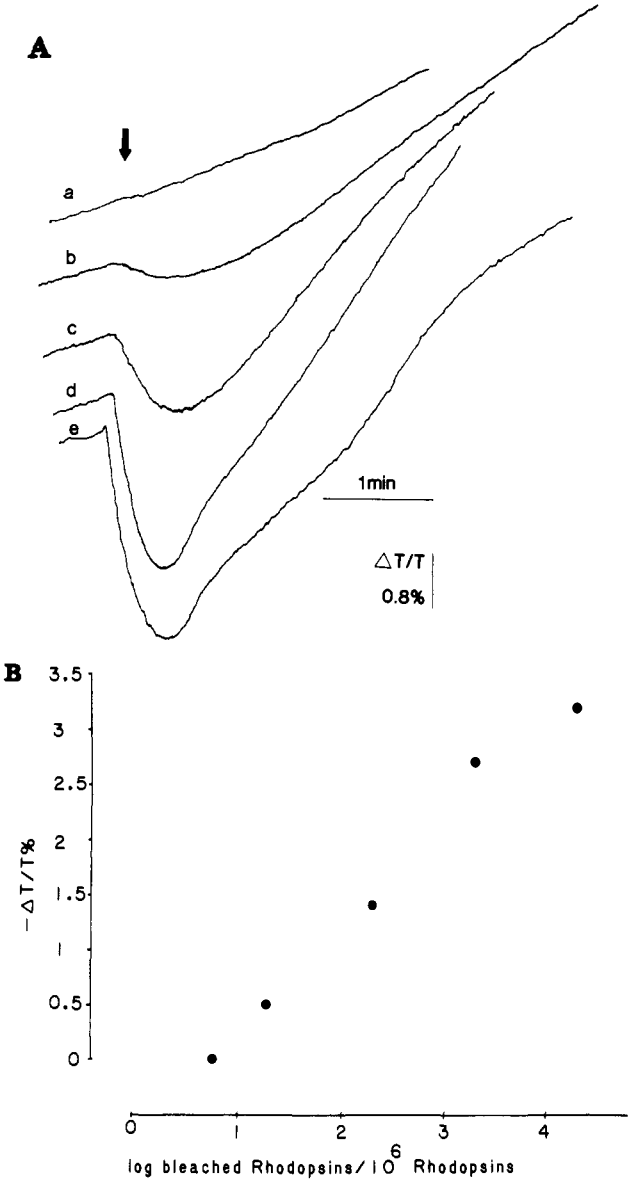

FIGURE 6: (A) Near-infrared (740 nm) light-scattering changes at different bleaching intensities in disk membranes reconstituted with G protein and PDE. The flash is indicated by the arrowhead. [Rhodopsin] = 2 μM, [G protein] = 600 nM, [PDE] = 60 nM, [cGMP] = 1 mM, and [GTP] = 5 μM. Bleaching intensities: trace a, 0.0006% rhodopsin; trace b, 0.002% rhodopsin; trace c, 0.02% rhodopsin; trace d, 0.2% rhodopsin; trace e, 2% rhodopsin. (B) Amplitude of the decrease in transmission after a flash of light as a function of rhodopsin bleached.

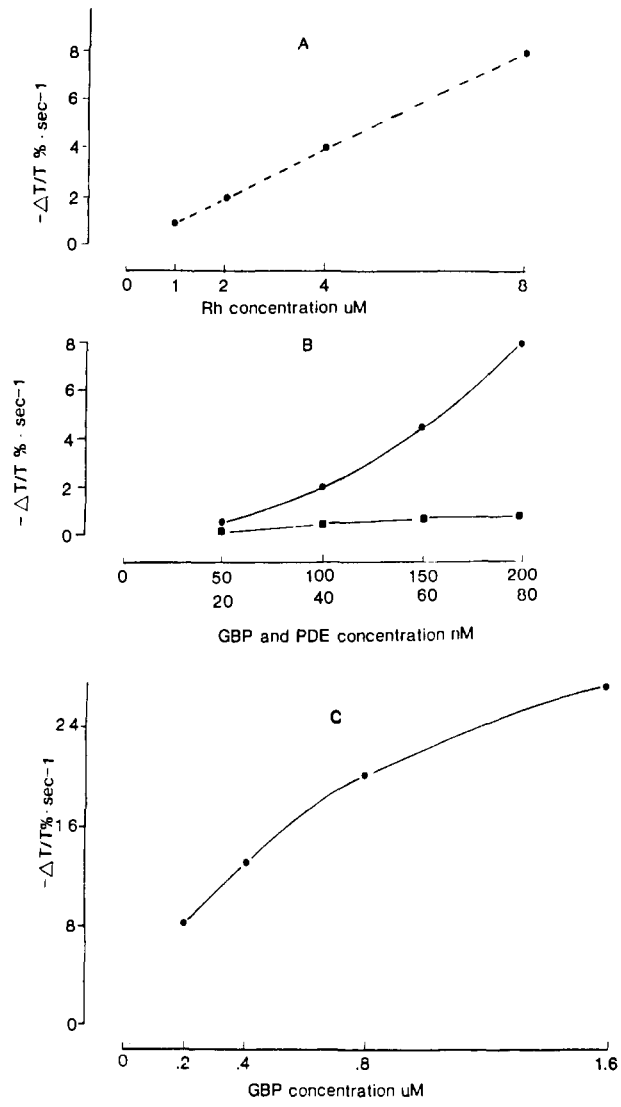

FIGURE 7: Initial speed of the transmittance decrease at 710 nm of disk membranes reconstituted with G protein and PDE. The flash bleached 0.2% rhodopsin. Every point, mean of three experiments. Standard deviation is less than 10%. (A) Initial speed as a function of rhodopsin concentration. [G protein] = 100 nM, [PDE] = 30 nM, [cGMP] = 1 mM, and [GTP] = 3 μM. (B) (Closed circles) Initial speed as a function of G protein and PDE concentrations: [rhodopsin] = 2 μM, [cGMP] = 1 mM, and [GTP] = 3 μM. (Closed squares) Initial speed as a function of G protein and PDE concentrations in the absence of cGMP. (C) Initial speed as a function of G protein concentration: [rhodopsin] = 2 μM, [PDE] = 60 nM, [cGMP] = 1 mM, and [GTP] = 3 μM.

of the infrared transmission change.

Using reconstitution experiments, we repeated and confirmed the results of Kuhn et al. (1981) showing the G protein binding and dissociation signals (Figure 2A, traces a and b). However, the addition of PDE and cGMP so decrease the latter signal that it becomes almost undetectable. This result demonstrates that the scattering signal we observed is almost as rapid as the dissociation signal. Since reconstitution of the system without PDE allows observation of the G protein signal, while reconstitution without G protein or GTP blocks our signal almost completely (Figure 2A, trace b), the rhodopsin–G protein interaction seems to be the initial event.

The full expression of the light-scattering signal also requires both GTP and cGMP. There is no direct relationship between the kinetics of nucleotide hydrolysis and the kinetics of the cGMP- and PDE-dependent infrared transmission changes. Both 8Br-cGMP and GTPγS are effective in supporting the initial transmission decrease (D phase), so the energy of nucleotide hydrolysis is not necessary to generate the D phase of the scattering signal. However, there is a relationship between nucleotide hydrolysis and two phases of the signal: the E and L phases only occur when cGMP (Figure 3A) and GTP (Figure 4), respectively, are almost completely hydrolyzed. Furthermore, the hydrolysis-resistant analogues 8Br-cGMP and GTPγS block the E and L phases, respectively.

Since electron and light microscopic observations indicate that changes in infrared light transmission are correlated with vesicle aggregation, it seems likely that the aggregation process is the physical basis for the cGMP- and PDE-dependent change in infrared transmission. The cGMP- and PDE-dependent light-scattering changes are of sufficient magnitude (10–50%) to rule out changes in the refractive index of the membranes as the sole causative factor. The change in vesicle aggregation is sufficiently large to account for the optical signal

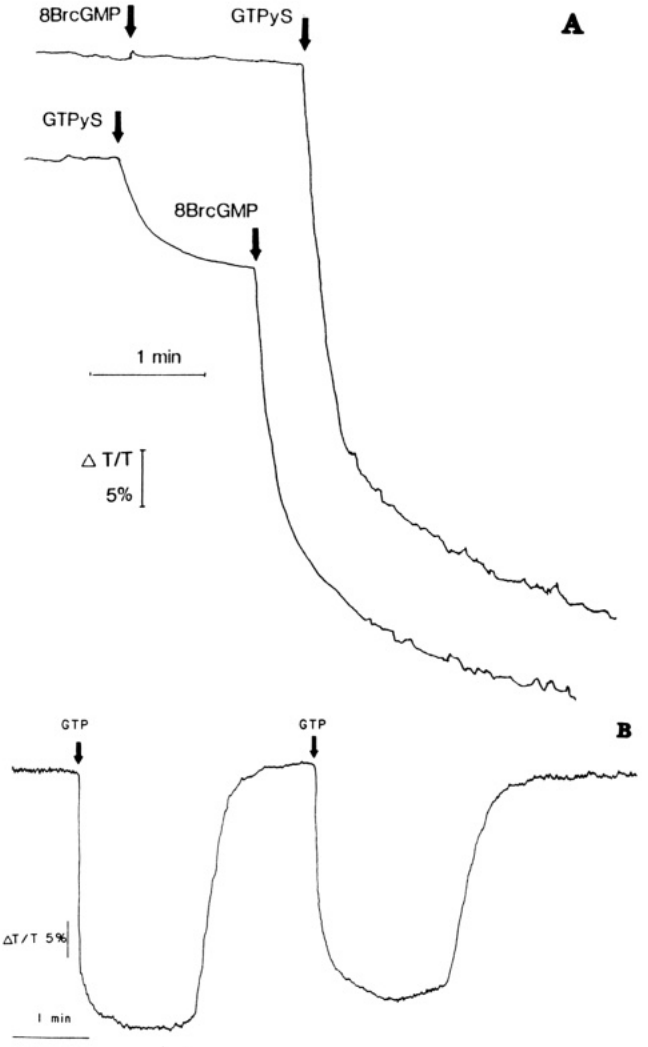

FIGURE 8: Addition of nucleotides to fully bleached disk membranes reconstituted with G protein and PDE: effect on near-infrared light scattering at 710 nm. [Rhodopsin] = 2.5 μM, [G protein] = 200 nM, and [PDE] = 60 nM. Membranes were stirred at 60 rpm. (A) After the addition, [8Br-cGMP] = 1 mM and [GTPγS] = 8 μM. Note that the addition of 8Br-cGMP in the absence of GTPγS is completely ineffective. (B) Effect of repeated GTP additions. After the addition, [GTP] = 5 μM. From the beginning of the experiment, 1 mM 8Br-cGMP is present in the cuvette.

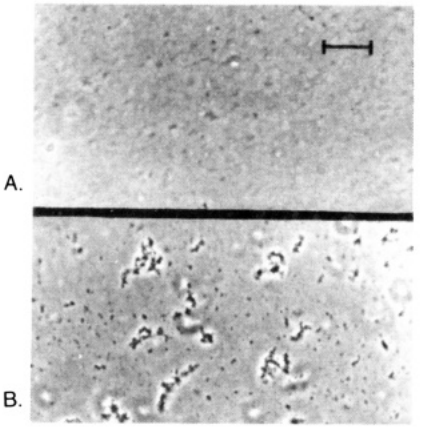

FIGURE 9: Light micrographs of disk membranes reconstituted with G protein and PDE. Bar = 7.5 μM. Both samples were fully bleached. [Rhodopsin] = 2.5 μM, [G protein] = 200 nM, and [PDE] = 80 nM. (A) No nucleotide added; (B) [GTPγS] = 8 μM, [8Br-cGMP] = 1 mM.

(increase in turbidity) observed (Benedek, 1971). The aggregation process requires the presence of G protein, PDE, cGMP, and GTP as does the light-scattering change. It is supported by hydrolysis-resistant GTP and cGMP analogues as is the light-scattering change. The observation that both vesicle aggregation and the cGMP- and PDE-dependent scattering changes are reversible when GTP is used (rather than GTPγS) supports the idea of a causal relationship between the two processes. Therefore, it appears likely that the light-activated cyclic nucleotide cascade controls the vesicle aggregation phenomenon. In this system, 8Br-cGMP has an apparent K_m of 125 μM, which is close to the K_m of the catalytic site but far removed from the high-affinity cGMP binding sites of PDE (Yamazaki et al., 1980, 1982). The K_m for GTPγS is 0.4 μM, close to the known K_m for the G protein (Wheeler et al., 1977).

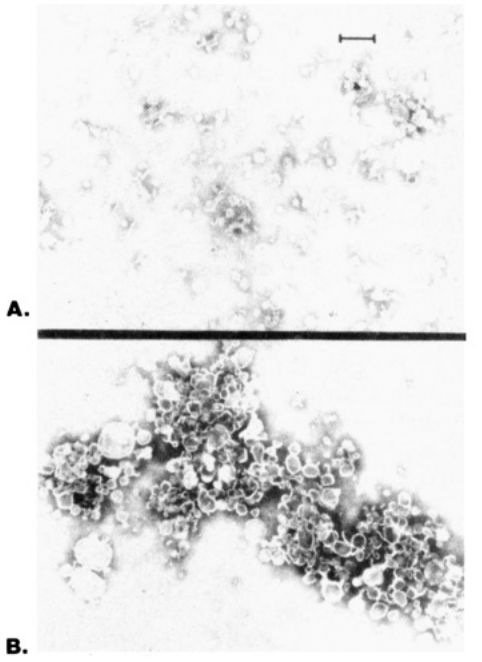

FIGURE 10: Electron micrographs of disk membranes reconstituted with G protein and PDE. Bar = 0.38 μM. Both samples were fully bleached. [Rhodopsin] = 2.5 μM, [G protein] = 200 nM, and [PDE] = 80 nM. (A) No nucleotides added; (B) [GTPγS] = 8 μM, [8Br-cGMP] = 1 mM.

In addition to infrared light-scattering changes which occur in suspensions of disk membranes, there have been reports of infrared transmission changes elicited by light flashes in isolated, superfused retinas (Harary et al., 1978; Akimoto, 1982). The relationship of the cGMP- and PDE-dependent infrared transmission changes observed in our study to those which occur in the intact retina is unclear. If there is a relationship between the signals observed in membrane suspensions and those in the intact retina, it might be possible to develop a method for studying biochemical parameters of the cyclic nucleotide system in intact photoreceptors.

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Registry No. PDE, 9068-52-4; cGMP, 7665-99-8; GTP, 86-01-1; 8Br-cGMP, 31356-94-2; GTPγS, 37589-80-3.

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