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Light- and nucleotide-dependent increase in apparent viscosity in a suspension of retinal disks

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Light triggers the cyclic nucleotide cascade in photoreceptor disk membranes. We report here that light-induced changes in the apparent viscosity of disk membrane suspensions can also be observed using either native disk membranes or washed membranes reconstituted with G protein and PDE. The viscosity changes are light- and GTP-dependent and require the presence of G protein and PDE. The magnitude of the viscosity change increases with increasing membrane concentration. Under the same conditions in which light elicits a change in viscosity, we observe a large increase in light scattering by the disk membrane suspension.

Photoreceptor; Viscosity; Rhodopsin; G-protein; Phosphodiesterase; Guanosine triphosphate; Disk membrane

1. INTRODUCTION

The cyclic nucleotide system of the rod photoreceptor is a well-established model for the study of receptor-mediated biochemical responses to receptor activation. The extensive analogy between the photoreceptor cGMP PDE and adenylate cyclase [1,2] has been extremely useful for extending our understanding of the molecular mechanisms which control both light-activated PDE and hormone or neurotransmitter sensitive adenylate cyclase. In both enzyme cascades, activation of the catalytic moiety requires receptor activation, a GTP binding protein (G protein), and GTP (or GTP analog). Fluoride activation of PDE and adenylate cyclase mediated by G protein is a feature of both systems [3]. Similarly, cholera and pertussis toxin labelling of G proteins in both systems regulates GTPase activity in similar ways [4,5]. Finally, both opsin and the β-receptor can be phosphorylated by kinases which specifically identify the activated receptor [6]. It is apparent that novel observations in the photoreceptor PDE system may be relevant to understanding the adenylate cyclase system and vice versa. Therefore, the present description of a light- and nucleotide-dependent increase in the apparent viscosity of disk membrane suspensions should be considered within the broader framework of reports which document changes in cell structure which occur in a variety of cell types following either receptor activation or electrical stimulation.

In rod disk membranes, light activation of GTPase and PDE alters their binding and release from disk membranes in a nucleotide-dependent manner [7,8]. G protein binding and release are correlated with infrared light scattering changes in disk vesicle suspensions [9,10]. We previously

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Abbreviations: G protein, three polypeptide subunits of molecular masses 39, 37 and 6 kDa which comprise the GTPase; PDE, three polypeptide subunits of 94, 92 and 13 kDa which comprise phosphodiesterase; GTP, guanosine triphosphate; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); cGMP, guanosine cyclic 3′,5′-monophosphate; DTT, dithiothreitol

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observed that under conditions in which a large light scattering increase occurs, PDE binding to the disk membrane increases, and disk membrane vesicles aggregate [8,11]. In this report, we show that in the presence of extrinsic membrane proteins (PDE and G protein), bleached rhodopsin and GTP (or its hydrolysis-resistant analogues), a large increase in the viscosity of the disk membrane suspension occurs. Since these conditions are similar to those necessary to observe light- and nucleotide-dependent increases in infrared light scattering, PDE binding and vesicle-vesicle aggregation, we believe that there is a relationship between these phenomena.

2. MATERIALS AND METHODS

Membranes were prepared as described [8,11]. Fresh bovine eyes were dissected under dim red light (Kodak Wratten no.1). The retinas were shaken in 40% sucrose containing 130 mM KCl, 20 mM Tris-HCl (pH 7.7), 2 mM $MgCl_2$, and 5 mM dithiothreitol (DTT), 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. Toad disk membranes were prepared according to the same procedure with assistance of infrared image converters. The toads (*Bufo marinus*) were dark-adapted overnight prior to the experiments. The harvested disk membranes were washed twice with 130 mM KCl, 20 mM Tris-HCl (pH 7.7), 2 mM $MgCl_2$, and 5 mM DTT (250 μl/bovine retina, 50 μl/toad retina). G protein and PDE were extracted as described [8,11]. Enzymes and membranes (at 1 mM rhodopsin concentration) were stored at 0°C in 5 mM Tris-HCl (pH 7.8), 5 mM DTT and 130 mM KCl, 20 mM Tris-HCl (pH 7.7) and 2 mM $MgCl_2$, respectively. Experiments were performed within 72 h of the completion of the preparation. Rhodopsin concentration was estimated as described in [11].

Viscosity measurements were made with the falling ball method [12]. Either fully bleached, native bovine membranes or dark-adapted toad membranes reconstituted with bovine peripheral enzymes (G protein and PDE) were used. Fully bleached bovine membranes at 1 mM rhodopsin concentration were diluted to different rhodopsin concentrations by adding KCl solution on a weight/weight basis. After equilibration at 25°C for 20 min, every sample was divided into 2 parts: control and GTP (1 mM) or GTPγS (100 μM) treated. The samples were then aspirated into a glass capillary (i.d. = 1.3 mm), and allowed to settle for 6 min. The apparent viscosity was measured with the falling ball method, using steel balls with a diameter of 0.64 mm. The apparatus was standardized using sucrose and glycerol solutions of known viscosity. Dark-adapted toad membranes were reconstituted with bovine PDE and G protein: reconstitution experiments were made by mixing bovine PDE and G protein with toad membranes washed twice at low ionic strength (2 mM Tris-HCl (pH 7.7), 5 mM DTT, using 10 ml/mg rhodopsin) to remove peripheral proteins. Concentrated KCl, Tris-HCl (pH 7.7) and $MgCl_2$ solutions, were added to yield final concentrations of 130, 20 and 2 mM, respectively. After reconstitution, the protein concentrations were: 500 μM rhodopsin, 80 μM G protein and 8 μM PDE. Lower rhodopsin concentrations were obtained by diluting the original sample with KCl solution. GTPγS was added to a final concentration of 100 μM. The samples were equilibrated at 25°C and then aspirated into a glass capillary and allowed to settle for 5 min. The viscosity was measured with the falling ball method. The rate of fall of 2 steel balls was measured in the dark sample with a 3 min interval between each ball. 2 min after a flash of 450 nM light (bleaching 0.5% rhodopsin) the fall of 2 additional balls was measured with a 3 min interval between tests. For the performance of the dark experiments all procedures were performed with aid of infrared image converters. For a discussion of the falling ball method for measuring the viscosity of non-Newtonian fluids see [12].

Light scattering measurements were performed as previously described [8,11] using a Shimadzu UV-3000 recording spectrophotometer. The measuring beam was 840 nm and a 1 mm optical path was used. The apparent absorbance of the samples was 0.8.

3. RESULTS

Fig.1A shows the viscosity of bleached bovine disk membranes in the presence and absence of GTPγS (an hydrolysis-resistant analogue of GTP).

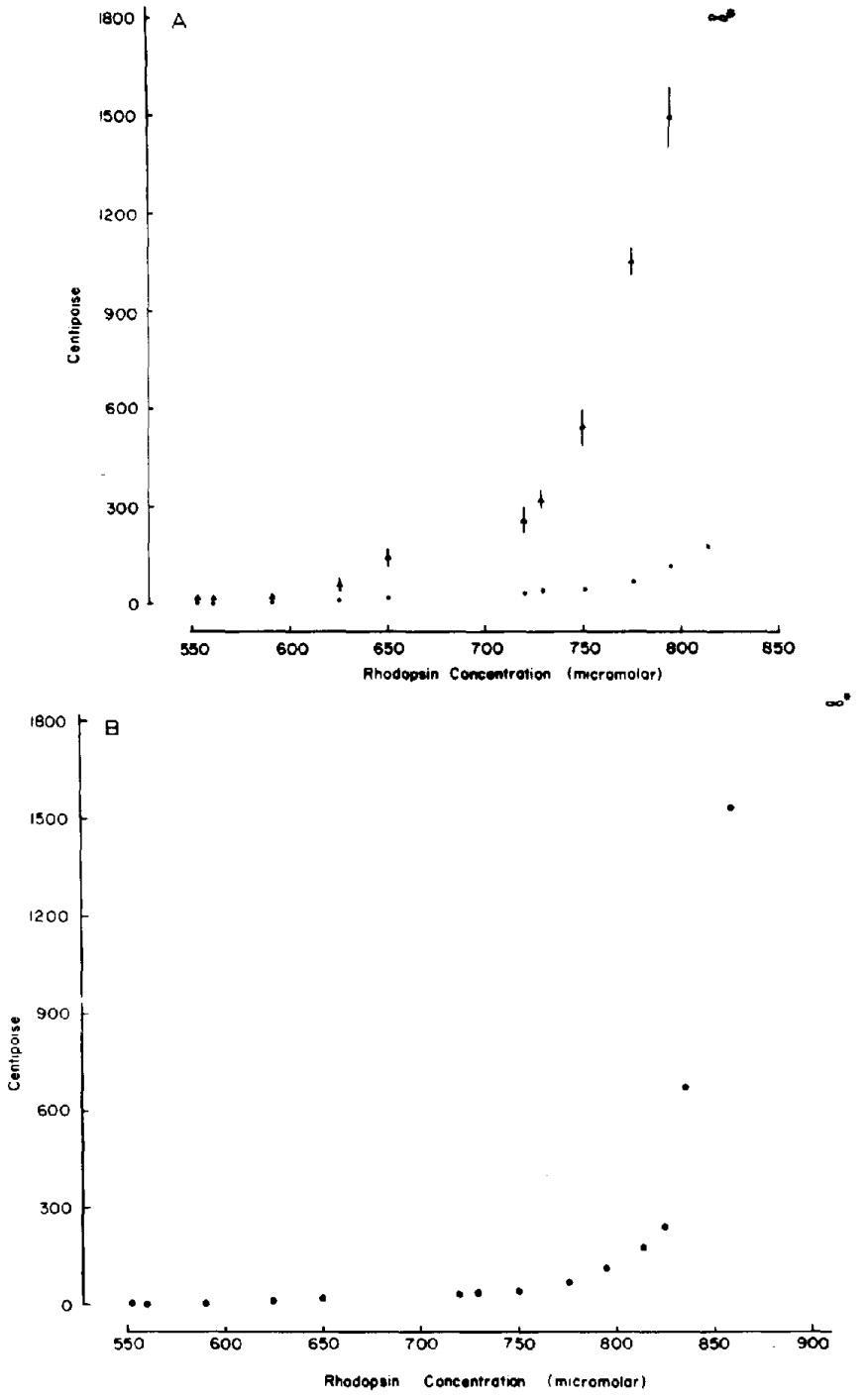

Fig.1. (A) Filled circles: apparent viscosity of a suspension of bovine bleached membranes in the absence of nucleotides. Triangles: apparent viscosity of bleached bovine disk membrane suspensions in the presence of 100 μM GTPγS or 1 mM GTP. Every point: mean ± SD of 3 experiments. The rhodopsin concentration of each sample was adjusted to the value indicated by adding KCl solution to the membranes on a weight/weight basis. (B) Apparent viscosity of bovine disk membranes in the absence of nucleotides, as a function of rhodopsin concentration.

Addition of this nucleotide increased the apparent viscosity of the disk membrane suspension. This increase became more evident at higher rhodopsin concentrations. This nucleotide-dependent increase in apparent viscosity was abolished by repeatedly washing disk membranes at low ionic strength (1 mM Tris-HCl, pH 7.7, 2 ml/mg rhodopsin, 3 times), by treatment of the membranes with 8 M urea, or by treatment of the membranes with proteolytic enzyme trypsin (2 μg trypsin/mg rhodopsin, 10 min). Readdition of extrinsic membrane proteins to low ionic strength washed membranes restores the GTP effect on viscosity. Both G protein and PDE are needed for this effect. Reconstitution with G or PDE only leads to a minimal viscosity increase upon addition of GTP or GTPγS (not shown). GTP at 1 mM concentration has an effect on viscosity similar to that of GTPγS, although with GTP the apparent viscosity increase was reversible. ATP addition did not increase the apparent viscosity. The presence of 1 mM $CaCl_2$ or 1 mM EGTA did not change the effect of GTP. Fig.1B shows the apparent viscosity of a suspension of bovine disks measured as a function of rhodopsin concentration, in the absence of nucleotides. It is important to consider that the rhodopsin concentration employed in this experiment was far below the 2–3 mM concentration present inside the rod photoreceptor [13,14]. Thus, if there is an increase in viscosity in intact photoreceptor cells it is likely to be quite large.

In order to demonstrate that the viscosity change was light-dependent, we employed disk membranes from dark-adapted toad retinas prepared with infrared image converters to avoid photobleaching. Fig.2A shows the effect of visible light (λ = 450 nm, bleaching 0.5% rhodopsin) on the apparent viscosity of a preparation of toad disk membranes reconstituted with bovine extrinsic membrane proteins. In the presence of GTPγS or GTP, a light flash caused an increase of the apparent viscosity of the suspension. No effect was observed in the absence of nucleotides. It should be noted that the apparent viscosity of toad membranes was higher than those of bovine membranes at similar rhodopsin concentrations. Furthermore, the relationship between apparent viscosity and rhodopsin concentration varied between different toad membrane preparations (at rhodopsin = 500 μM, apparent viscosities in the dark were 11, 15, and 20 cp). The amplitude of the light-induced increase in these preparations also varied (at rhodopsin = 500 μM, apparent viscosities in the light were 24, 60 and 78 cp, respectively). Under the same experimental conditions (using a cuvette with a 1 mm optical path) a large light-dependent increase in turbidity was observed (fig.2B).

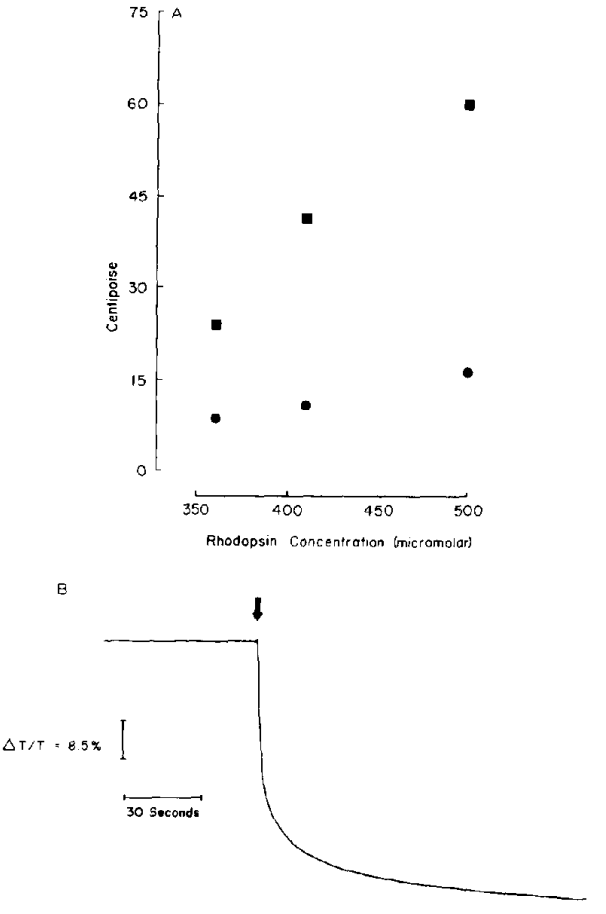

Fig.2. (A) Effect of light on viscosity of a suspension of dark-adapted toad disk membranes reconstituted with bovine G protein and PDE. The squares and circles indicate the apparent viscosity values measured in the bleached and dark-adapted samples, respectively. (B) Light-dependent turbidity change in the same membrane preparation used for experiments in A. Rhodopsin (350 μM), G protein (56 μM), PDE (5.6 μM), GTPγS (100 μM). The stimulating flash (λ = 450 nm) bleached 0.5% rhodopsin. Light scattering measurements were made at 840 nm, as described [8] except that a 1 mm optical pathlength cuvette was used. The apparent absorbance of the sample was 0.8.

In our previously published results [8,11], we observed an effect of cGMP (or its hydrolysis resistant analogues) on our cGMP- and PDE-dependent scattering signal. We were unable to observe any effect of cGMP on either light scattering or viscosity in these experiments. We believe that the much higher membrane and enzyme concentrations used in these experiments may account for this difference.

4. DISCUSSION

The data presented above demonstrate that a change in the apparent viscosity of rod disk suspensions occurred under conditions identical to those in which infrared light scattering changes were measured. The protein and nucleotide requirements for both phenomena were similar. Both phenomena were elicited by light in dark-adapted membranes or by nucleotide addition to bleached membranes. These effects were observed in disk membranes with their original complement of peripheral membrane proteins or with low ionic strength washed membranes reconstituted with peripheral membrane proteins. Our previous work indicated that these changes in infrared light scattering properties of disk membrane suspensions reflected light-induced activation of PDE, which resulted in increased disk vesicle aggregation [8,11]. We suggest that these phenomena regulate the increase in apparent viscosity of the disk membrane suspensions.

Since the GTP-dependent viscosity increase appeared to depend on protein-protein interactions which occurred between disk vesicles (that is, particles whose radii are much larger than the radii of ions like Na, K, Ca, or nucleotides) a significant decrease in the diffusion coefficient of small molecules would not be predicted to result from the increase in viscosity. We were unable to observe a change in the conductivity of a disk suspension (130 mM NaCl or KCl, 20 mM Tris-HCl (pH 7.7), 2 mM $MgCl_2$) under conditions in which a large GTP-dependent increase in viscosity occurred (not shown). However, it is possible that the increase in viscosity which we measured might affect the diffusion of proteins involved in visual excitation. If viscosity changes occur in vivo, as they appear to occur in vitro, then viscosity changes might regulate the kinetics of protein-protein interactions of the cyclic nucleotide cascade and participate in regulation of photoreceptor cell electrophysiology.

A variety of studies have demonstrated that cells can respond to activation of cell membrane receptors by altering their morphology. Application of β-adrenergic agonists to cultured cilial epithelium results in a profound rearrangement of cytoskeletal proteins and the appearance of a stellate morphology in these cells [15]. Application of follicle-stimulating hormone to cultured Sertoli cells results in similar morphological and cytoskeletal changes [16]. Electrical stimulation of cochlear hair cells produces changes in their length [17]. These cells became longer in response to hyperpolarizing currents and shorter in response to depolarizing currents. Light elicits mechanical responses in the isolated squid retina which must reflect an underlying change in the structure of the cell [18]. Light-induced changes in the infrared transmission properties of the isolated toad retina have their origin in the outer segment [19]. Furthermore, a light-dependent decrease in the interdiskal spacing occurs [20]. While we have no direct evidence for a viscosity change occurring in the intact photoreceptor cell, it is clear that receptor-mediated changes in cellular structures occur in rod photoreceptors as well as other cell types and that the physiological relevance of these changes is not well understood. Further investigation of these receptor-mediated events are necessary to fully understand their functional significance.

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