Rotational Diffusion of Rhodopsin in the Visual Receptor Membrane: Effects of Temperature and Bleaching†

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ABSTRACT: Rhodopsin present in bovine rod outer segments was covalently labeled with the triplet probe erythrosinyliodoacetamide. The rotational diffusion of the protein in the membrane was studied by measuring the time dependence of the phosphorescence emission anisotropy. In this way it was possible to monitor directly the rotational diffusion of both bleached and unbleached rhodopsin. Nonlinear least-squares analysis of the resulting anisotropy decay curves revealed changes in the rotational dynamics of the protein as a function of temperature and photobleaching. By assuming the coexistence of mobile and immobile protein fractions, it was shown that a decrease in temperature is capable of inducing aggregation of both bleached and unbleached rhodopsin. Differences in the time dependence of the emission anisotropy between bleached and unbleached rhodopsin are interpreted as providing evidence that rhodopsin undergoes a conformational change upon bleaching. Information on the specific binding site of the erythrosin probe indicates that this change is localized in the C-terminal region of the protein. The steady-state fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene was measured as a function of temperature. A close agreement was found between the activation energy of the fluorescence depolarization and the activation energy of the relaxation of bleached rhodopsin.

Rhodopsin comprises 80–90% of the membrane-bound protein content of vertebrate rod outer segments (ros)[1] (Papermaster & Dreyer, 1974). This, combined with the relative ease of purification of rhodopsin and its importance in the visual cycle, has led to extensive research into all aspects of the protein's behavior. In particular, because of its importance as the primary event in transduction, photobleaching of rhodopsin and its immediate consequences have been studied with a wide range of techniques.

Following light absorption by rhodopsin, which causes isomerization of the bound retinal chromophore from the 11-cis to the all-trans form, there is a series of spectrally well-defined reactions terminating in dissociation of the chromophore from the protein (Wald, 1968). These spectral changes are, in turn, believed to result in a number of conformational and chemical changes in rhodopsin leading to the catalysis of certain biochemical events in the ros (Kuhn et al., 1982; McDowell & Kuhn, 1977). Due to its membrane-bound characer, it has proven difficult to obtain details of any light-induced changes in the molecular structure of rhodopsin. Evidence that changes do occur has come primarily from various enzymatic and chemical modification studies (Kuhn et al., 1982; Pellicone et al., 1985; Sale et al., 1977). Increases in the sulfhydryl reactivity (DeGrip et al., 1973) and an enhanced susceptibility to enzymatic digestion (Kuhn et al., 1982; Hargrave et al., 1982) have suggested the exposure of a number of amino acid residues to the extradiscal space possibly associated with partial unfolding of the protein. It has recently been shown, by studies of cyanogen bromide (CNBr) cleavage of methionine residues, that light-induced conformational changes may be occurring in particular extradiscal loops (Pellicone et al., 1985).

By investigating the rotational dynamics of membrane proteins, it has been shown (Restall et al., 1985) that it is possible to detect conformational changes occurring within the protein. Rhodopsin was first shown to exhibit rapid rotational diffusion in the plane of the membrane by photodichroic studies carried out by Cone (1972). While it is possible to look at the rotation of unbleached rhodopsin by such optical methods using the intrinsic chromophore, to investigate rotational diffusion of the protein in the bleached state, it becomes necessary to attach an extrinsic probe molecule.

The use of triplet probes to investigate the rotational dynamics of membrane proteins that lack an intrinsic chromophore was first proposed by Razi-Naqvi et al. (1973). Such probes have subsequently been used to study the rotation of a number of different membrane proteins [reviewed by Cherry (1979) and Kinosita et al. (1984)]. By covalently labeling rhodopsin with the phosphorescent probe erythrosinyliodoacetamide, we have been able to investigate the rotational mobility of the protein in the membrane, as a function of temperature, in both the bleached and unbleached state. Direct evidence has been obtained for a light-induced conformational change that is most likely associated with the C-terminal region of rhodopsin that extends into the extradiscal space.

Materials and Methods

Isolation of ros Membranes. Cattle eyes were obtained fresh from a local slaughterhouse and stored in an ice box within 30 min of enucleation. All subsequent operations were carried out under dim red illumination or in total darkness, at 0 °C.

Broken rod outer segments (ros) were prepared and purified by the method given by Papermaster & Dreyer (1974). All buffers used in the preparative stages contained 2 mM dithiothreitol to help prevent oxidative damage to ros components. Purified ros fragments were stored at −20 °C, in 10

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[1] Abbreviations: ros, rod outer segments; DPH, 1,6-diphenyl-1,3,5-hexatriene; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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mM sodium phosphate buffer, pH 7.4, containing 5% glycerol.

The concentration of unbleached rhodopsin, as a percentage of the total protein present, was determined as the difference in absorbance, at 500 nm, between a sample of purified ros before and after bleaching (extinction coefficient 40 000 L mol^{-1} cm^{-1}) (Zimmerman & Godchaux, 1982). For these measurements and for all subsequent experiments requiring bleached material, bleaching was carried out by exposing unbleached material to white light from a 100-W bulb, at a distance of 1 m, for at least 10 min.

Total protein concentration was assayed by the method of Lowry et al. (1951) modified such that the alkaline copper reagent contained 0.1% (w/v) sodium dodecyl sulfate. The discontinuous polyacrylamide gel technique of Laemmli (1970) was used to check the purity of the ros preparations.

Labeling with Erythrosinyliodoacetamide. For a series of flash photolysis measurements, purified ros membranes, containing approximately 1 mg of protein, were washed and resuspended in 10 mM sodium phosphate buffer, pH 7.4. Sufficient erythrosinyliodoacetamide (Molecular Probes, Inc., Junction City, OR 97448) was added to give a 2:1 molar ratio of probe to protein. This mixture was incubated, at room temperature, for 1 h in the dark, after which the material was washed 4 times in 10 mM sodium phosphate buffer, pH 7.4, to remove all the unreacted iodoacetamide. All washings were carried out by centrifugation at 20000*g* for 20 min. The final pellet was resuspended in 10 mM sodium phosphate buffer, pH 7.4, containing 66% glycerol and 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA). The presence of high glycerol concentrations has previously been used (Muhlebach & Cherry, 1985; Restall et al., 1984) to slow vesicle tumbling and prolong the triplet lifetime of the probe. The sample was thoroughly deoxygenated with nitrogen to prevent oxygen quenching of the triplet state of the probe, prior to performing the flash photolysis measurements.

Flash Photolysis Measurements. The flash photolysis apparatus used is essentially the same as that described by Restall et al. (1984, 1985). The laser-induced phosphorescence was collected from both sides of the sample cuvette, perpendicular to the direction of the laser beam, and focused through polarizers oriented parallel on one side and perpendicular on the other side to the electric vector of the laser excitation. This was oriented vertically, normal to the plane of excitation and emission, yielding signals $I_{VV}(t)$ and $I_{VH}(t)$, respectively. Collection of these data sets was alternated with that of the same two emission components excited by horizontally polarized radiation. The collection of these two components [$I_{HV}(t)$ and $I_{HH}(t)$] was necessary in order to allow correction for differences in the efficiency of registration of the orthogonally polarized emission components.

Ideally, $I_{HV}(t)$ and $I_{HH}(t)$ are identical, and their ratio, $G = \sum I_{HH}(t)/\sum I_{HV}(t)$, is independent of both the decay of the excited state population and its depolarization. In practice, small variations in the value of the G parameter occur during the data collection. However, after application of the ratio to correct the relative values of $I_{VV}(t)$ and $I_{VH}(t)$, consistent experimental phosphorescence anisotropy decays, $R(t)$, defined by

$$R(t) = [I_{VV}(t)G - I_{VH}(t)]/[I_{VV}(t)G + 2I_{VH}(t)] \quad (1)$$

were obtained.

$I_{VV}(t)$ and $I_{VH}(t)$ signals from 64 laser shots were averaged to obtain an anisotropy decay curve for each sample of bleached rhodopsin. $I_{HV}(t)$ and $I_{HH}(t)$ curves were obtained by averaging data from 32 laser shots before and 32 shots after the anisotropy measurements. Final anisotropy decay curves were obtained by combining data from three separate samples. These were, in turn, derived from at least three different ros preparations.

In the case of measurements carried out on unbleached rhodopsin, an anisotropy decay curve was obtained from a single laser shot. This curve was corrected with a G signal from 32 laser shots fired after the anisotropy measurement had been taken. This was necessary since the action of irradiating the sample with the laser was sufficient to cause some bleaching of the rhodopsin present in the sample. In order to obtain final decay curves significantly free of noise, at least 10 preparations of unbleached rhodopsin had to be used.

Purified ros membranes were labeled with 1,6-diphenyl-1,3,5-hexatriene (DPH) by incubation, for 1 h at room temperature, with enough of the probe to give a final lipid:probe ratio of 500:1. The ros were suspended in 10 mM sodium phosphate buffer, pH 7.4, containing 66% glycerol and 0.5 mM EGTA. Fluorescence polarization measurements were performed on an Elscint Model MV1a microviscosimeter.

Results

Gel electrophoresis has revealed that the protein content of the purified ros is almost entirely accounted for by rhodopsin, apparent as a distinct band of approximate M_r 40 000. Bleaching had no effect on the band pattern obtained. Absorbance data at 500 nm showed that some 80% of the protein present, as determined by the method of Lowry et al. (1951), in the purified ros comprised unbleached rhodopsin.

Probe Location. In all studies of rotational diffusion utilizing extrinsic probe molecules it is important to determine that the probe is binding to the protein and not the lipid component of the membrane. A small quantity of purified ros, after incubation with erythrosinyliodoacetamide in a 2:1 molar ratio of probe to protein, was extracted with excess chloroform–methanol (2:1 v/v). Water was added and the mixture vigorously shaken. After settling, the mixture separated into a two-phase system containing the lipid in the lower organic phase and any unreacted probe in the upper aqueous layer. Denatured protein partitioned at the interface of the two layers. No significant amounts of probe were found to be associated with the ros lipids, whereas about 10% of the probe was covalently bound to the protein fraction. The other 90% remained in the aqueous phase. Overall, this indicates that about 20% of the protein became labeled with probe after the incubation. The low level of probe that becomes covalently bound to rhodopsin makes it necessary to ensure that samples are thoroughly washed prior to the flash photolysis measurements. Following our washing procedure, no significant amount of probe was found to be noncovalently bound to the protein.

Flash Photolysis Measurements. Data for the rotational diffusion of bleached and unbleached rhodopsin were obtained from three separate ros preparations. For each preparation, measurements were taken from three separate samples of bleached and ten separate samples of unbleached material. Phosphorescence depolarization of erythrosin-labeled bleached rhodopsin was investigated over a range of temperatures between 5 and 45 °C. Unbleached material was examined at 5 and 35 °C.

Theoretically, the time dependence of the anisotropy for a simple system comprising a protein rotating uniaxially about the membrane normal is given by

$$R(t) = A_1 \exp(-t/\phi) + A_2 \exp(-4t/\phi) + A_3 \quad (2)$$

where $A_1 = (6/5)(\sin^2\theta \cos^2\theta)$, $A_2 = (3/10)(\sin^4\theta)$, $A_3 = (1/10)(3\cos^2\theta - 1)^2$, and the relaxation time $\phi = 1/D$, where

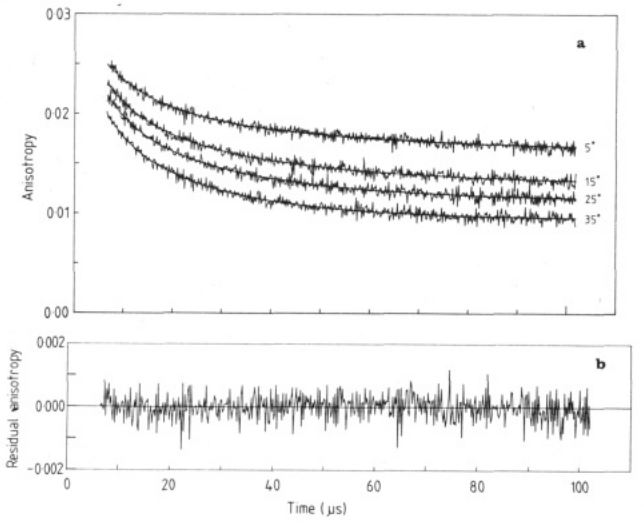

FIGURE 1: (a) Temperature dependence of $R(t)$ curves for bleached rhodopsin labeled with erythrosinyliodoacetamide present in the ros membrane. The data are shown for each temperature with the solid lines representing the best fit to eq 2. (b) Residuals for the fit to the curves for $R(t)$ for bleached rhodopsin at 5 °C. Similar results were obtained for all the temperatures examined.

Table I: Results of a Nonlinear Least-Squares Analysis of Cumulated Anisotropy Data Sets for Bleached Rhodopsin[a]

temp (°C)	A_1	A_2	A_3	ϕ (μs)	R_0
5	0.005 6	0.0076	0.0163	38.7	0.0295
15	0.007 8	0.0083	0.013	32.4	0.0291
25	0.009 1	0.0087	0.0113	27.7	0.0291
35	0.001 04	0.0089	0.0094	22.8	0.0287
45	0.009 1	0.0089	0.0089	23.5	0.0269

[a] Parameters are for best fits to eq 2.

D is the diffusion coefficient for rotation about the membrane normal (Cherry, 1978). However, in many cases involving studies of proteins using triplet probes, the rotational diffusion has proved to be more complex because of either the presence of different populations of proteins (Nigg & Cherry, 1979) or the existence of polypeptide segments on the protein (Burkli & Cherry, 1981; Restall et al., 1984). Nevertheless, the decay of anisotropy for rhodopsin proved to be well described by eq 2. This is illustrated in Figure 1a, which shows the decay of the phosphorescence emission anisotropy for bleached rhodopsin at different temperatures. In each case, the decay curves begin at 5 μs as data obtained at times earlier than this were found to be unreliable due to the transient overloading of the detection system following the laser flash (Restall et al., 1984). The fitted curves are shown as the solid lines through the data with the associated residuals for the data set at 5 °C shown in Figure 1b and serve to illustrate the good fit of eq 2 to the data. As the temperature is increased, the decay becomes more rapid and $R(t)$ decays to a lower time-independent value. These changes are reflected in the values of the constants obtained from the nonlinear least-squares analysis (Table I). Two distinct trends are apparent; first, there is a progressive decrease in the time-independent term (A_3) as the temperature is increased, and second, the relaxation time (ϕ) is getting shorter with increasing temperature. Exposure of the ros membranes to periods of illumination of up to 2 h induced no further changes in any of the parameters.

To investigate further the temperature dependence of the relaxation time, the data were plotted in the form of an Arrhenius representation. The results are shown in Figure 2. It is evident that the process occurs with a single apparent activation energy of −2.97 kcal mol^{-1} up to 35 °C. A similar

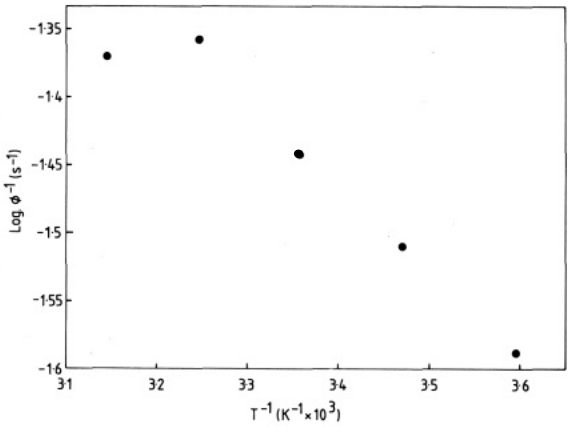

FIGURE 2: Arrhenius plot of the relaxation time (ϕ) for bleached rhodopsin obtained from the results of the nonlinear least-squares analysis of the $R(t)$ data. An apparent activation energy of −2.97 kcal mol^{-1} is obtained between 5 and 35 °C.

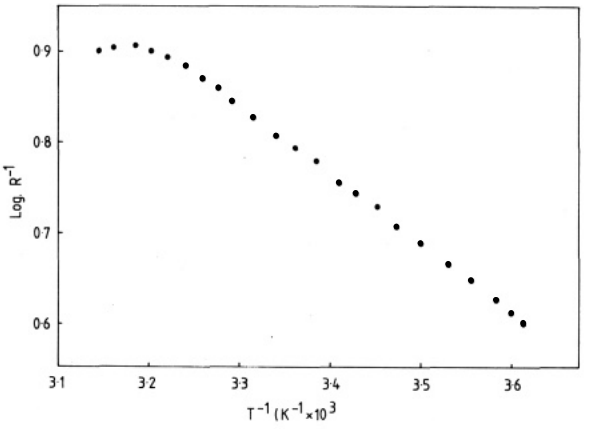

FIGURE 3: Arrhenius plot of the steady-state fluorescence anisotropy of DPH incorporated into ros membranes suspended in 10 mM sodium phosphate buffer, pH 7.4, containing 0.5 mM EGTA and 66% glycerol. An apparent activation energy of −3.16 kcal mol^{-1} is found between 5 and 38 °C.

Arrhenius plot of the steady-state fluorescence anisotropy of DPH in the ros membrane is shown in Figure 3. Once again, the anisotropy shows a constant activation energy, −3.16 kcal mol^{-1}, up to 38 °C. However, the results obtained at higher temperatures are somewhat anomalous. The relaxation time associated with the protein at 45 °C is slightly higher than at 35 °C, a reversal of the trend observed between 5 and 35 °C. Similarly, the fluorescence anisotropy of DPH no longer continues to decrease at temperatures above 38 °C. In both cases, the changes seen at high temperatures most likely represent thermal denaturation of the sample and are not assigned any significance.

The rotational dynamics of unbleached rhodopsin have also been examined. Figure 4a shows the time dependence of the emission anisotropy at 5 and 35 °C. The 35 °C data are shown only as the fitted curve for reasons of clarity, the signal to noise ratio being similar to that presented for the data set at 5 °C. The associated residuals for 5 °C are presented in Figure 4b. The higher level of noise present in these signals results from the fact that the data have to be accumulated as a series of single-shot measurements on independent samples. Nevertheless, it is immediately apparent that the decay of the anisotropy is similar to that of the bleached material but the time-independent value is lower than that observed after bleaching.

Table II shows the parameters derived from the nonlinear least-squares analysis of the data derived from the unbleached

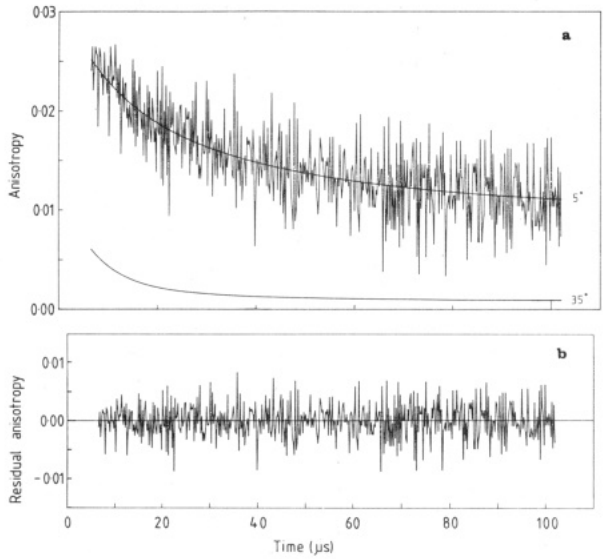

FIGURE 4: (a) $R(t)$ curves for unbleached rhodopsin at 5 and 35 °C. Best fits to eq 2 are shown at each temperature. For clarity, experimental data are shown only for 5 °C. A similar signal to noise ratio was obtained for data at 35 °C. (b) Residuals for the fit to the curve obtained at 5 °C for unbleached rhodopsin. A similar fit was obtained for the data at 35 °C.

Table II: Results of Nonlinear Least-Squares Analysis of the Cumulated Anisotropy Data Sets for Unbleached Rhodopsin[a]

temp (°C)	A_1	A_2	A_3	ϕ (μs)	R_0
5	0.0118	0.0094	0.01	42.0	0.0312
35	0.0018	0.0122	0.001	23.5	0.015

[a] Parameters are for the best fits to eq 2.

rhodopsin samples. As with the bleached samples, the value of ϕ is observed to get smaller with an increase in temperature due to more rapid diffusion of the protein, and there is a decrease in the constant A_3 term. Changes in both the relaxation times and the A_3 value were shown to be reversible with temperature, indicating that no permanent alterations had been invoked in the ros.

Discussion

In this study we have successfully labeled bovine rhodopsin with the phosphorescent probe erythrosinyliodoacetamide and investigated the rotational dynamics of the protein in the ros membrane. Previous studies (Wu & Stryer, 1972) have shown that iodoacetamide derivatives react preferentially with one particular sulfhydryl group on unbleached rhodopsin. Elucidation of the complete amino acid sequence of rhodopsin (Ovchinikov, 1982; Hargrave et al., 1983) and site-specific modification of the protein with (iodoacetamido)salicylate (McDowell & Griffith, 1978) have revealed the probable location of this sulfhydryl group at cysteine residue 316. This residue is situated within the 40 or so C-terminal amino acids of rhodopsin that are believed to be exposed to the extradiscal space (Hargrave et al., 1982). Work with iodoacetamide derivatives of spin-labels has indicated these reagents have a low reactivity with rhodopsin (Sale et al., 1977; Wu & Stryer, 1972), and this is reflected in our studies. Following incubation of erythrosinyliodoacetamide with unbleached ros, in a 2:1 molar ratio, it was observed that about 20% of the protein became labeled. It proved difficult to improve on this value either by increasing the molar ratio of probe to protein used in the incubation or by increasing the time of incubation. Rhodopsin was always labeled in the unbleached state as the sulfhydryl reactivity has been shown to increase upon bleaching (DeGrip et al., 1973; Fung & Hubbell, 1978).

Most of the postulated conformational and chemical changes associated with rhodopsin bleaching are thought to occur during the meta 1 to meta 2 transition (Kemp, 1984). This transition has a half-time in the order of milliseconds at room temperature (Yoshizawa & Shichida, 1982). By investigating the first 100 μs after exposure to light, we have been able to study, primarily, bleaching intermediates prior to the formation of meta 2. The rotational dynamics of this system, containing essentially unbleached rhodopsin, have been compared with one comprising entirely bleached material. Our studies have shown that both photobleaching and temperature variation are capable of altering the nature of the rotational dynamics of rhodopsin in native ros membranes. The temperature dependence of the phosphorescence emission anisotropy for the samples of bleached rhodopsin is typical of the results obtained for intrinsic proteins in lipid bilayers. As such, they closely resemble the data obtained with other systems such as the band 3 anion transport system of the erythrocyte membrane and the calcium-dependent adenosine-5′-triphosphatase from sarcoplasmic reticulum membranes (Nigg & Cherry, 1979; Burkli & Cherry, 1981; Restall et al., 1984). The lower molecular weight of rhodopsin and the fluid nature of the ros membrane combine, however, to give decay kinetics that are somewhat faster than those obtained with either the Ca^{2+}-ATPase or the anion transport system.

In the absence of a complete, theoretically sound, description of the data, it is difficult to make definative statements about the meanings of the parameters derived from the curve fitting. Nevertheless, direct analysis of the $R(t)$ curves can be used to give useful information about the general trends occurring in the data. Where comparisons have been made between a full vector analysis and a direct analysis of the $R(t)$ curve, very similar results were obtained (Restall et al., 1984).

Studies with immobilized erythrosinyl isothiocyanate (Garland & Moore, 1979) have shown the phosphorescence polarization to be $p_f = 0.25$, corresponding to a fundamental zero-point anisotropy of $R_f = 2p_f/(3 - p_f) = 0.18$. The measured R_0 value in these studies is less than 0.05, suggesting that rapid (submicrosecond) movement has already occurred prior to the start of data collection. The source of this rapid movement is likely to be either independent movement of the probe molecule or rapid movement of a small polypeptide segment to which the probe is attached. Such segmental movements have previously been reported for the Ca^{2+}-ATPase in sarcoplasmic reticulum membranes (Burkli & Cherry, 1981; Restall et al., 1984). It seems likely in this case that the segmental movement associated with rhodopsin is even faster than that reported for the Ca^{2+}-ATPase, and consequently, the observed decay in the anisotropy results solely from movement of the whole protein. This may well explain why it is possible to fit an equation for simple molecular movement to the data.

The relaxation time, ϕ, is observed to decrease with increasing temperature, indicating greater protein mobility. There is also a steady decrease in the value of the A_3 term. Looking first at the changes occurring in ϕ, it is evident that the relaxation times measured compare favorably with those reported by other workers for this system (Cone, 1972; Downer & Cone, 1985). The relaxation times show a steady decrease with increasing temperature up to 35 °C. At 45 °C, there is a marginal increase in the relaxation time, most probably because of thermal denaturation. Plotting the relaxation times in the form of an Arrhenius plot shows that, at least for temperatures up to 35 °C, the protein mobility shows a single activation energy that is comparable with the apparent acti-

vation energy of DPH fluorescence anisotropy in the same membrane. The value of the steady-state DPH fluorescence anisotropy is affected by both the dynamics of the probe and the static constraints upon its movement. It would therefore be wrong to place too much emphasis on the similarity between the activation energies of the two processes. Nevertheless, it does provide more evidence that the relaxation time we have measured is a good index of rhodopsin mobility and that the overiding feature influencing the protein movement is the fluidity of the lipid bilayer.

The changes occurring in the value of A_3 are suggestive of protein aggregation. An increase in the proportion of protein that is rotationally immobile on the time scale of the experiment would be expected to cause an increase in the A_3 term. Following an approach given by Hoffmann and co-workers (1980), the fraction of immobile protein (f) can be calculated from the equation:

$$f = (R - MR_0)/[R_0(1 - M)] \quad (3)$$

where M is the ratio between the time-independent anisotropy ($R_\infty = A_3$) and the initial anisotropy R_0.

Assuming that no immobile protein is present at 35 °C and taking R_0 from the results of the curve fitting at 5 °C (where the best estimate of R_0 is obtained due to the slower movement of the protein), it can be shown that the proportion of immobile protein rises from 11% at 25 °C to 18% at 15 °C and 34% at 5 °C.

The data obtained for the sample of unbleached rhodopsin also show the characteristic decay in anisotropy to a time-independent value. Curve fitting to eq 2 shows that the relaxation times are similar to those obtained for the bleached protein. However, it is evident that the anisotropy decays to a lower A_3 term than that seen in the case of the bleached material.

Two possible explanations exist for this behavior; either the unbleached material is less aggregated than the bleached material, or there has been a change in the conformation of the protein upon bleaching that has affected the angle the phosphor makes with the rotational diffusion axis. Assuming that the results are caused totally by differences in the state of protein aggregation, it is first necessary to assume that only in unbleached rhodopsin at 35 °C is there an absence of immobile protein. Thus, the true R_∞ has a value of 0.001. Calculation of the fraction of immobile protein according to eq 3 shows that 30% of the protein is immobile in the unbleached sample at 5 °C. Extending the calculation to the results obtained for bleached rhodopsin now shows that at 35 °C 29% of the protein must now be stationary on the time scale of the measurement, this figure rising to 54% at 5 °C.

Alternatively, the results can be interpreted in terms of a light-induced conformational change in the protein that results in a different R_∞ term for the bleached material compared with the unbleached. Under these circumstances, the extent of protein aggregation seen on lowering the temperature from 35 to 5 °C is about the same for both bleached and unbleached protein, i.e., 30–35%. Furthermore, it is now possible to estimate the possible change in orientation of the phosphor with respect to the rotational diffusion axis from the relation

$$\theta = \arccos [(1 \pm 2M^{1/2})/3]^{1/2} \quad (4)$$

where M is again the ratio R_∞/R_0 (Hoffmann et al., 1980). In the case of bleached rhodopsin, this angle is found to be 33° whereas for unbleached material it has two possible values, 48° or 62° depending on whether the (+) or the (−) sign is used in eq 4.

While it is not possible to say unequivocably that the differences in the time-dependent anisotropy of bleached and unbleached rhodopsin are due to a conformational change, it would appear to be the most likely explanation. The similarities between the relaxation times and the temperature-induced aggregation both suggest the rhodospin molecule has not changed its dynamics following bleaching.

Recent work (Downer & Cone, 1985) has shown that oligomerization of rhodopsin does not appear to occur during photoexcitation of native ros and is therefore unlikely to be important in transduction. Baroin et al. (1977, 1979), using spin-labeled rhodopsin, have reported no effects immediately after bleaching but progressive protein immobilization at prolonged light exposures. However, this is probably not physiologically significant and is most likely the result of photooxidative damage to either the membrane lipids or the protein (Korchaga et al., 1980; Pogozheva et al., 1981). In our studies, longer exposure of the ros membranes to light was not observed to cause any protein aggregation, possibly because the irradiation was done after thorough flushing of the sample with nitrogen, effectively rendering the sample oxygen free.

We conclude that, in both the bleached and unbleached state, native ros contain rhodopsin in an equilibrium, over the temperature range investigated, between mobile and immobile protein. At 35 °C it is reasonable to assume that most, if not all, of the protein is mobile in samples of both bleached and unbleached rhodopsin. It would appear then, that the difference in the rotational dynamics between bleached and unbleached rhodopsin is indicative of a light-induced conformational change in the protein. If the erythrosin probe is indeed binding to cysteine residue 316, it would follow that this change is most likely associated with the C-terminal region of rhodopsin. Previous studies (Hargrave et al., 1980; Miller & Dratz, 1984; Kuhn & Chabre, 1983) have revealed that this part of the protein undergoes multiple phosphorylation at a number of serine and threonine residues. Proteolytic studies using thermolysin (Hargrave et al., 1980; Yamamoto & Shichi, 1983) show that increased phosphorylation by rhodopsin kinase occurs after the decay of meta 1. Our results provide direct evidence for a light-induced conformational change in the C-terminal region of rhodopsin that may be associated with an enhanced interaction with the kinase.

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