THE ELECTRIC DIPOLE MOMENT OF RHODOPSIN SOLUBILIZED IN TRITON X-100

DANIEL C. PETERSEN *and* RICHARD A. CONE

From The Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218. Dr. Petersen's present address is the Physics Department, Harvey Mudd College, Claremont, California 91711.

ABSTRACT The electric dipole moment of solubilized rhodopsin was determined with dielectric dispersion measurements. Rhodopsin was extracted from disc membranes of cattle rod outer segments with the nonionic detergent Triton X-100. The dipole moment of rhodopsin at its isoionic point in the detergent micelle is 720 D (150 charge-Å). This value is comparable to dipole moments of nonmembrane proteins, especially those which tend to aggregate or polymerize. Flash irradiation of the rhodopsin results in an increase in the dipole moment of about 25 D (5 charge-Å). The light-induced increase in dipole moment appears to be composed of two parts—a faster component related to a change in the number of protons bound by rhodopsin and a slower component apparently independent of the change in proton binding.

INTRODUCTION

The dielectric properties of several aqueous proteins have been studied extensively with dielectric dispersion measurements. The frequency dependence of the dielectric constant (dielectric dispersion) of a protein solution yields information about the nature and magnitude of the electric dipole moment of the protein molecule, as well as the molecular size and shape (reviews: Oncley, 1943; Takashima, 1969). Recently, dielectric dispersion measurements have also been used to characterize functional states of a protein (Rosseneu-Motreff et al., 1971), the self-aggregation of protein molecules (Gerber et al., 1972), and protein-lipid interactions (Soetewey et al., 1972).

While water-soluble proteins have been studied by dielectric dispersion, no similar observations have been reported for another large class of proteins—those found in membranes. Since membrane proteins can be expected to have amphiphilic or hydrophobic exteriors, their charge configurations (hence, electric dipole moments) could differ considerably from those of aqueous proteins. Moreover, the interaction of the dipole moment of a membrane protein with the electric field in the membrane could strongly influence the conformation, orientation, and activity of the protein.

This paper reports observations of the electric dipole moment of rhodopsin,[1] the principal membrane protein of rod outer segments (Bownds et al., 1971; Heitzmann,

[1] A preliminary report of these observations appears in Petersen and Cone (1973).

1972) and the molecule which initiates visual excitation. The mechanism of the excitation process is not yet known, but the spectral changes which rhodopsin undergoes after absorbing a photon indicate that a series of conformational changes take place in the molecule (reviews: Hubbard et al., 1965; Abrahamson and Wiesenfeld, 1972; Morton, 1972). Moreover, the early receptor potential occurs in synchrony with the spectral changes (Cone, 1969). This fast electrical response appears to arise from charge displacements in or near the rhodopsin molecule (Cone, 1967, 1969; Hagins and McGaughy, 1967). Therefore, it is of interest to know whether rhodopsin also exhibits light-induced charge displacements after being extracted from the membrane. In order to study this phenomenon we have measured the light-induced change in the dipole moment of rhodopsin in a detergent solution.

THEORY

Many explanations have been proposed for the dielectric dispersion of solutions of proteins and suspensions of larger particles (see Appendix). The most successful interpretation for solutions of aqueous proteins having molecular weights of less than 10^5 daltons is that due to Oncley (1938, 1943). In interpreting the first observations of the dielectric properties of protein solutions in alternating electric fields, Oncley (1938, 1943) assumed that the dielectric dispersion of the solution in the frequency range of 10^3–10^8 Hz is a measure of the degree of orientation of the permanent dipole moment of the protein in the applied field. In this analysis, based on a model by Debye (1929), the tendency for the permanent dipole to align with the electric field is opposed by the rotational Brownian motion of the molecule. For sufficiently low field strengths (as is the case in this experiment) the distribution of molecular orientations is only slightly perturbed by the applied field, and the molecules approach the electric field-induced equilibrium condition at a rate determined by the rotational diffusion of the molecules and essentially independent of the field strength. For low frequencies of the applied field, the equilibrium distribution is attained before the direction of the field is reversed, and the dipole orientation is maximal. But as the applied frequency approaches the rotational relaxation frequency of the molecule, the average molecular alignment declines, and the "dielectric constant" of the solution decreases. With sufficiently high frequencies, the applied field no longer perturbs the random orientation of the molecules, and the permanent dipole moment of the molecules no longer contributes to the dielectric constant of the solution.

In this analysis the dipole moment μ of the protein is given by the expression[2]

$$\mu = [(1/h)(9000\,kT/4\pi N)(1/c)(\epsilon_0 - \epsilon_\infty)]^{1/2}, \qquad (1)$$

where k is the Boltzman constant, T is the absolute temperature, N is Avogadro's number, c is the protein concentration in moles per liter, ϵ_0 and ϵ_∞ are the dielectric

[2] With these units, μ is in Debyes (D), where 1 D = 10^{-18} statcoulomb-cm. Thus, an electron and a proton separated by 1 Å produce a dipole moment of 4.8 D. In other words, 1 charge-Å = 4.8 D.

constants (dimensionless) at low and high frequencies, respectively, and h is an empirical parameter. Estimates of h range from 4.5 to 8.5; the commonly accepted value of 5.8, based on the dipole moment of glycine (Oncley, 1938), will be used here.

In Debye's model for a spherical particle, the real part of the dielectric constant of the solution follows a dispersion curve given by

$$\epsilon = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/[1 + (\nu/\nu_c)^2], \tag{2}$$

where ν is the frequency of the applied electric field and ν_c is the "critical frequency." Thus, for $\nu \ll \nu_c$, the dielectric constant ϵ approaches the value ϵ_0 while for $\nu \gg \nu_c$ the dielectric constant approaches ϵ_∞; $(\epsilon_0 - \epsilon_\infty)$ is the dielectric increment, which provides a measure of the dipole moment from Eq. 1. The critical frequency, which is the frequency at which $\epsilon = (\epsilon_0 + \epsilon_\infty)/2$, is related to the rate of rotational diffusion of the particle and, hence, to the particle size by

$$\nu_c = 1/2\pi\tau = kT/8\pi^2\eta r^3, \tag{3}$$

where τ is the rotational relaxation time, η is the viscosity of the solution, and r is the radius of the particle. (The effects of nonspherical shapes will be discussed below.)

In this general approach, the dielectric dispersion critical frequency is related to the rotational diffusion relaxation time of the hydrated protein. In fact, Oncley's interpretation has been used to estimate the water of hydration of various aqueous proteins (Oncley, 1943); that is, the number of water molecules which are, in effect, rigidly attached to the protein, insofar as its rotational diffusion is concerned. Also, the permanent dipole moment deduced for the protein in this case is actually the value for the hydrated protein. That is, having subtracted the dielectric constant of the aqueous solvent from the dielectric constant of the solution containing the protein, one assumes that the remainder is due to the protein only and is not affected by any protein-solvent interaction. While this procedure is not rigorously correct, it has become the generally accepted method of analysis (see, e.g., Smyth, 1955; Oncley, 1938).

In the case of rhodopsin, a detergent is needed to solubilize the protein, and the result is an aqueous dispersion of rhodopsin-detergent micelles. To remove the contribution of the detergent to the dielectric dispersion of the rhodopsin-detergent solution, we simply subtract that part of the dielectric increment which occurs for the detergent solution alone. (The dielectric increment of a solution of the nonionic detergent Triton X-100 shows no indication of any permanent dipole moment for the detergent micelles.) We then assume that the remaining dielectric increment results from the dipole moment of the rhodopsin, i.e., the contribution of the detergent micelle has been properly removed—a procedure analogous to the method for removing the contribution of the water to the dipole moment of soluble proteins.

Assuming Oncley's interpretation to be correct, then the relaxation time for rotational diffusion of the permanent dipole moment of rhodopsin should be related to the dielectric dispersion critical frequency, as noted above. The question is, then, whether

rhodopsin is in an essentially rigid micelle on the time scale of these measurements. In this case the micelle would rotate with the rhodopsin molecule, and the rotational diffusion of the micelle as a whole should be considered. On the other hand, the micelle may be sufficiently fluid that rotational diffusion of the rhodopsin molecule within the micelle must also be considered. As will be seen, the dielectric dispersion of rhodopsin solubilized by Triton X-100 can be explained in terms of Oncley's interpretation, with the rhodopsin-Triton X-100 micelle considered to be essentially a rigid structure on the time scale of rotational diffusion. And, as is shown in the Appendix, alternative explanations for the dielectric dispersion fail to explain the observations of this experiment.

EXPERIMENTAL APPARATUS AND PROCEDURES

Capacitance and Conductance Measurement Apparatus

Capacitance and conductance of the rhodopsin solutions were measured with a Model 75C Direct Capacitance Bridge (Boonton Electronics Corp., Parsippany, N.J.). The oscillator in this instrument has a continuously variable frequency range of 5–500 kHz. Peak field strength in the solution was limited to less than 1 V/cm. For the capacitances and conductances in this experiment, the bridge sensitivity was ± 0.005 pF and ± 0.1 μmho from 15 kHz to 500 kHz. Below 15 kHz the capacitance sensitivity fell, reaching ± 0.02 pF at 5 kHz. While the capacitance sensitivity was typically around 3% of the total dispersion of the capacitance for the rhodopsin solution, the conductance sensitivity was about one-half the maximum expected conductance dispersion—the variation in conductance due to the dispersion of the imaginary part of the dielectric constant (Takashima, 1969). Consequently, conductance dispersion measurements could not be made. However, as will be discussed below, changes in conductance could be detected following flash irradiation of the rhodopsin solution.

The capacitance cell consisted of two electrodes made of discs of fine-grained impermeable graphite (G-90 Graphite, Carborundum Co., Sanborn, N.Y.) separated by a 0.9 mm thick washer of Sylgard 184 Encapsulating Resin (Dow Corning Corp., Midland, Mich.) which contained a 0.9 cm diameter hole for the rhodopsin solution. The measured cell volume was 55 μl. G-90 Graphite was chosen for its low electrode polarization and its ease of machining and maintenance. The Sylgard washer produced a watertight seal with the electrodes without the need for any potentially contaminating sealant. Also the Sylgard is optically clear, permitting actinic (bleaching) and monitoring light beams to pass through the rhodopsin solution.

The cell was isolated electrically and thermally in a lighttight aluminum enclosure encircled by cooling coils. A Lauda/Brinkmann Model K-4/R circulator (Brinkmann Instruments Inc., Westbury, N.Y.) kept the temperature stable to 0.01°C between reference measurements (see below), and to ± 0.05°C during a complete measurement of the dielectric dispersion before and after bleaching. All measurements reported here were done between 0.8° and 2.7°C.

The rhodopsin solution was flash irradiated with an M3 flash bulb (General Electric,

Cleveland, Ohio) through a Wratten 8 filter (Eastman Kodak Co., Rochester, N.Y.) which removed visible wavelengths less than 475 nm, and two IR filters (KG3, BG18; Jena Glaswerk Schott and Gen., Mainz, Germany; distributors: Fish-Schurman Corp., New Rochelle, N.Y.). With these filters a single flash bleached approximately 75% of the rhodopsin in the cell, for the rhodopsin concentrations used in this experiment. The flash did not produce a detectable bridge imbalance with control solutions containing salts, detergents, and/or bleached rhodopsin. The absorption of the rhodopsin solution was monitored by a light beam which passed through the cell at right angles to the actinic beam and was detected with a photomultiplier (Model EU-701-93, Heath Co., Benton Harbor, Mich.). The spectrum of this monitoring beam was limited by interference filters (Baird Atomic, Cambridge, Mass.) to 403 ± 5 nm in order to observe the accumulation of the spectral intermediate metarhodopsin II (Matthews et al., 1963) following the flash. The time courses of both conductance and capacitance changes elicited by the actinic flash were observed with a PAR 129 Two Phase Lock-In Amplifier (Princeton Applied Research Corp., Princeton, N.J.). The changes in optical absorbance, conductance, and capacitance were displayed on an oscilloscope, allowing simultaneous measurements of the time courses of these three parameters following a flash.

Rhodopsin Preparation

Rhodopsin was obtained from frozen cattle retinas (George A. Hormel Co., Austin, Minn.) by Procedure C of Hubbard et al. (1971). To reduce the conductance of the rhodopsin solution the final stages of the extraction procedure were modified to include several washes in deionized distilled water and extraction with the nonionic detergent Triton X-100 at a concentration of 2% wt/vol in deionized distilled water. The conductance of the solution was still too high for capacitance measurements, necessitating the final step of deionizing the rhodopsin-Triton X-100 solution on a mixed-bed ion exchange column according to the method of Dintzis (1952), as modified by Fukami et al. (1959) for rhodopsin solutions. Such a column can remove inorganic ions and small molecular-weight peptides without denaturing rhodopsin (Albrecht, 1957). The pH of the rhodopsin solution deionized in this way was between 5.0 and 5.2, close to the isoelectric point of cattle rhodopsin in the detergent digitonin (pH = 5.3) (Fukami, 1960; Radding and Wald, 1956). The conductance of the solutions was within a factor of three of that to be expected from H^+ ions alone, indicating a few other ions at most were present per rhodopsin molecule. As there were some hints of aggregation at such low conductances, a very small amount of KCl (< 0.1 mM) was added to some of the solutions before making the dielectric dispersion measurements. Doubling the conductance in this way apparently had little effect on the pH, as all pH values were in the 5.0–5.2 range, whether or not extra salt was added.

The choice of detergents was an important consideration in this experiment. Digitonin is the gentlest detergent which can extract rhodopsin, as judged by regenerability of bleached rhodopsin on addition of 11-*cis* retinal (Wald and Brown, 1950), thermal stability (Johnson and Williams, 1970), and the time-course of bleaching—which

closely resembles the time course in situ and in vivo (Sengbusch, 1970; Sengbusch and Stieve, 1971 *a*, 1971 *b*; Cone and Cobbs, 1969). However, dielectric dispersion measurements of rhodopsin-digitonin solutions showed instabilities, probably resulting from the well-known problem of precipitation of rhodopsin-digitonin solutions at low temperatures and acid pH (Crescitelli, 1967). For this reason digitonin could not be used as the detergent. Triton X-100 was chosen as the substitute, since solutions of this detergent are much more stable against aggregation in low salt concentrations at low temperatures and acid pH (Crescitelli, 1967). Furthermore, Triton X-100 extracts rhodopsin readily, while giving the same optical absorbance and circular dichroism spectra seen in digitonin (Crescitelli, 1967; Shaw, 1972). Finally, although rhodopsin solubilized in Triton X-100 is not regenerable by the addition of 11-*cis* retinal, it is photoregenerable, at least through the metarhodopsin I intermediate, indicating that the chromophore region of rhodopsin is essentially undenatured during the early stages of photobleaching (Johnson and Williams, 1970).

Rhodopsin concentration was determined spectrophotometrically (Hubbard et al., 1971) by measuring the difference spectrum of rhodopsin at 500 nm before and after bleaching in the presence of hydroxylamine, and using 42,000 liter/cm-mol for the molar extinction coefficient of rhodopsin (Wald and Brown, 1953; Matthews et al., 1963; Daemen et al., 1970; Shichi, 1970; Bridges, 1971; Zorn and Futterman, 1971; Heitzmann, 1972). The purity of rhodopsin can be judged by the ratio of absorptions at the minimum between the near-ultraviolet and visible bands and the maximum of the visible absorption band (about 400 and 500 nm, respectively) (Hubbard et al, 1971; Crescitelli, 1967). Rhodopsin samples in this experiment typically had a 400/500 absorption ratio of about 0.25, a typical value for the type of extraction and purification procedure employed here (Albrecht, 1957, Johnson and Williams, 1970).[3] The other spectral monitor of purity, the ratio of absorbance at 280 nm and 500 nm cannot be used with Triton X-100 because of the large absorbance of this detergent at 280 nm.

Cell Constant

The cell constant is needed to convert observations of capacitance and conductance to the dielectric constant and conductivity, which are properties of the solution alone.

[3] While reduction of the 400/500 ratio to around 0.17–0.18 can be achieved by various techniques (Shichi et al., 1969; Zorn and Futterman, 1971), the regenerability of the resulting rhodopsin in digitonin has not been demonstrated, and there are indications that these additional purification steps render the rhodopsin more unstable against denaturation, especially when these procedures are coupled with the removal of even relatively small amounts of phospholipids. Similarly, removal of phospholipids intimately associated with the rhodopsin molecule also result in greater instability of the rhodopsin, and one cannot rule out damage to the rhodopsin resulting from such delipidation procedures (see Daemen, 1973). Thus, no attempts were made to reduce the 400/500 ratio below 0.25, or to extract those phospholipids which are intimately associated with the rhodopsin molecule. Extraction of loosely associated lipids with petroleum ether removed about half the phospholipids, leaving at most about 50 phospholipids per rhodopsin molecule (estimated from assaying the total phosphate content of a rhodopsin-Triton X-100 sample by the procedure of Ames and Dubin (1960). The dipole moment determined in this experiment therefore refers to this rhodopsin-phospholipid complex. (In this regard, the addition of fatty acids to completely delipidated bovin serum albumin results in a 25% reduction in the dipole moment (Soetewey et al., 1972).)

The cell constant C_c can be determined by measuring the capacitance of the cell when it is empty and when it contains a solution of known dielectric constant:

$$C_c = (C_x - C_e)/(\epsilon_x - 1), \tag{4}$$

where C_c is given in dimensions of capacitance, C_x is the measured capacitance of the cell containing a solution of dielectric constant ϵ_x, and C_e is the capacitance of the empty cell. Given C_c, the dielectric increment $(\epsilon_0 - \epsilon_\infty)$ in Eq. 1 becomes

$$\epsilon_0 - \epsilon_\infty = (C_0 - C_\infty)/C_c, \tag{5}$$

where C_0 and C_∞ are the capacitances measured at the low and high frequency plateaus, respectively.

The observed value of the conductance G can be converted to the conductivity of the solution σ by the relation

$$\sigma = (0.08854/C_c)G, \tag{6}$$

where C_c is in picofarads, and the term 0.08854 is the permittivity of free space in units of picofarads per centimeter.

The cell constant was determined by measuring the capacitance of the cell when it contained deionized distilled water. This calibration was checked by measuring the capacitance of the cell filled with ethanol and comparing the calculated dielectric constant with the known value. The two numbers agreed to 0.15%. The cell constant was $C_c = 0.643 \pm 0.001$ pF.

Electrode Polarization

A major problem in determining the dielectric dispersion of rhodopsin solutions is electrode polarization. The contribution of electrode polarization to the measured capacitance scales approximately as $G^2\nu^{-3/2}$ (Oncley, 1938, 1943; Shaw, 1942), which means that at low frequencies and high conductances electrode polarization can predominate. In fact, with the conductances and rhodopsin concentrations used in this experiment, the electrode polarization contribution to the capacitance at 5 kHz could be as much as 10 times that due to the dielectric dispersion of the protein. To correct for this effect, the capacitance was measured for control solutions of 2% Triton X-100 adjusted to nearly the same conductance as the rhodopsin solution (by addition of KCl). The capacitances of these solutions gave straight lines when plotted against $\nu^{-3/2}$. By interpolating between solutions having conductances very near to and bracketing that of the rhodopsin solution, the capacitance due to electrode polarization could be determined, and this contribution was subtracted from the capacitance of the rhodopsin solution. This correction procedure worked very well down to 10–15 kHz. Below this frequency the corrections fluctuated considerably depending on the control runs chosen. Since the dielectric dispersion curve had leveled off to the low frequency plateau by the 15 kHz point, the corrections below this frequency could be improved

by requiring the 5 kHz point to have the same capacitance as that seen at 15 kHz. With this constraint a subtraction curve which scaled as $\nu^{-3/2}$ could be obtained. The resulting corrected capacitances at 7.5 and 10 kHz also fell on the 5–15 kHz plateau, supporting the use of this technique.

Given the electrode polarization effects, small changes in conductance could result in sizable changes in measured capacitance. Since it was very difficult to eliminate slow conductance changes during the course of the experiment, each measurement of capacitance and conductance at a given frequency was bracketed by measurements of capacitance and conductance at 50 kHz, a frequency at which changes in electrode polarization had negligible effects on the capacitance. All capacitances were determined relative to these 50 kHz values, and the conductance of the reference measurement was considered in determining the electrode polarization correction for each frequency.

Light-induced capacitance and conductance changes were determined by measuring the capacitance and conductance at 50 kHz before and within 1 min after the actinic flash. Flashes were repeated until no further capacitance and conductance changes were seen. Typically, no change was detectable after three flashes.

Glycerol Run

To obtain a high frequency plateau in the dielectric dispersion curves, it was necessary to extend the measurements in this region by slowing down the rotational diffusion rate of the rhodopsin-Triton X-100 micelle. This was done by adding glycerol to the rhodopsin solution to increase its viscosity. Glycerol was added to a final concentration of approximately 45% wt/vol; this was found to increase the viscosity by a factor of 7.6. The glycerol dispersion curve for unbleached rhodopsin was fitted to the non-glycerol curve of capacitance vs. frequency by multiplying the oscillator frequencies for the glycerol data by 7.6 and then sliding the glycerol curve vertically along the capacitance axis to give the best agreement with the non-glycerol curve over their common frequency range. Thus, only one adjustable parameter was used to merge the two curves. The dispersion curves obtained after flash irradiation in both the glycerol and non-glycerol solutions were measured with respect to the light-induced increments observed at 50 kHz.

Translational Diffusion

An independent experiment was performed to test whether the dielectric dispersion of rhodopsin arises from the partial orientation of the permanent dipole moment of rhodopsin in the applied electric field. If this assumption is correct, then from Eq. 3 the critical frequency of the dielectric dispersion should agree with the relaxation frequency for rotational diffusion of the rhodopsin-Triton X-100 micelle, assuming the micelle to be rigid for this time scale (see above). This rotational relaxational frequency could not be measured directly but was inferred from the translational diffusion coefficient of the micelle, which was determined by laser "optical beating" spectroscopy (Cummins et al., 1969). If the micelle is assumed to be spherical, its radius can be ob-

tained from the translational diffusion coefficient D_T by Stokes' law:

$$r = kT/6\pi\eta D_T. \tag{7}$$

This value for the radius can be inserted in Eq. 3 to give the expected dielectric dispersion critical frequency due to the rotational diffusion of the micelle.

RESULTS

The dielectric dispersion of rhodopsin solubilized in 2% Triton X-100 is shown in Fig. 1 *a*. Application of Eqs. 5 and 1 to the capacitance increment between the low and high frequency plateaus gives a dipole moment for rhodopsin of 720 D (150 charge-Å). The error for this value is estimated to be less than 20%. This estimate is based on (*a*) the variability of μ in the four experiments depicted in Fig. 2 *a*—which had a total spread of -7% to $+10\%$ relative to the mean value of μ, (*b*) estimates of electrode polarization errors, (*c*) uncertainty in rhodopsin concentration, and (*d*) uncertainties in merging the non-glycerol and glycerol data. (An analysis of the possible effects of glycerol on the dielectric increment indicates these effects contribute less than a 10% error to the dipole moment (Takashima, 1962; Hendrickx et al., 1968; Matthews et al., 1963; Burke et al., 1973).

Flash irradiation ($\lambda > 475$ nm) of the rhodopsin solution produced an increase in capacitance. After three flashes no further change was detectable; the upper dispersion curve in Fig. 1 *a* gives the final capacitance after irradiation. The capacitance of both high and low frequency plateaus increased, but the change was greater at low frequencies; therefore, the permanent dipole moment of rhodopsin increased on irradiation. (The small increment seen at high frequencies may reflect an increase in the polarizability of rhodopsin after irradiation.) The light-induced increase in capacitance, as observed in four experiments (Fig. 2 *b*), corresponds to an increase in dipole moment of 25 ± 8D (5 ± 2 charge-Å). To observe this small increase in dipole moment, we determined the light-induced change in capacitance at a single frequency (50 kHz) by making several measurements on each sample before and after flash irradiation. In this way errors resulting from sample-to-sample variation were minimized and all systematic errors except that arising from the setting error of the bridge were eliminated. The observed capacitance changes were much larger than this setting error. (For example, Fig. 1 *a* shows a change at 50 kHz of about 0.05 pF, which is 10 times larger than the setting error of the bridge.) Thus the limiting source of error in determining the magnitude of the capacitance increase arose from variabilities in the samples, as shown in Fig. 2 *b*.

Fig. 1 *a* shows the increment in capacitance resulting from the addition of rhodopsin to the Triton X-100 solution. This increment is less than 0.5% of the total measured capacitance, most of the rest being due to the dielectric constant of water. The increment could have been increased by raising the rhodopsin concentration; but, in order to limit aggregation while keeping conductance at a minimum, the rhodopsin concentration was held to about 10^{-5} M.

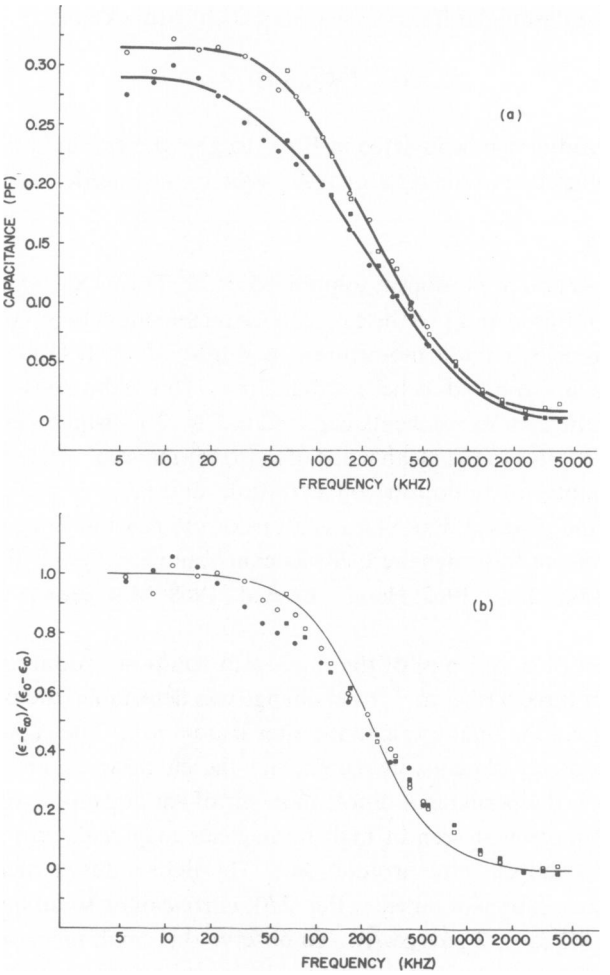

FIGURE 1 (*a*) Dielectric dispersion of rhodopsin in 2% Triton X-100. The data show the increment in capacitance resulting from the addition of 6.7 μM rhodopsin to Triton X-100. Temperature 2.3°C; pH = 5.0. Solid symbols: before illumination; open symbols: after illumination ($\lambda > 475$ nm). Circles: no glycerol; squares: 45% wt/vol glycerol added. Curves were drawn by eye. (*b*) Normalized dielectric dispersion of rhodopsin before and after illumination. Data of Fig. 1 *a* presented in terms of the fractional dielectric increment $(\epsilon - \epsilon_\infty)/(\epsilon_0 - \epsilon_\infty)$. Symbols defined as in Fig. 1 *a*. Solid curve: dielectric dispersion given by Eq. 2 for a rotationally diffusing spherical particle with a critical frequency of 225 kHz. (Note: at the critical frequency, $(\epsilon - \epsilon_\infty)/(\epsilon_0 - \epsilon_\infty) = 1/2$.)

The light-induced capacitance increase was about one part in 2,000 with respect to the total capacitance of the solution. In addition to this capacitance increase, there was also a 1% decrease in the conductance of the solution following irradiation. These light-induced changes in capacitance and conductance were observed in a total of eleven experiments on samples from three separate detergent extractions. Complete

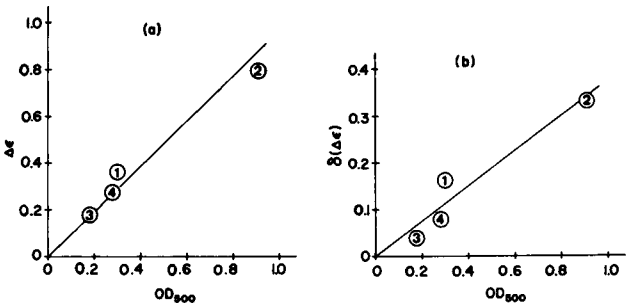

FIGURE 2 (*a*) The dielectric increment $\Delta\epsilon$ for rhodopsin in Triton X-100 vs. rhodopsin concentration. The dielectric increment presented is the increment between 400 kHz and 30 kHz, which is about two-thirds of the total dielectric increment ($\epsilon_0 - \epsilon_\infty$) between 4,000 kHz and 5 kHz. This measure of the dielectric increment is independent of the glycerol data and avoids the uncertainties which result from electrode polarization effects at low frequencies. Rhodopsin concentration is given in terms of the optical density of the rhodopsin-Triton X-100 solution at 500 nm (OD_{500}). $OD_{500} = 1$ corresponds to a rhodopsin concentration of 23.8 μM. Data for four separate experiments (circled numbers) *1:* $OD_{500} = 0.30$, pH = 4.3, $T = 0.8$°C. *2:* $OD_{500} = 0.91$, pH = 5.1, $T = 2.7$°C. *3:* $OD_{500} = 0.18$, pH = 5.2, $T = 2.3$°C. *4:* $OD_{500} = 0.28$, pH = 5.0, $T = 2.3$°C. (Sample *4* was used for Fig. 1.) The straight line was fit by eye, as was the line in Fig. 2 *b*. (*b*) The change in dielectric increment following irradiation $\delta(\Delta\epsilon)$ vs. rhodopsin concentration. The change was measured at 50 kHz after the actinic flashes produced no further detectable change in capacitance or conductance (typically, after three flashes). Numbered data points refer to the same experiments shown in Fig. 2 *a*.

analyses of the light-induced capacitance changes were performed on four of these experiments, and the results are shown in Figs. 1 and 2. That the observed light-induced changes in capacitance and conductance reflect changes in the rhodopsin molecule may be argued from the following considerations: (*1*) The conductance and capacitance changes for successive flashes were linearly proportional to the amount of rhodopsin activated by each flash, as determined by the absorbance increase at 403 nm. (*2*) The conductance decreased while the capacitance increased, thus ruling out electrode polarization effects or changes in the cell constant, either of which would have caused both changes to be of the same sign. (*3*) Heating of the solution by the flash would have resulted in a conductance increase and a capacitance decrease, the opposite of the observed effects. (*4*) Finally, no change in conductance or capacitance occurred following flash irradiation of rhodopsin-free Triton X-100 solutions adjusted to the same conductance as the rhodopsin solution.

Fig. 1 *b* gives the dielectric dispersion of the rhodopsin solution in terms of the fractional dielectric increment $(\epsilon - \epsilon_\infty)/(\epsilon_0 - \epsilon_\infty)$. The bleached and unbleached curves superimpose within errors, giving the same critical frequency of 225 ± 25 kHz. This implies that the effective radius of the micelle for rotational diffusion, as determined from Eq. 3, changes less than 4% on irradiation (cf. Incardona et al., 1971; Heller and Ostwald, 1972).

The dielectric dispersion critical frequency can also be inferred from the *translational* diffusion coefficient D_T of the rhodopsin-Triton X-100 micelle, as discussed above.

Application of Eqs. 7 and 3 to the value for D_T obtained from laser light scattering leads to a critical frequency of 210 ± 15 kHz, in excellent agreement with the critical frequency obtained from the dielectric dispersion measurements.[4] The agreement of these two independent determinations of the size of the rhodopsin-Triton X-100 micelle strongly supports the conclusion that the frequency dependence of the dielectric dispersion curve arises from the rotational diffusion of a permanent dipole, in accord with Oncley's interpretation for soluble proteins. (By "permanent" dipole we mean that the charge configuration of the rhodopsin is effectively constant over the frequency range of this experiment, that is, above 5 kHz.) If this interpretation is correct, then the micelle may be considered to be essentially rigid on this time scale.[5]

Fig. 1 *b* also contains the dispersion curve (solid line) obtained from Eq. 2 for a spherical particle with a critical frequency of 225 kHz. This curve has a steeper frequency dependence near the critical frequency than that obtained in the rhodopsin-Triton X-100 solution. This might reflect the presence of some variation in the rhodopsin-Triton X-100 micelle size. Indeed, some larger micelles were observed in the light scattering experiments. The proper combination of ellipsoidal shapes for the micelle and the rhodopsin molecule, micelle fluidity, etc., could probably also account for the shape of the observed dispersion curve, as nonspherical shapes tend to spread the dispersion curve (Oncley, 1943), but at present not enough information is available for such an analysis.

Fig. 2 *a* shows the effect of rhodopsin concentration on the dielectric increment $\Delta\epsilon$, as observed in four different rhodopsin solutions. The dielectric increment is seen to vary linearly with rhodopsin concentration over a fivefold range, in agreement with Eq. 1. The change in dielectric increment following irradiation, $\delta(\Delta\epsilon)$, should also depend linearly on concentration; Fig. 2 *b* shows this to be the case. On the other hand, the change in conductivity of the solution following irradiation was found to be quite complex, showing a strong dependence on the pH of the solutions in qualitative agreement with previous observations by T. Hara (1958) and R. Hara (1963).

The time course of the changes in dielectric increment and conductivity following flash irradiation can be seen in Fig. 3. This figure presents simultaneous oscilloscope traces for changes in capacitance (C), conductance (G), and optical transmittance (T) at 403 nm (which monitors the accumulation of metarhodopsin II). Immediately following the flash there is an initial rapid increase in capacitance ($\ll$ 1 s) in conjunction

[4] The effective radius for the micelle inferred from the dielectric dispersion measurements is about 50 Å and that from the laser light-scattering experiment is about 51 Å. These values should be compared with a value 20–22 Å for the radius of the rhodopsin molecule alone, assuming rhodopsin to be spherical.

[5] There are other possible interpretations, because of the complex nature of the micelle. For example, the micelle may not be spherical, in which case the critical frequency inferred from the translational diffusion measurements should be slower than 210 kHz (see Perrin, 1934, 1936). The resulting discrepancy between this critical frequency and that measured by dielectric dispersion could then be removed by assuming that the micelle is fluid and the rhodopsin can rotationally diffuse within the micelle. In addition, one could invoke a nonspherical shape for the rhodopsin molecule, etc. Such complications cannot be resolved with the present measurements. For now it is sufficient to note that the simplest interpretation leads to a consistent picture.

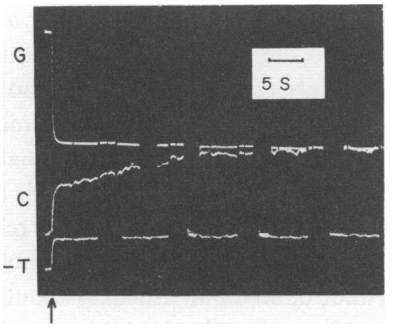

FIGURE 3 Simultaneous oscilloscope traces of the changes in capacitance (C), conductance (G), and optical transmittance at 403 nm (T) of a rhodopsin-Triton X-100 solution following an actinic flash (arrow). (Note: The decrease in T is a measure of the accumulation of meta-rhodopsin II.) OD_{500} = 0.28; pH = 5.1; temperature = 2.5°C. The gaps in the oscilloscope traces result from periodic checks and calibrations performed during the experiment.

with a rapid conductance decrease, both apparently concurrent with the decrease in transmittance (within instrumental time resolution). After this initial transient, G and T remain essentially constant, but C undergoes a second, much slower increase, with a half-time of about 10 s. No further changes are observed in G, C, or T for the next several hundred seconds. The light-induced capacitance change in Fig. 1 *A* is the sum of both these fast and slow capacitance increases. Additional experiments of the type shown in Fig. 3 were performed at various pH values (pH 5–7). In these experiments the magnitude of the fast component of the light-induced change in capacitance followed roughly the magnitude of the light-induced conductivity change, and thus varied considerably for different pH values. The slow capacitance change seemed to be much less sensitive to the change in pH. Thus there appear to be at least two mechanisms of light-induced changes in the dielectric increment, one associated with the light-induced decrease in conductivity, the other independent of such conductivity changes.

DISCUSSION

The Magnitude of the Dipole Moment

As mentioned earlier, Oncley's interpretation of the dielectric properties of protein solutions was based on the assumption that the dielectric dispersion resulted from the partial orientation of the permanent dipole moment of the protein molecules in an alternating electric field. Several other interpretations of the dielectric dispersion have been proposed subsequently. As discussed above and in the Appendix, Oncley's interpretation appears to account for most of the dielectric dispersion of rhodopsin solubilized in Triton X-100. We therefore conclude that the dipole moment calculated from Eq. 1 arises predominantly from the permanent dipole moment of the lipoprotein rhodopsin in Triton X-100.

Eq. 1 gives a dipole moment of 720 D or 150 charge-Å near the isoionic point of rhodopsin. If rhodopsin were a sphere with a diameter of 40–45 Å, it could achieve

such a dipole moment by having about four positive charges at one pole and four negative charges at the other. If rhodopsin were 75 Å long, the minimum length proposed by Wu and Stryer (1972), then only two charges at each end would be needed for the observed dipole moment. For the pH values in this experiment, rhodopsin has about 35 normally charged amino acid residues, divided approximately evenly between positive and negative groups (Shields et al., 1967; Heller, 1968; Shichi et al., 1969; Zorn and Futterman, 1971; Radding and Wald, 1956). Thus, a relatively small asymmetry in the arrangement of these groups can account for the dipole moment. (In addition, the dipole moment of the peptide bond for an α-helix is about 4 D per peptide, but the folding of the polypeptide backbone should reduce this contribution to a minor term in the overall dipole moment for a globular protein (Wada, 1959, 1960, 1967; Applequist and Mahr, 1966).

The dipole moment of rhodopsin is comparable to that found in other proteins (Oncley, 1943; Gerber et al., 1972). Gerber et al. separated globular soluble proteins into two groups with regard to their dipole moments: those with $\mu < 450$ D and those with $\mu > 650$ D. According to this classification, rhodopsin fits into the larger moment group. On dividing the dipole moment of each protein by its molecular weight (W), we find that the two groups become even more clearly separated, the first group tending to cluster around a μ/W value of 5×10^{-3} D/dalton, the second around 20×10^{-3}. Rhodopsin, which has a molecular weight of 35,000 ± 2,000 (Lewis et al., 1974), again fits into the larger dipole group, having a μ/W value of about 20×10^{-3}.

Gerber et al. note that the proteins with the larger dipole moments all tend to aggregate and suggest that the dipole moments aid in the aggregation process by orienting closely apposed monomers. In the case of rhodopsin, the dipole moment may help orient the molecule in the membrane, either by interacting with adjacent phospholipids or with the electric field across the photoreceptor membrane. For example, if a potential drop of 30 mV over 50 Å is experienced by a dipole moment of 150 charge-Å, an orientation energy of about 4 kT results. The magnitude of the dipole moment of rhodopsin in the membrane could, of course, be quite different from that seen in Triton X-100 at the isoionic point.

The Light-Induced Dipole Moment Change

Illumination of rhodopsin results in a small increase in the dipole moment. Near the isoelectric point of rhodopsin the increase is about 25 D and is apparently composed of two components, one associated with a decrease in conductivity of the solution and the other independent of conductivity changes. The latter, slower, component may reflect a general conformational change in the protein, similar to the loosening-up of the rhodopsin structure inferred from bleaching kinetics (see, e.g., Hubbard et al., 1965; Abrahamson and Wiesenfeld, 1972; Morton, 1972). It is of interest to note that deliberate denaturation of bovine serum albumin with urea results in an increase in the dipole moment, although to a much greater extent than observed here (Takashima, 1964). Alternatively, this dipole moment change might reflect an alteration of some

specific part of the protein, just as the visible absorption spectrum of rhodopsin reveals changes in the vicinity of the chromophore following absorption of a photon.

The faster component of the dipole moment increase, which is associated with the light-induced change in conductance, could be directly related to the binding of a proton by rhodopsin. Measurements of the pH of rhodopsin-Triton X-100 solutions done at room temperature showed a fast increase in pH on flash irradiation. For an initial pH value of 5, this increase corresponded to the uptake of on the order of one hydrogen ion per rhodopsin molecule—consistent with the H^+ uptake seen during the metarhodopsin I to metarhodopsin II transition in more extensive experiments on rhodopsin in digitonin (Radding and Wald, 1956; Wong and Ostroy, 1973) and in whole and sonicated rod outer segments (Falk and Fatt, 1966; Emrich, 1971). This faster dipole moment increase could therefore reflect a conformational change required for proton binding and/or simply the binding of charge to rhodopsin.

When the eye is subjected to an intense flash, a rapid electrical response, the early receptor potential (ERP) can be detected. The charge displacements which generate the ERP occur in synchrony with the spectral transitions of rhodopsin and probably arise in or near the rhodopsin molecule (Cone, 1967, 1969; Hagins and McGaughy, 1967; Hagins and Rüppel, 1971). The dominant component of the ERP occurs during the metarhodopsin I to metarhodopsin II transition (Cone and Cobbs, 1969); a single charge must be displaced approximately 0.1–50 Å perpendicular to the membrane surface to produce the observed electrical response (Cone, 1969; Murikami and Pak, 1970; Hagins and Rüppel, 1971; Rüppel and Hagins, 1973). The corresponding dipole moment change observed in this experiment is 5 charge-Å, in good agreement with the estimates for the ERP. However, to establish the relationship between dipole moment changes and the ERP, it will be necessary to determine the direction of the dipole moment change with respect to the membrane surface and demonstrate that the spectral transition and dipole moment change are synchronous. Moreover, it should be noted that the light-induced changes in dipole moment observed for rhodopsin solubilized in Triton X-100 may differ considerably from the dipole moment changes which occur for rhodopsin in situ.

APPENDIX

Alternative Interpretations of Dielectric Dispersion

As mentioned above, Oncley interpreted the frequency dependence of the dielectric constant of protein solutions as resulting from the partial orientation of rotationally diffusing permanent dipoles in the applied electric field. Several other theories have been proposed for the dielectric dispersion of protein solutions (see South and Grant, 1972). The principal explanations involve proton fluctuations (Kirkwood and Shumaker, 1952; Scheider, 1965, 1970; Schwarz, 1970), the Maxwell-Wagner effect (Maxwell, 1873; Wagner, 1914; Schwan, 1957), surface conductivity (O'Konski, 1960), ion migration (Schwarz, 1962; Scheider, 1972), and the ion atmosphere (Debye and Falkenhagen, 1928).

The strongest argument for the predominance of the rotational diffusion of permanent dipoles as the source of dielectric dispersion in rhodopsin-Triton X-100 solutions is the agreement

between the observed critical frequency and the value predicted by the translational diffusion coefficient of the micelle. None of the other theories mentioned can predict this relationship. (Note that here a dipole is considered permanent if it is effectively constant on the time scale of the rotational diffusion of the molecule.) Nevertheless, it is worth considering the alternative mechanisms in more detail to see whether these mechanisms can contribute to the dielectric dispersion.

The proton fluctuation model explains dielectric dispersion in terms of a nonvanishing mean square electric dipole moment. This effect arrises from fluctuations in the configuration of protons on the protein surface as they associate with and dissociate from ionizable amino acid residues. Kirkwood and Shumaker (1952) treated the case of dipole fluctuations in static fields. For a spherical protein of radius r the mean square fluctuating dipole moment $\langle \Delta\mu^2 \rangle$ is given by

$$\langle \Delta\mu^2 \rangle = e^2 r^2 \sum_i \frac{n_i}{2 + K_i/[H^+] + [H^+]/K_i},$$

where the e is the proton charge, n_i is the number of ionizable groups of type i with dissociation constant K_i, and $[H^+]$ is the proton concentration of the solution. From the amino acid composition of rhodopsin (Shields et al., 1967; Heller, 1968; Shichi et al., 1969; Zorn and Futterman, 1971), the rms fluctuating dipole moment is found to be 130 D at a pH value of 5 and 200 D at pH 4.3.

Scheider (1965, 1970) shows how this rms fluctuating dipole moment contributes to the dielectric increment when the protein also has a permanent dipole moment. If the protein has a permanent dipole moment $\langle \mu \rangle$ with a critical frequency due to rotational diffusion ν_{rot}, and a fluctuating dipole moment $\langle \Delta\mu^2 \rangle^{1/2}$ associated with a proton fluctuation critical frequency ν_{pf}, then the resulting dielectric dispersion is proportional to

$$\{\langle \mu \rangle^2 (1 + \nu^2/\nu_{rot}^2)^{-1} + \langle \Delta\mu^2 \rangle (1 + \nu_{pf}/\nu_{rot})^{-1} [1 + \nu^2/(\nu_{rot} + \nu_{pf})^2]^{-1}\}.$$

For rhodopsin the rotational diffusion critical frequency is 225 kHz, and the fluctuating dipole moment is 130–200 D, as determined above. The proton fluctuation critical frequency can be estimated from the dissociation of the ionizable amino acid groups of the protein (Scheider, 1965). For rhodopsin the proton fluctuation critical frequency is about 25 kHz. The critical frequency for the dielectric dispersion curve resulting from the combined effects of $\langle \mu \rangle^2$ and $\Delta\mu^2$ is essentialy the same as that for the permanent dipole moment alone. However, since the fluctuating dipole moment adds in quadrature with the permanent dipole moment, it contributes at most only a few percent of the total observed dipole moment of 720 D.

The Maxwell-Wagner effect occurs for a suspension of particles (proteins) if the ratio of the dielectric constant to the conductivity of the particles differs from that for the surrounding solvent. This leads to a change in the dielectric constant of the solution caused by the polarization (charge buildup) at the particle-solvent interface. The surface conductivity explanation of O'Konski is equivalent to the Maxwell-Wagner effect, where a term is added to the conductivity of the particle in order to include a conducting layer which reflects the presence of a higher concentration of ions at the protein-solvent interface. The dispersion of the dielectric increment in both cases is due to the fact that at low frequencies ionic conduction dominates the electric field distribution, whereas at high frequencies displacement (capacitative) currents dominate (O'Konski, 1960).

The relaxation time for the Maxwell-Wagner effect for spherical particles is (O'Konski, 1960) $\tau = (1/4\pi)(\epsilon_p + 2\epsilon_s)/(\sigma_p + 2\sigma_s)$, where ϵ is the dielectric constant, σ is the conductivity, and

the subscripts p and s refer to protein and solvent, respectively. The relaxation time can be estimated crudely by assuming $\epsilon_p \ll \epsilon_s$ and $\sigma_p \ll \sigma_s$ (i.e., nonconducting particles whose dielectric constant is small relative to that of water). This yields $\nu_c \cong 110$ kHz, close to the critical frequency observed. However, in this case the dielectric increment is proportional to $(\sigma_p/\sigma_s - \epsilon_p/\epsilon_s)^2$, and therefore is strongly dependent on solvent conductivity. According to this formula, the dielectric increments per unit rhodopsin concentration should vary several-fold over the conductance range of the experiments shown in Fig. 2*a*, contrary to what is seen. Alternatively, one can choose σ_p to satisfy exactly the experimental relaxation time. This assumption results in a dielectric increment which would vary several fold over the conductance range for the data in Fig. 2*a*, again contrary to the observed conductance insensitivity of the dielectric increment.

Concerning the surface conductivity model of O'Konski, reasonable choices of surface conductivity (O'Konski, 1960; Moser et al., 1966) give a critical frequency several hundred times higher than the observed value. Arbitrarily fixing surface conductivity to satisfy the observed relaxation time results again in the variation in dielectric increment with conductance which disallowed the Maxwell-Wagner explanation.

The ion migration effect refers to the polarization of the counterion layer surrounding the protein by the applied electric field. This displacement of counterions produces a dielectric increment with a relaxation time determined by the counterion mobility. Schwarz (1962) originally proposed this mechanism to explain the dielectric dispersion of polystyrene spheres in a well-conducting medium. Good agreement with experimental data resulted from assuming that the counterion mobility was about one-third the free mobility (for potassium ions in water). Using this assumption in the case of rhodopsin results in a critical frequency which is at least a factor of 30 too high.

A more realistic treatment of ion migration for the case of proteins is given by Scheider (1972). He considers the migration of protons along the protein surface. (Proton migration differs from proton fluctuations in that proton fluctuations refer to dipole fluctuations caused by protons in the solvent alternatively binding to and dissociating from ionizable sites, whereas proton migration refers to the jumping of protons from one site to another on the protein surface under the influence of an applied field [Scheider, 1970].) The rate-limiting factor for ion mobility in this case involves the dissociation of protons from ionizable groups on amino acids. Applying Scheider's methods to rhodopsin results in a critical frequency for proton migration of 125 kHz, within a factor of two of the observed value. The magnitude of the dielectric increment resulting from proton migration can also be obtained from Scheider's analysis. As was the case for the surface conductivity model of O'Konski (see above), the dielectric increment depends on the surface conductance. The surface conductance λ is a function of many factors, including the rms jump distance for protons migrating along the protein surface, the dissociation rate constant for the carboxyl and imidazole groups in the protein, the number of protons bound by the protein at its isoelectric point, and the radius of the protein molecule. Inserting reasonable values for these terms in the case of rhodopsin results in a surface conductance of about 2×10^{-13} mho at the isoelectric point. (For comparison, Scheider found that $\lambda = 2.6 - 3.8 \times 10^{-13}$ mho for serum albumin at its isoelectric point.) This value for surface conductance may be inserted in the appropriate equations for dielectric constant in the surface conductivity model of O'Konski (1960). The resulting dielectric increment is less than 1% of the experimentally observed dielectric increment for rhodopsin in Triton X-100. (Similarly, Scheider concluded that proton migration contributed less than 10% of the observed dielectric increment of serum albumin at its isoionic point.)

As noted by South and Grant (1972), the ion atmosphere model relies on a large number of ions surrounding the protein molecule, which is not the case for the low ionic strengths used in this experiment. Moreover, the relaxation time in this model is inversely proportional to the

product of the solvent conductivity and the concentration of ions around the protein. As a result, the experiments noted in Fig. 2*a* should have critical frequencies varying at least a factor of 2.5, whereas the observed critical frequencies are all within 20% of 225 kHz.

We conclude, therefore, that the frequency-dependent partial orientation of the permanent dipole moment of rhodopsin appears to account for most of the observed dielectric dispersion, in accordance with Oncley's interpretation.

The laser light-scattering measurements were performed with R. F. Bonner and H. Carney on an instrument kindly made available by F. D. Carlson.

We thank Mu-ming Poo and Ete Szüts for helpful suggestions on the manuscript.

This research was supported in part by a grant from the National Eye Institute (USPHS) and a U.S. Public Health Service Postdoctoral Research Fellowship (D. Petersen).

Received for publication 29 March 1974 and in revised form 9 June 1975.

REFERENCES

ABRAHAMSON, E. W., and J. R. WIESENFELD. 1972. The structure, spectra, and reactivity of visual pigments. *In* Handbook of Sensory Physiology. Vol. VII/1. H. J. A. Dartnall, ed. Springer-Verlag, New York. 69.

ALBRECHT, G. 1957. Terminal amino acids of rhodopsin. *J. Biol. Chem.* **229**:477.

AMES B. N., and D. T. DUBIN. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. *J. Biol. Chem.* **235**:769.

APPLEQUIST, J., and T. G. MAHR. 1966. The conformation of poly-l-tyrosine in quinoline from dielectric dispersion studies. *J. Am. Chem. Soc.* **88**:5419.

BOWNDS, D., A. GORDON-WALKER, A. C. GAIDE-HUGUENIN, and W. ROBINSON. 1971. Characterization and analysis of frog photoreceptor membranes. *J. Gen. Physiol.* **58**: 225.

BRIDGES, C. D. B. 1971. The molar absorbance coefficient of rhodopsin. *Vision Res.* **11**:841.

BURKE, J. J., D. C. PRATT, T. R. FAULKNER, A. MOSCOWITZ. 1973. An analysis of the absorption and circular dichroism of some visual pigments. *Exp. Eye Res.* **17**:557.

CONE, R. A. 1967. Early receptor potential: photoreversible charge displacement in rhodopsin. *Science (Wash. D.C.).* **155**:1128.

CONE, R. A. 1969. The early receptor potential. *In* Proceedings International School Physics, "Enrico Fermi." Course 43. W. Reichardt, ed. Academic Press, Inc., New York. 187.

CONE, R. A., and W. H. COBBS III. 1969. Rhodopsin cycle in the living eye of the rat. *Nature (Lond.).* **221**: 820.

CRESCITELLI, F. 1967. Extraction of visual pigments with certain alkyl phenoxy polyethoxy ethanol surface-active compounds. *Vision Res.* **7**:685.

CUMMINS, H. Z., F. D. CARLSON, T. J. HERBERT, and G. WOODS. 1969. Translational and rotational diffusion constants of tobacco mosaic virus from Rayleigh linewidths. *Biophys. J.* **9**:518.

DAEMEN, F. J. M. 1973. Vertebrate rod outer segment membranes. *Biochim. Biophys. Acta.* **300**:255.

DAEMEN, F. J. M., J. M. P. M. BORGGREVEN, and S. L. BONTING. 1970. Molar absorbance of cattle rhodopsin. *Nature (Lond.).* **227**:1259.

DEBYE, P. 1929. Polar molecules. New York Chemical Catalog Co., Reinhold Publishing Corp., New York.

DEBYE, P., and H. FALKENHAGEN. 1928. Dispersion der Leitfähigkeit und der Dielektrizitätskonstante starker Electrolyte. *Phys. Z.* **29**:121, 401.

DINTZIS, H. W. 1952. Ph.D. thesis. Harvard University, Cambridge, Mass.

EMRICH, H. M. 1971. Optical measurements of the rapid pH-change in the visual process during the meta-rhodopsin I–II reaction. *Z. Naturforsch.* **26B**:352.

FALK, G., and P. FATT. 1966. Rapid hydrogen ion uptake of rod outer segments and rhodopsin solutions on illumination. *J. Physiol.* **183**:211.

FUKAMI, I. 1960. On the electrophoresis of cattle rhodopsin. *Jpn. J. Physiol.* **10**:666.

FUKAMI, I., B. L. VALEE, and G. WALD. 1959. Does rhodopsin contain a trace metal? *Nature (Lond.).* **183**:28.

GERBER, B. R., L. M. ROUTLEDGE, and S. TAKASHIMA. 1972. Self-assembly of bacterial flagellar protein: Dielectric behavior of monomers and polymers. *J. Mol. Biol.* **71**:317.

HAGINS, W. A., and R. E. MCGAUGHY. 1967. Molecular and thermal origins of fast photoelectric effects in the squid retina. *Science (Wash. D.C.).* **157**:813.

HAGINS, W. A., and H. RÜPPEL. 1971. Fast photoelectric effects and the properties of vertebrate photoreceptors as electric cables. *Fed. Proc.* **30**:64.

HARA, R. 1963. Changes in electrical conductance of rhodopsin on photolysis. *J. Gen. Physiol.* **47**:241.

HARA, T. 1958. The effect of illumination on the electrical conductance of rhodopsin solutions. *J. Gen. Physiol.* **41**:857.

HEITZMANN, H. 1972. Rhodopsin is the predominant protein of rod outer segment membranes. *Nat. New Biol. (Lond.).* **235**:114.

HELLER, J. 1968. Structure of visual pigments. I. Purification, molecular weight, and composition of bovine visual pigment$_{500}$. *Biochemistry.* **7**:2906.

HELLER, J. and T. OSTWALD. 1972. Rhodopsin: conformational changes in a membrane protein. *Ann. N.Y. Acad. Sci.* **195**:439.

HENRICKX, H., R. VERBRUGGEN, M. Y. ROSSENEU-MOTREFF, V. BLATON, and H. PEETERS. 1968. The dipolar origin of protein relaxation. *Biochem. J.* **110**:419.

HUBBARD, R., D. BOWNDS, and T. YOSHIZAWA. 1965. The chemistry of visual photoreception. *Cold Spring Harbor Symp. Quant. Biol.* **30**:301.

HUBBARD, R., P. K. BROWN, and D. BOWNDS. 1971. Methodology of vitamin A and visual pigments. *Methods Enzymol.* **18**(C):615.

INCARDONA, N. L., K. MILES, and B. N. BAKER. 1971. Sedimentation of bovine rhodopsin-digitonin micelles. *Nat. New Biol. (Lond.).* **229**:252.

JOHNSON, R. H., and T. P. WILLIAMS. 1970. Thermal stability of rhodopsin extracted with Triton X-100 surfactant. *Vision Res.* **10**:85.

KIRKWOOD, J. G., and J. B. Shumaker. 1952. The influence of dipole moment fluctuations on the dielectric increment of proteins in solution. *Proc. Natl. Acad. Sci. U.S.A.* **38**:855.

LEWIS, M. S., L. C. KRIEG, and W. D. KIRK. 1974. The molecular weight and detergent binding of bovine rhodopsin. *Exp. Eye Res.* **18**:29.

MATTHEWS, R. G., R. HUBBARD, P. K. BROWN, and G. WALD. 1963. Tautomeric forms of metarhodopsin. *J. Gen. Physiol.* **47**:215.

MAXWELL, J. C. 1873. A treatise on electricity and magnetism. Oxford University Press, London.

MORTON, R. A. 1972. The chemistry of the visual pigments. *In* Handbook of Sensory Physiology. Vol. VII/1. H. J. A. Dartnall, ed. Springer-Verlag, New York. 33.

MOSER, P., P. G. SQUIRE, and C. T. O'KONSKI. 1966. Electric polarization in proteins—dielectric dispersion and Kerr effect studies of isoionic bovine serum albumin. *J. Phys. Chem.* **70**:744.

MURIKAMI, M., and W. L. PAK. 1970. Intracellularly recorded early receptor potential of the vertebrate photoreceptors. *Vision Res.* **10**:965.

O'KONSKI, C. T. 1960. Electric properties of macromolecules. V. Theory of ionic polarization in polyelectrolytes. *J. Phys. Chem.* **64**:605.

ONCLEY, J. L. 1938. Studies of the dielectric properties of protein solutions. I. Carboxyhemoglobin. *J. Am. Chem. Soc.* **60**:1115.

ONCLEY, J. L. 1943. The electric moments and the relaxation times of proteins as measured from their influence upon the dielectric constants of solutions. *In* Proteins, Amino Acids and Peptides. E. J. Cohn and J. T. Edsall, eds. Reinhold Publishing Corp., New York. 543.

PERRIN, F. 1934. Mouvement Brownien d'un ellipsoide (I). Dispersion diélectrique pour des molécules ellipsoidales. *J. Phys. Radium* (7). **5**:497.

PERRIN, F. 1936. Mouvement Brownien d'un ellipsoide (II). Rotation libre et dépolarisation des fluorescences translation et diffusion des molécules ellipsoidales. *J. Phys. Radium* (7). **7**:1.

PETERSEN, D. C., and R. A. CONE. 1973. The effect of light on the dipole moment of rhodopsin. Association for Research in Vision and Opthalmology. Abstracts. 3.

RADDING, C. M., and G. WALD. 1956. Acid-base properties of rhodopsin and opsin. *J. Gen. Physiol.* **39**: 909.

ROSSENEU-MOTREFF, M. Y., F. SOETEWEY, R. LAMOTE, and H. PEETERS. 1971. Size and shape determination of apotransferrin and transferrin monomers. *Biopolymers.* **10**:1039.

RÜPPEL, H., and W. A. HAGINS. 1973. Spatial origin of the fast photovoltage in retinal rods. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, ed. Springer-Verlag, New York. 257.

SCHEIDER, W. 1965. Dielectric relaxation of molecules with fluctuating dipole moment. *Biophys. J.* **5**:617.
SCHEIDER, W. 1970. On models of dielectric relaxation due to steady-state chemical processes. *J. Phys. Chem.* **70**:4296.
SCHEIDER, W. 1972. The two-body diffusion problem and applications to reaction kinetics. *J. Phys. Chem.* **76**:349.
SCHWAN, H. P. 1957. Electrical properties of tissue and cell suspensions. *In* Advances in Biological and Medical Physics. Vol. 5. J. H. Lawrence and C. A. Tobias, eds. Academic Press, Inc., New York. 147.
SCHWARZ, G. 1962. A theory of the low-frequency dielectric dispersion of colloid particles in electrolyte solution. *J. Phys. Chem.* **66**:2636.
SCHWARZ, G. 1970. Acid-base catalysis of dielectric relaxation of zwitterions. *J. Phys. Chem.* **74**:645.
SENGBUSCH, G. V. 1970. Ph.D. thesis. Technische Hochschule, Aachen, Germany.
SENGBUSCH, G. V., and H. Stieve. 1971 *a*. Flash photolysis of rhodopsin. I. Measurements on bovine rod outer segments. *Z. Naturforsch.* **26B**:488.
SENGBUSCH, G. V., and H. STIEVE. 1971 *b*. Flash photolysis of rhodopsin. II. Measurements on rhodopsin digitonin solutions and fragments of rod outer segments. *Z. Naturforsch.* **26B**:861.
SHAW, T. I. 1972. The circular dichroism and optical rotatory dispersion of visual pigments. *In* Handbook of Sensory Physiology. Vol. VII/1. H. J. A. Dartnall, ed. Springer-Verlag, New York, 180.
SHAW, T. M. 1942. The elimination of errors due to electrode polarization in measurements of the dielectric constants of electrolytes. *J. Chem. Phys.* **10**:609.
SHICHI, H. 1970. Spectrum and purity of bovine rhodopsin. *Biochemistry.* **9**:1973.
SHICHI, H., M. S. LEWIS, F. IRREVERRE, and A. L. STONE. 1969. Biochemistry of visual pigments. I. Purification and properties of bovine rhodospin. *J. Biol. Chem.* 244:529.
SHIELDS, J. E., E. C. DINOVO, R. A. Henriksen, R. L. KIMBEL, JR., and P. G. MILLAR. 1967. The purification and amino acid composition of bovine rhodopsin. *Biochim. Biophys. Acta.* **147**:238.
SMYTH, C. P. 1955. Dielectric behavior and structure: dielectric constant and loss, dipole moment and molecular structure. McGraw-Hill, New York. Chap. 1, 7.
SOETEWEY, F., M. ROSSENEU-MOTREFF, R. LAMOTE, and H. PEETERS. 1972. Size and shape determination of native and defatted bovine serum albumin monomers. II: Influence of the fatty acid content on the conformation of bovine serum albumin monomers. *J. Biochem.* **71**:705.
SOUTH, G. P., and E. H. GRANT. 1972. Electric dispersion and dipole moment of myoglobin in water. *Proc. R. Soc. Lond. A Math. Phys.* **328**:371.
TAKASHIMA, S. 1962. Dielectric dispersion of protein solutions in viscous solvent. *J. Polymer Sci.* **56**:257.
TAKASHIMA, S. 1964. Dielectric dispersion of albumins. Studies of denaturation by dielectric measurement. *Biochim. Biophys. Acta.* **79**:531.
TAKASHIMA, S. 1969. Dielectric properties of proteins. I. Dielectric relaxation. *In* Physical Principles and Techniques of Protein Chemistry. Part A. S. J. Leach, ed. Academic Press, Inc., New York. 291.
WADA, A. 1959. Dielectric properties of polypeptide solutions. IV. Several problems on the electric dipole moment of the α-helix. *J. Chem. Phys.* **31**:495.
WADA, A. 1960. Dielectric properties of polypeptide solutions. Measurement of the dielectric properties of the α-helix by the low frequency bridge method. *Bull. Chem. Soc. Jpn.* **33**:822.
WADA, A. 1967. Dielectric properties of polypeptide solutions. *In* Poly-α-amino acids: protein models for conformational studies. G. D. Fasman, ed. Marcel Dekker, Inc., New York. 369.
WAGNER, K. W. 1914. Erklärung der dielektrischen Nachwirkungsvorgänge auf Grund Maxwellscher Vorstellungen. *Arch. Elektrotech.* **2**:371.
WALD, G., and P. K. BROWN. 1950. The synthesis of rhodopsin from retinene$_1$. *Proc. Natl. Acad. Sci. U.S.A.* **36**:84.
WALD, G., and P. K. BROWN. 1953. The molar extinction of rhodopsin. *J. Gen. Physiol.* **37**:189.
WONG, J. K., and S. E. OSTROY. 1973. Hydrogen ion changes of rhodopsin. I. Proton uptake during the metarhodopsin I_{478} metarhodopsin II_{308} reaction. *Arch. Biochem. Biophys.* **154**:1.
WU, C. W., and L. STRYER. 1972. Proximity relationships in rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **69**:1104.
ZORN, M., and S. FUTTERMAN. 1971. Properties of rhodopsin dependent on associated phospholipid. *J. Biol. Chem.* **246**:881.