INTERFACIAL ELECTRIC POLARIZABILITY OF PURPLE MEMBRANES IN SOLUTION

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ABSTRACT An investigation of the scattered light ($\lambda = 632.8$ nm) from purple membrane suspensions with different concentrations subjected to external AC and DC electric fields has been carried out. The electric pulses used were in the field strength range 0–3.2×10^4 Vm^{-1} and the frequency range 10 Hz–1 MHz, the pulse duration being ≤ 0.5 s. A concentration dependence of the relative changes in the scattered light intensity was obtained, the effect being positive on orienting the suspensions by an AC field at 1 and 10 kHz, and negative in the case of a DC field. The negative effects in the diluted samples decrease and turn positive as the strength of the field increases. The positive effects show the existence of an interfacial polarizability along the plane of the membrane, and the negative ones suggest the presence of a permanent dipole moment (p), perpendicular to the plane of the membrane. The values of γ (induced polarizability) and p were found to be on the order of 10^{-28}–10^{-29} Fm^2 and 10^{-24} Cm, respectively. An explanation in terms of membrane aggregation for the observed dependence on concentration is given.

INTRODUCTION

The electro-optics of dispersed systems (1–5) have been widely used for the investigation of biological systems (3–5). Recently electro-optic work on energy transducting fragments of the plasma membrane of *Halobacterium halobium* (purple membrane) has been reported (6–12). Purple membranes are ideal for the study of basic problems of photochemical biology and membrane structure and function (13–15).

Optical properties of suspensions of purple membranes oriented by an electric field provide information on the geometric, optical, and electrical parameters of purple membranes (6–12), and also reveal possible conformational changes in the purple membrane's bacteriorhodopsin caused by the applied electric field (6–9). Excitation of an oriented suspension of purple membranes with a pulsed laser beam provides new possibilities for studying the bacteriorhodopsin photocycle (16, 17). The preparation of dipole oriented dried layers of purple membranes is also of considerable interest (18–20).

Up to now, mainly DC electric fields have been used for electro-optical studies on purple membranes; purple membrane suspensions of relatively high optical density were used in which rather strong electrodynamical interactions between the particles are possible. Electro-optical measurements of aqueous colloids and biocolloids over a wide concentration range have shown that a significant induced dipole moment (1, 2, 4), due to interfacial electric polarizability of the particles, is a very general occurrence. On this basis a considerable interfacial electric polarizability of purple membranes is expected. Studies of electric dichroism (8, 11, 12) and electric-light scattering (10) in AC and DC fields suggest that this is in fact the case.

This paper presents electro-optical investigations of aqueous purple membrane suspensions oriented by DC fields and AC fields of different frequencies. Suspensions of relatively high optical density as well as of lower optical density (dilution up to 1:100) were used.

THEORY

If an external electric field induces only orientation of the disperse system's particles leading to a change of its optical properties (e.g., the intensity of the scattered light), the change will depend on the applied field strength and, in general, can be characterized by a dimensionless quantity (1, 2)

$$\alpha = (I_E - I_0)/I_0 \tag{1}$$

I_E and I_0 being the intensities of scattered light when the external orienting field is switched on or off, respectively.

At low degrees of orientation ($U \ll kT$, where k is the Boltzman constant, T the absolute temperature, and U the energy of orientation [1]) with the plane of observation perpendicular to the direction of the electric field, the following expression is obtained for the relative effect α in the case of disk-shaped particles in Rayleigh-Debye-Gans (RDG) approximation (21)

$$\alpha = \frac{A(KB)}{I_0(KB)}(\mu^2 + \delta)E^2 \tag{2}$$

B being the diameter of the particle, $K = (2\pi/\lambda')\sin(\theta'/2)$ (λ' is the wavelength of the light in the solution, θ' the angle of observation), $\mu = p_1/kT$ ($p_1 \gg p_2 = p_3$; p_1 is the component of the permanent dipole moment along, and p_2 and p_3 are the cmponents perpendicular to the symmetry axis[1] of the particle, $\delta = (\gamma_1 - \gamma_2)/kT$, ($\gamma_1$ and γ_2 are the electrical polarizabilities along, and perpendicular to, the symmetry axis of the particle, respectively), and $A(KB)$ and $I_0(KB)$ are the following functions

$$A(KB) = (1/3)\sum_{n=1}^{\infty}\frac{(-1)^n (KB)^{2n}\, n}{(n+1)!\,(n+2)!\,(2n+3)} \tag{3}$$

$$I_0(KB) = \frac{2}{(KB)^2}\left[1 - \frac{J_1(KB)}{KB}\right]. \tag{4}$$

$J_1(KB)$ is the cylindrical Bessel function of first order.

From the initial slope of the experimental curve $\alpha = f(E^2)$ using Eq. 2 for a given value of the particle diameter B, the sum of the permanent and induced moments is obtained.

In the case of disk-shaped particles following (21, 22), one obtains, at full orientation, for the relative variation of the scattered light intensity

$$\alpha_\infty^{\parallel} = \frac{4J_1^2(KB)}{(KB)^2 I_0(KB)} - 1 \tag{5}$$

$$\alpha_\infty^{\perp} = \frac{2}{I_0(KB)}\sum_{n=0}^{\infty}\frac{(-1)^n (KB)^{2n}(2n+1)!!\,(2n+1)!!}{(n+1)!\,(n+2)!\,2n!!\,2n!!}. \tag{6}$$

The superscripts $\parallel$ and $\perp$ denote the orientation of the particle symmetry axis with respect to the field direction. From Eqs. 5 and 6 one can calculate the values of B for the experimentally obtained values of $\alpha_\infty^{\parallel}$ and $\alpha_\infty^{\perp}$.

At low degrees of orientation, the disorientation of the particles after switching off the electric field (at $t = 0$) leads to an exponential relaxation decay (1, 2, 22)

$$\alpha_t = \alpha_0 \exp(-t/\tau) \tag{7}$$

where α_0 is the stationary value and α_t is the instant value at any $t > 0$. The relaxation time τ related to the rotational diffusion coefficient D (2, 4) by

$$D = 1/6\tau. \tag{8}$$

According to Perrin's equation (23) for strongly flattened rotational ellipsoids ($b \gg a$)

$$D = (3kT/16\pi\eta b^3)/(q^{-1} + tg^{-1}q) \tag{9}$$

$q = b/a$, a and b being the symmetrical and its perpendicular semiaxes of the ellipsoid, η the viscosity of the solution. For $b \gg a$ one obtains a much simpler relation (24)

$$B = (3kT/4\eta D)^{1/3}. \tag{10}$$

Assuming disk-shaped particles as a good approximation for a strongly flattened ellipsoid one can use Eqs. 9 or 10 to calculate the diameter of the disks when the value of D is known from the experimental curve (Eq. 7). The discrepancy between the two values is <2% when $q \geq 5$.

[1]In the case of disk-shaped particles, the symmetry axis is identical with the axis normal to the plane of the disk.

MATERIALS AND METHODS

Purple membranes of *Halobacterium halobium* were isolated using a standard procedure (25). Aqueous suspensions of the membranes were placed in an external electric field at different concentrations. The stock suspension had an absorbance of 0.7 at 562 nm; measurements were also carried out on suspensions diluted 20 and 100 times with doubly distilled water.

Relative changes in intensity of light scattering were measured at 90° with the electric field perpendicular to the plane of observation. The light source was a standard He-Ne gas laser (HNA 50, Carl-Zeiss-Jena, GDR), $\lambda = 632.8$ nm. The scattered light intensity was detected by a photomultiplier whose output was displayed on a storage oscilloscope (C8-13, USSR). A Zopan pulse generator type PGP 3 was used in the case of the DC field (Zopan, Warszawa Zaklad Opracovan Produkcji Aparatury Naukowej Warszawa, ul. Bródnowska 8). An AC electric field (10 Hz–1 MHz) was applied using a sine generator, GF 11 (Clamann & Grahnert, Dresden, GDR). Pulses of various duration (≤0.5 s) were used in both cases. All measurements were carried out at 293 K. The electric light scattering method and the apparatus used have been described elsewhere (1, 2).

RESULTS

Fig. 1 shows the dispersion dependence of α in the frequency range 10 Hz–1 MHz for the undiluted suspension (curve I) and for a diluted one in ratios 1:20 (curve II). The mesurements were made at 3.2×10^3 Vm^{-1}. At this field strength, α is on the linear part of the curve $\alpha = f(E^2)$, so the condition of a low degree of orientation is fulfilled ($U \ll kT$).

The influence of AC and DC fields up to 3.2×10^4 V m^{-1}—in the case of AC field at frequencies of 1 and 10 kHz, which are in the plateau region of the dispersion curves—was studied. Positive effects are found on orienting the purple membrane by means of an AC field, the effects being greater for diluted samples. Fig. 2 shows the dependence of α as a function of E^2. The same dependence in the case of the DC field is shown in Fig. 3. In this case

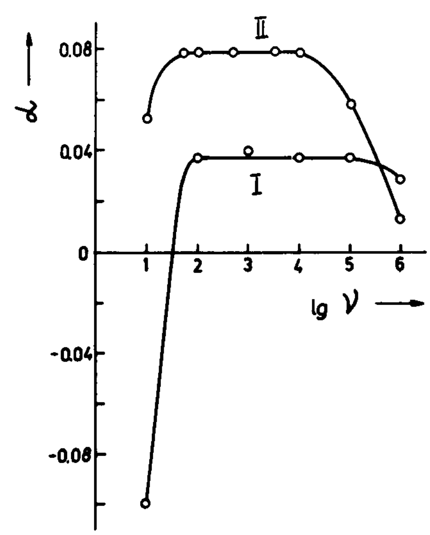

FIGURE 1 Dispersion dependence of the relative effect on the applied electric field of 3.2×10^3 Vm^{-1}. *I*, undiluted suspension (absorbance 0.7 at 562 nm); *II*, diluted suspension 1:20.

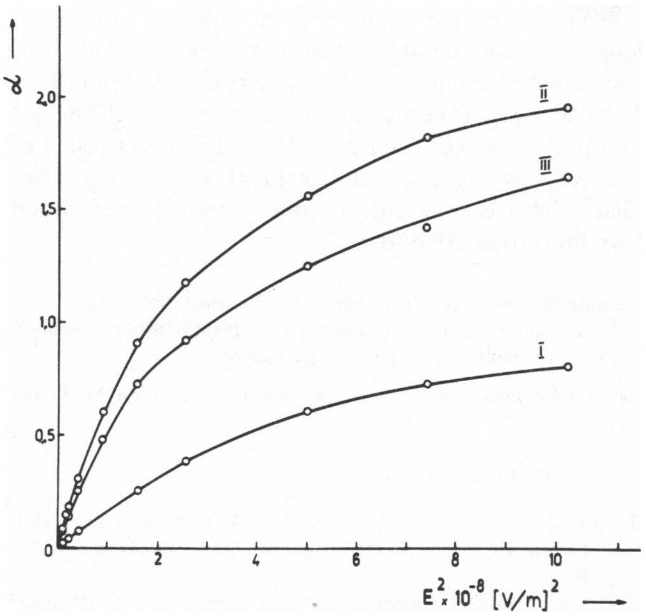

FIGURE 2 Electric light scattering vs. E^2 (AC applied field, ν = 1 kHz). *I*, undiluted suspension; *II*, diluted 1:20; *III*, diluted 1:100.

for the undiluted suspension, α is negative ($I_E < I_o$) in the whole range of electric fields used (0–3.2 × 10^4 Vm^{-1}). For the diluted samples the negative effect pases through minimum and turns positive at field strength values greater than 1.6 × 10^4 Vm^{-1}.

The data for both AC and DC orienting fields were used to separate the influence of permanent and induced dipole moments with the use of Eq. 2 (Table I).

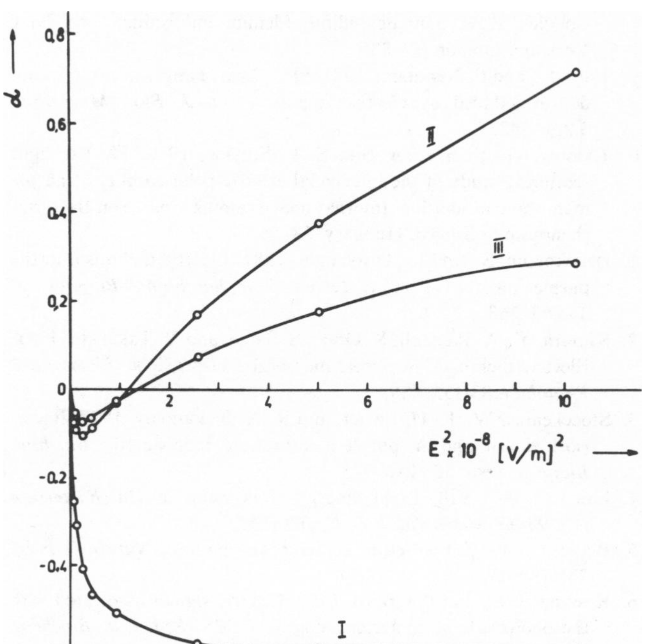

FIGURE 3 Electric light scattering vs. E^2 (DC applied field). *I*, undiluted suspension; *II*, diluted 1:20; *III*, diluted 1:100.

TABLE I
ELECTRIC PARAMETERS OF PURPLE MEMBRANES

Suspension	$10^{24} \times p_1$	$10^{29} \times (\gamma_2 - \gamma_1)$
	Cm	*Fm^2*
undiluted	3.9 ± 0.1	6.2 ± 0.2
dilute 1:20	2.6 ± 0.1	25.6 ± 0.2
dilute 1:100	1.7 ± 0.1	20.6 ± 0.5

An average value $\bar{\tau} \simeq$ 19 ms was found for the decay of the electro-optical effect and a value $\bar{B}(\bar{\tau})$ = 700 nm was calculated from Eq. 10 for the average diameter of the particles. (This value of $\bar{B}$ was used to determine p_1 and $\gamma_2 - \gamma_1$ values in Table I.)

The diameter of the particles was also obtained from Eqs. 5 and 6 and the experimental values of $\alpha_\infty^\perp$ and $\alpha_\infty^\parallel$. For $\alpha_\infty^\perp$ = 1.95 (AC electric field) $\bar{B}(\alpha_\infty^\perp)$ = 580 nm; and for the assymptotical value of $\alpha_\infty^\perp$ 2.5, $-B(\alpha_\infty^\perp)$ = 685 nm. In the case of DC fields, the saturated $\alpha_\infty^\parallel$ value gives $B(\alpha_\infty^\parallel)$ = 275 nm.

DISCUSSION

It is obvious from the dispersion curves (Fig. 1) that the effect α is independent of the electric frequency in the range 100 Hz–10 kHz. If one assumes that there is only an orientational effect, the conclusion that the orientation of the purple membranes is due only to the induced dipole moment $p_{ind} = (\gamma_2 - \gamma_1)\, E$ follows. Many investigations of colloids (1, 2) show that the electrical polarizability is of interfacial origin and is due to the electric field deformation of the particle double electric layer. It can be seen from our curves that in this case the electrical polarizability of the purple membranes is connected with a movement of interfacial charge, which leads to the induced moment. This conclusion is confirmed by the observation that the effect decreases at frequencies > 10 kHz (100 kHz for the undiluted suspension) because of the limited mobility of the ions responsible for the electrical polarizability of the particles.

The positive values of the effect in this frequency range indicate that the purple membranes orient with the symmetry axis perpendicular to the direction of the electric field, which is to be expected because the component of the electrical polarizability in the disk plane is much greater than that perpendicular to it ($\gamma_1 << \gamma_2$). Because the orientation is essentially due only to the induced dipole moment at frequencies in the plateau of the dispersion curve, from Eq 2 one obtains $\gamma_2 - \gamma_1$ from the initial slope of AC field curves $\alpha = f(E^2)$. The values obtained by us (Table I) agree with those of Kimura et al. (12), taking into account the differences in preparation of the samples and also different experimental conditions.

Because the curves shown in Fig. 2 were obtained using AC electric fields with frequencies from the plateau of the dispersion curves, the effects are strictly positive. The magnitudes of the effects are larger for the dilute suspensions than for the undiluted one and are indicative of the greater interfacial polarizability of the particles in the first case. This is also seen from the data in Table I.

The fast decay of the dispersion curves in the low frequency range (10–100 Hz) and the passage to negative values (for the undiluted suspension) indicate that at these frequencies the membranes are oriented with the symmetry axis along the applied field. The same orientation was observed when a DC electric field was applied (Fig. 3).

A possible explanation of this orientation is the existence of a permanent dipole moment in the purple membranes, which is oriented along the symmetry axis of the disk. The order of magnitude of p_1 values calculated from the experimental data (Table I) agrees well with the data of Kimura et al. (12) and with the later measurements of Keszthelyi (4).

The concentration dependence of the negative effect and of the p_1 value in our investigations could be explained by aggregation of the purple membranes in the undiluted suspension leading to the superposition of their permanent moments resulting in a different total moment responsible for the observed orientation. Diluting this solution presumably brings about a dissociation of the aggregates that leads to a decrease of the permanent moment, from which a more exact value of p_1 could be calculated.

When the strength of the orienting field increases, the induced dipole moment also increases, and at some magnitude of the applied field it becomes predominant, leading to a reorientation of the particles. We have observed such reorientation only for our diluted suspensions. Saturation of the negative effect was observed with undiluted suspension. We suppose that because of the competitive influence of the permanent and induced moments this saturation is only apparent. This opinion is supported by the discrepancies between $\bar{B}(\alpha_\infty^\parallel) = 270$ nm ($\alpha_\infty^\parallel$ is the saturated value of curve, *I*, Fig. 1) and $\bar{B}(\tau) = 700$ nm, although $\bar{B}(\alpha_\infty^\perp) = 685$ nm for the assymptotical value of $\alpha_\infty^\perp$ (curve *II*, Fig. 2) is in good agreement with $\bar{B}(\tau)$. This "false" saturation at low DC electric fields leads to a smaller calculated value for the angle between the transition moment and the normal to the membrane plane.

Although the electric light-scattering technique has been widely used for the investigation of heterogenous systems (1, 2), it could be regarded as a new method for the study of purple membrane. At appropriate wavelengths this technique is not subject to interference by other phenomena. If one uses the scattering method the information on the orientation of the membrane fragments does not depend on the existence and orientation of chromophores. Also, compared with other techniques, a very large concentration range can be studied, which is particularly useful in investigating processes of aggregation and interaction of membrane fragments. A possible drawback of the light scattering method is that it requires much more complicated analysis.

In conclusion we should like to point out that the dipole moments of purple membranes in aqueous suspensions depend to a considerable extent on the concentration. The electric polarizability is the measured quantity from which the orientation of the membrane fragments at high DC and high frequency AC electric fields can be understood. The relatively low frequency (10–100 kHz) of relaxation of this polarizability is good evidence for its interfacial nature and deserves further attention.

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