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ORIENTATION OF MEMBRANE FRAGMENTS BY ELECTRIC FIELD

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Summary

Purple membrane fragments from *Halobacterium halobium* were oriented by a static electric field in a water suspension. It was found that an electric field of approx. 20 V/cm is sufficient to achieve practically complete orientation; the purple membranes have a permanent electric dipole moment of $(6 \pm 1) \cdot 10^{-23}$ C · m, the orientation of the retinal transition moment relative to the direction of the electric dipole moment, θ, is $(59 \pm 1)^0$, and the purple membrane rotational diffusion constant $D_{rot} = 0.65\ s^{-1}$. It was found that because of the electrophoretic movement of the particles a hydrodynamic velocity gradient builds up which also orients the purple membranes.

Introduction

The orientation of macromolecules by an electric field is a well-known phenomenon [1]. Larger organelles like chloroplasts were first oriented by magnetic fields [2] and later by a 50 Hz alternating electric field [3]. In the latter work also sub-chloroplast fragments were oriented. As for various items of valuable information like the existence and size of permanent or inducible electric dipole moments of the particles [1], the direction of the transition moment of different chromophores relative to the dipole moments [2,3], rotational diffusion constants [1] and possible conformational changes [4] which can be obtained in such experiments, a detailed study of the orientation of purple membrane of *Halobacterium halobium* [5] has been undertaken. Research works using high-voltage pulses on a purple membrane suspension and static electric field of approx. 100 V/cm on purple membranes in a special gel system have already been reported [6,7].

In this paper we report that a static electric field of approx. 20 V/cm is sufficient to achieve practically complete orientation, the purple membranes have a

permanent electric dipole moment, and together with the dipole orientation a shear orientation appears because of the electrophoretic movement of the membrane fragments.

Materials and Methods

The purple membrane fragments used in the investigations were obtained by a standard procedure from *H. halobium* strain NRL R_1M_1 [8]. The average diameter of the purple membrane fragments is approx. 0.5 μm, their thickness approx. 5 nm [9]. The solution was further purified by washing it in water with a specific resistance of 10 M$\Omega \cdot$ cm.

The purple membrane suspension (pH 6) was poured into a cuvette of 0.1 cm thickness with platinum electrodes at a distance of 0.4 cm. The resistance of a 4—5 mm high column was approx. $2 \cdot 10^4$ Ω, the absorbance $A \approx 1.8$ (at 562 nm). Voltages of 0—10 V were applied through a resistance of 2 kΩ to the suspension in the following time order: T (positive), $2T$ (zero), T (negative) (Fig. 1e). T was generally 3 s. Voltage vs. time curves on the 2 kΩ resistance, recorded with a Tektronix T912 Storage Scope, showed a slow (time constant 0.5—1 s depending on voltage) time-dependence of approx. 20%. This was due to electrode effects. The voltages on the suspension were calculated assuming ohmic resistance only. The variation of the voltage fell to very small values between $t = 2.5$ and 3 s, therefore quantitative evaluations were performed for this time region.

As the light source, a stabilized high-pressure xenon lamp of 450 W with a Carl Zeiss monochromator and a Spectraphysics He—Ne laser of 1 mW were used. The diameter of the light beam was always smaller than 1 mm. The photocurrent from an RCA 6819A photomultiplier was amplified by a Keithley 417 high-speed picoamperemeter and recorded by a fast analog-digital converter and multichannel analyser (ICA 70).

All the experiments were performed at room temperature ($T \approx 23°$C).

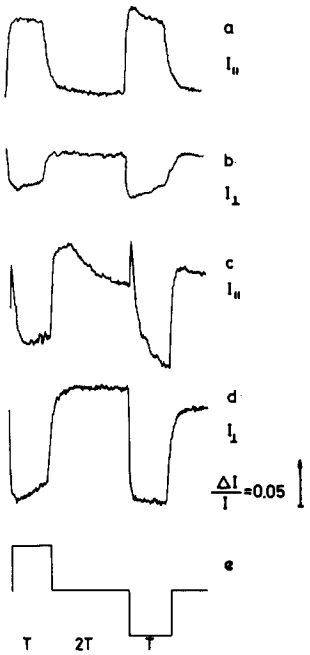
Results

The purple membranes contain as chromophore, bacteriorhodopsin molecules. the measurements to determine orientation were performed at $\lambda = 562$ nm. Changes in light intensity behind the sample were recorded with polarized beams (perpendicular to electric field $I_\perp$ and parallel $I_{/\!/}$) dependent on the electric field strength. Some of the results are reproduced in Fig. 1.

The results show that the purple membranes are oriented by the electric field, therefore they must have a permanent or inducible electric dipole moment. It is seen that the records always contain two practically symmetric deviations (for positive and negative fields) from the zero line I (absorption of the solution at 'rest'). The light intensity without absorber (I_0) was measured too. It is a straightforward matter to calculate the reduced dichroisms from the intensities

$$\frac{\Delta A_\perp}{A} = -\frac{1}{A} \log\left(1 + \frac{I_\perp}{I}\right) = \Phi(E)\left(\tfrac{3}{2}\sin^2\theta - 1\right) \tag{1a}$$

$$\frac{\Delta A_{/\!/}}{A} = -\frac{1}{A} \log\left(1 + \frac{I_{/\!/}}{I}\right) = \Phi(E)(3\cos^2\theta - 1) \tag{1b}$$



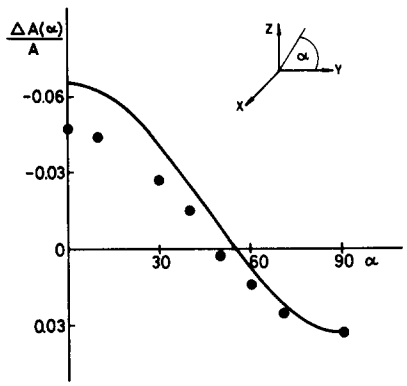


Fig. 1. Time course of light intensity changes behind the absorbing purple membrane suspension. λ = 562 nm. (a) Light polarized parallel to the direction of the electric field ($I_{\parallel}$), field strength E = 4 V/cm; (b) light polarized perpendicular to the direction of the electric field ($I_{\perp}$), E = 4 V/cm; (c) $I_{\parallel}$, E = 18 V/cm; (d) $I_{\perp}$, E = 18 V/cm and (e) time course of the applied voltage, T = 3 s.

Fig. 2. Dependence of the reduced dichroism, $\Delta A/A$, from the angle, α, between the direction of the field and of the plane of the polarized light. The insert shows the definition of α. ———, theoretical curve (Eqn. 3), $\Theta = 59°$; points, measured data. λ = 562 nm, E = 4 V/cm.

where Θ is the angle between the directions of the chromophore transition moment vector and of the electric field, $A = \log I_0/I$ and $\Phi(E)$ is the orientation function [1] which depends on the electric field strength E. From Eqns. 1a and b it follows that the requirement

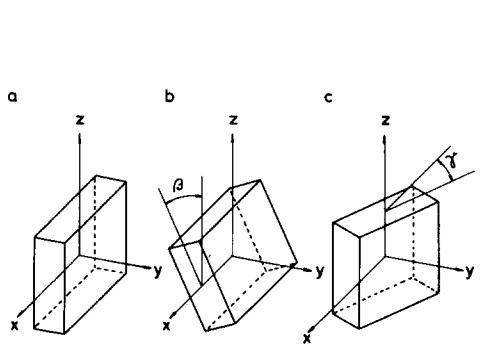
$$\frac{\Delta A_{\perp}}{A} = -\frac{1}{2}\frac{\Delta A_{\parallel}}{A} \tag{2}$$

must be satisfied independently of Θ, which means that in our case where $\Theta \approx 67°$ [13], $\Delta A_{\perp}/A > 0$ and $\Delta A_{\parallel} < 0$; consequently $I_{\perp} < 0$ and $I_{\parallel} > 0$ for any value of the orienting field. In this argument it is assumed that the electric dipole moment and the normal of the purple membrane are parallel. It can be seen in Fig. 1a and b that, at a field strength of 4 V/cm, Eqn. 2 is approximately fulfilled.

The result of the measurement of $\Delta A(\alpha)/A$, where α is the angle of the polarization vector relative to the field direction, is given in Fig. 2. The theoretical curve:

$$\frac{\Delta A(\alpha)}{A} = \Phi(E)(3\cos^2\theta\cos^2\alpha + \tfrac{3}{2}\sin^2\theta\sin^2\alpha - 1) \tag{3}$$

is near to the measured points. This result means that the purple membranes, as



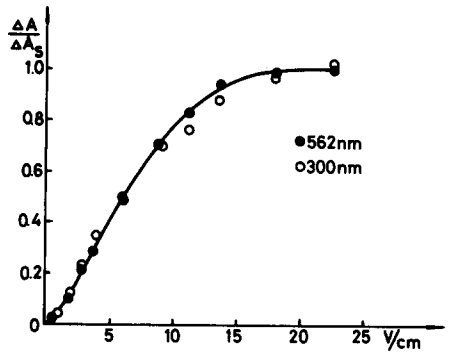


Fig. 3. Positions of purple membrane discs (represented by parallelepiped) during different orientations: (a) dipole orientation, (b) additional tilt around X-axis and (c) additional tilt around Z-axis. The direction of the orienting electric field coincides with the Y-axis.

Fig. 4. Dependence of $\Delta A_{\perp}/A$ on the electric field strength. The data are related to the saturation value of $\Delta A_{\perp}/A$. λ = 562 and 300 nm.

discs, are oriented perpendicularly to the field direction (Fig. 3a).

It was observed at higher field strength that $I_{\perp}$ behaves normally, i.e., it always remains smaller than 0; $I_{/\!/}$, however, is smaller than expected from Eqn. 1b (this is also apparent in Fig. 2), and even after having a small peak for a short time with $I_{/\!/} > 0$ it goes over to $I_{/\!/} < 0$. The calculated $\Delta A_{\perp}/A$ values reach saturation at a field strength of E = 18—20 V/cm (Fig. 4) whereas $\Delta A_{/\!/}/A$ values even change sign.

It is well known that oriented particles relax freely after the field is switched off with relaxation time:

$$\tau_{rel} = \frac{1}{6D_{rot}} \tag{4}$$

where D_{rot} is the rotational diffusion constant of the particles [1]. At least two relaxation modes can be observed for the $I_{/\!/}$ curves at higher field strengths with half-times $t_1 \approx 180$ ms and $t_2 \approx 1.2$ s. The directions of the relaxation modes are opposite. The relaxation time, t_1, coincides with those of $I_{\perp}$ curves. Another timing is also seen in the 'falling' part of the $I_{/\!/}$ curve, producing a peak at 18 V/cm with $t_3 \approx 300$ ms. The data clearly show that separate phenomena appear, but they can be observed mainly in the case of parallel polarization. The two relaxation times for purple membranes were already noticed by Shinar et al. [6] without explanation.

There are many reported cases when two relaxation times appeared for DNA molecules [1] and for RNA molecules [4]. In the latter case the explanation was that, in addition to the electric orientation, conformational changes of the molecules occur. In our case t_1 is related to the rotational diffusion because it appears at all field values, t_2 and t_3 could be related to some hypothetical conformational change following Ref. 4. This seems, however, very improbable.

The purple membranes have electric charge in addition to the electric dipole moment, therefore they are not only oriented perpendicularly to the field direction but they also move electrophoretically. The shearing forces are able

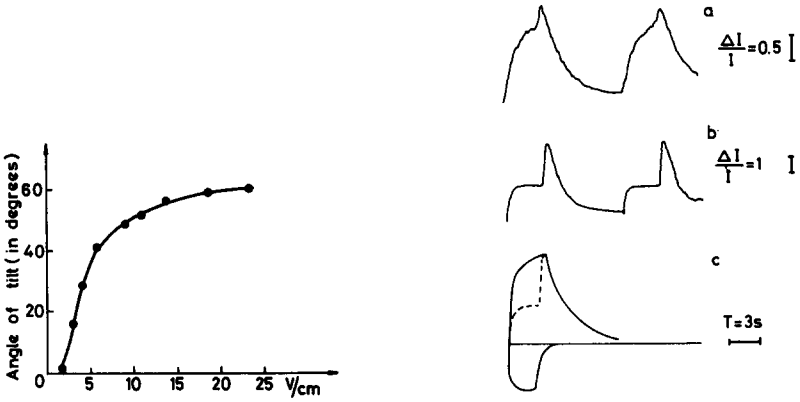


Fig. 5. Dependence of the angle of tilt around the Z-axis, γ from the electric field.

Fig. 6. Time course of the excess light scattering due to orientation. (a) $\lambda = 632$ nm, width of solid angle 10°, $E = 18$ V/cm; (b) $\lambda = 300$ nm, 5°, $E = 22$ V/cm; (c) components of the excess light scattering (only for explanation).

to tilt the purple membrane discs around Z- and X-axes (see Fig. 3) by angles β and γ, respectively.

The angles β and γ are, in principle, equal and are determined by both the holding force of dipole orientation and the hydrodynamic forces of the tilting. In our geometry, however, the probability of tilting around the X-axis is improbable.

Tilting around the Z-axis does not change the expression of $\Delta A_\perp/A$ (Eqn. 1): it changes, however, $\Delta A_\parallel/A$. The final value of $\Delta A_\parallel/A$ in the field in some cases can be expressed by putting γ instead of α into Eqn. 3 (the angle γ is measured from the x-axis). γ has been calculated from the measured data and given, depending on the field strength in Fig. 5. t_3 is the time needed for the electrophoretic flow orientation.

To understand the relaxation time constant, t_2, light-scattering experiments were performed. Excess scattering of $\lambda = 632$ nm light (from He-Ne laser) and $\lambda = 300$ nm were measured depending on field strength integrated in solid angles of widths 10 and 5°, respectively. Representative curves are shown in Fig. 6.

Two relaxation time constants, t_1 and t_2, appear when the field is switched off: the excess scattering increases with t_1 and then decreases with t_2. The curves clearly show that the purple membrane solution contains larger aggregates with longer relaxation time t_2 in addition to the single discs with t_1. According to the light-scattering theories [10], the large particles scatter light to small angles and the small particles to large angles: (the leading term of angular distribution is proportional to $(r/\lambda \cdot \sin(\psi/2))^2$, where r is the size of the particle, λ the wavelength and ψ the angle of scattering.) Therefore, in the given solid angle more scattered light is collected from large particles than from small ones. Moreover, small particles scatter the light out from the solid angles, their contribution appears mainly as a lack in excess scattering (see Fig. 6c).

Discussion

Size distribution of the particles

The results of light-scattering measurements is evaluated as the demonstration of the existence of two discrete components of the sizes of particles. The small particles with time constant, t_1, are the purple membranes with approx. 500 nm average diameter according to the literature [9]. The theoretically expected relaxation time:

$$\tau_{rel} = 1.4t = 2\pi\eta r^3/3kT \tag{5}$$

where η is the viscosity of the solution, r the diameter of the discs, k the Boltzmann constant and T the temperature in K [11]. Using the measured t_1 value and $r_1 = 500$ nm, a value of $\eta = 0.04$ P is obtained. This seems to be reasonable because the purple membrane suspension was rather dense.

From the value of t_2 the size of the larger particles can be calculated as $r_2 = \sqrt{t_2/t_1} \cdot r_1 \approx 1.9r_1$. The 'quantized nature' of relaxation times is easily understood (accepting that r in Eqn. 5 means the largest dimension of the particle): in the case of one disc $\tau_{rel(1)}$ is obtained, in the case of aggregation of two discs $\tau_{rel(2)}$ appears. The total cross-section of light scattering depends on the square of the number of molecules in the particles [10], therefore the large particles scatter four times more light than the small ones. The separation of light scattering of small and large particles can be considered complete in Fig. 6b ($\lambda = 300$ nm), therefore this measurement allows the estimation of the ratio of large particles to be approx. 15%.

The t_2 time constants in Fig. 1c and d have their origin in the relaxation of the oriented larger particles. They move much slower and the electrophoretic flow orientation (discussed below) does not appear during the measuring time. From the amplitude the ratio of large particles to small ones is again approx. 15%.

It is clear that the second time constant for relaxation reported for purple membranes in Ref. 6 and for broken chloroplasts [3] without explanation is due to some aggregates.

Dipole orientation

In the analysis of the linear dichroism data the contribution of larger particles (approx. 15%) is neglected.

The dipole orientation can be observed practically unperturbed in the data of $\Delta A_\perp/A$ (Fig. 3). The analysis of this curve can answer the question as to whether permanent or induced dipoles are responsible for the orientation [12]. In Fig. 7 plots of the quantity: $((\Delta A_\perp/E^2)/(\Delta A_\perp/E^2))_{E^2\to 0}$ vs. E^2 are presented.

The data are characteristic for permanent dipole orientation. (In the figure a curve is given for a pure induced dipole case too.) The orientation function for the permanent dipole case [12] is:

$$\Phi(\beta) = 1 - 3(\coth\beta - 1/\beta)/\beta \tag{6}$$

where $\beta = \mu BE/kT$. μ is the permanent dipole moment of the particle and $B = E_{int}/E$ is an internal field function which expresses that the internal field at the dipoles is different from the applied field E. From the data in Fig. 4 or Fig. 7,

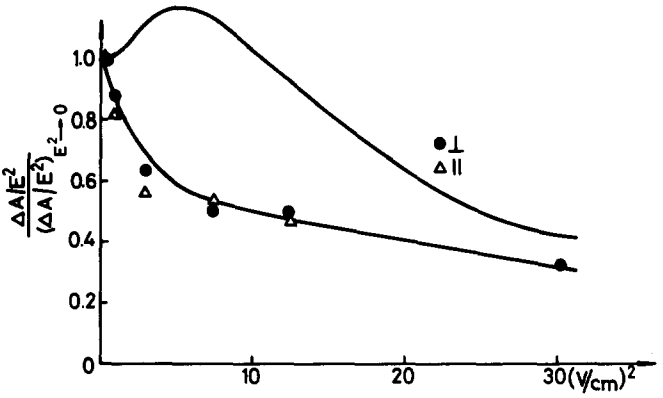


Fig. 7. Plots of the quantity $(\Delta A/E^2)/(\Delta A/E^2))_{E^2\to 0}$ vs. E^2. Line through points is the theoretical curve for permanent, the other for induced dipole moments.

$\mu B = (6 \pm 1) \cdot 10^{-23}$ C · m or $(2 \pm 0.3) \cdot 10^7$ debye. This huge dipole moment is the sum of many individual dipole moments in the purple membrane.

If it is assumed that the membrane-bound bacteriorhodopsin molecules are the only sources of the permanent dipole moment, $B \approx 1$ and the purple membrane contains approx. $1.8 \cdot 10^4$ bacteriorhodopsin molecules then:

$\mu_{BR}/e = n_e d = 18$ nm

where e is the elementary charge, n_e their number at a distance of d nm. If $d \approx 4.5$ nm, $n_e \approx 4$. To put it differently, $\mu_{BR} \approx 1000$ debye.

The saturation value of $\Delta A_1/A = 0.11 \pm 0.02$, therefore using Eqn. 1a, the orientation of the retinal transition moment relative to the electric dipole moment of the purple membrane plane is $\Theta = (59 \pm 1)^0$.

Reported values for the angle between the orientation of the retinal transition moment and the normal of the purple membrane plane Θ' vary from 71 to 63° [13—17]. Because the value of Θ is close to the mean of the Θ' values, we may state that the electric dipole moment is nearly perpendicular to the purple membrane disc as it has been assumed in Results.

The half-life of the fast decay (Fig. 1) is $t_1 = 180$ ms, therefore the rotational diffusion constant for the purple membranes is, from Eqn. 4, $D_{rot} = 0.65$ s^{-1}. The value is of the same order of magnitude as that reported in Ref. 6.

Electrophoretic flow orientation

A description of the electrophoretic flow orientation can be given as follows.

When the field is switched on the dipole orientation takes place within approx. 100 ms. The purple membranes, which are supposed as being rigid discs [9], begin to move in the direction of the field pushing the water in front of them. A velocity gradient between the moving and standing water particles arises which causes the discs to tilt. The tilt increases until the hydrodynamic forces and dipole forces become equal. This situation is represented by the angle γ. The appearance of the tilt takes time that is characterized by $t_3 \approx 300$ ms.

As an additional argument for electrophoretic flow orientation traces of highly damped rotation, which characterize the shearing effects of differential flow, were observed in Fig. 1c and d. According to the theory [18] the period of one rotation:

$$T = \frac{2\pi}{G}(r_e + r_e^{-1}) \tag{8}$$

where G is the velocity gradient and $r_e = a/b$. In our case a is the thickness and b is the diameter of the purple membrane discs. As a rough estimation for a single disc $G \approx v_0/b$, where v_0 is the drift velocity of the particles. v_0 has been estimated to be approx. 0.1 cm/s from the rate of increase of resistance between the electrodes in the case of a constant applied electric field of approx. 20 V/cm. Then, $T \approx 0.2$ s from Eqn. 8 whilst the observed value of T is about 0.4 s.

Conclusions

The study of electric orientation of purple membranes demonstrates that practically complete orientation can be achieved by a small, quasi-static electric field with careful experimentation in the case of asymmetric planar membrane fragments of approx. 100—1000 nm diameter. It is very important to have a symmetric voltage course as in Fig. 1, to consider the consequences of possible coagulation of particles, to have low conductance of the solution and to control the electrode effects.

The oriented membrane fragments render the possibility of studying the function and probably the structure of membrane-bound proteins.

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