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Electro-optical investigation of lipid-depleted purple membranes

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Abstract

The effect of delipidation on the electric surface properties of purple membranes was investigated by electric light scattering and microelectrophoresis. Natural membrane lipids were partially removed (approximately 75% of the phospholipids are extracted) from purple membrane using mild treatment with the zwitterionic bile salt 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate. Partial delipidation of purple membrane fragments causes: (i) a strong reduction of the permanent dipole moment from 6.10×10^{-24} Cm for native to 0.56×10^{-24} Cm for delipidated purple membranes, and (ii) a two-fold decrease in the electric polarizability. The ionic strength effect on the electric surface properties of delipidated purple membranes confirms the electrokinetic nature of a part of the membrane charge asymmetry. The contribution of the native membrane lipids to the electro-optically observed permanent dipole moment, i.e. to the membrane charge asymmetry, of purple membrane is demonstrated.

Keywords: Electric light scattering; Bacteriorhodopsin; Delipidated purple membranes; Permanent dipole moment; Electric polarizability; Electrophoretic mobility

1. Introduction

Purple membranes (PM) from *Halobacterium halobium* consist of a single protein bacteriorhodopsin (bR) [1,2] and lipids (60% phospholipids and 30% glycolipids) [3,4] and are flat disc-shaped membranes with crystalline structure [5,6]. The protein molecules are packed in trimers, the space between and within the trimers being filled with lipid molecules forming a typical bilayer structure [7,8].

The natural lipids can be partially extracted from PM using different extraction techniques [8–11]. The three-dimensional structure of lipid-depleted PM (LD-PM) has been determined [12]; the spectroscopic properties [9,10], the deprotonation of the Schiff base [13] and photodynamics of bR [11] of lipid-depleted PM have been investigated. It has been also shown that the lipids are extracted mainly from the lipid layer between the trimers [8].

Purple membranes possess a high charge asymmetry and orient in an electric field.

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Electro-optic methods have been applied to investigate the orientation of PM fragments by an external electric field [14–17] and have provided information on the electric moments (permanent and induced dipole moments) [14–18], the retinal tilt angle [14,18–21], etc. It has been also shown that the removal of the C-terminal tail of the polypeptide leads to a decrease of the value of the permanent dipole moment [18,20]. The removal of bound cations from PM (by deionization) has even a stronger effect on the membrane charge asymmetry [22,23].

In the present study, the effect of partial delipidation of PM on the electric moments was investigated. The results are compared with previous electro-optic data on enzyme-treated and deionized PM (dei-PM) so as to distinguish between the contribution of the different membrane components and the electric double layer in the electro-optically obtained permanent dipole moment.

2. Experimental

Purple membrane fragments from ET1001 strain were isolated according to the procedure described in [24]. Delipidation of PM by treatment with 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS, purchased from Sigma Chemical Co.) was performed as described by Szundi and Stoeckenius [9]. After three times incubation of PM with CHAPS in 5 mM sodium acetate buffer (pH 5.4) the suspension was washed four to five times with distilled water by centrifugation [9].

Delipidated PM were deionized on a cation exchange column following the same procedure as that used for deionization of native PM [25].

The microelectrophoretic measurements were performed with Rank Brothers Apparatus MK II using a flat rectangular cell. Electrophoretic mobility values which differ by more than 5% at the two stationary levels were ignored.

The electric light scattering measurements were performed on suspension of purple membrane fragments with [bR] = 1 μM, pH 6.54, conductivity of the solution $\rho = 5 \times 10^{-6} \Omega^{-1}$ cm^{-1}, at 22°C.

2.1 Electric light scattering

Suspensions of PM fragments behave as an isotropic media due to the random orientation of the membrane fragments. Application of an external electric field induces anisotropy of the dispersed membrane system and consequently changes in the intensity of the scattered light, as a result of interactions between the particles electric moments and the applied field.

The theoretical background of the optical phenomena and the measuring apparatus are described in [15,26]. Only a brief representation will be given here.

The degree of orientation of the membrane fragments (the energy of orientation smaller than the energy of the thermal motion) in an external electric field is followed by measuring the relative change in the scattered light intensity:

$$\alpha = (I_E - I_0)/I_0 \qquad (1)$$

where I_E is the intensity of the scattered light when an electric field of strength E is applied to the suspension, and I_0 is the light intensity without an external electric field.

For low degrees of orientation of rigid thin discs in the Rayleigh–Debye–Gans (RDG) approximation the electro-optic effect α is expressed by [15]:

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

where $K = (2\pi/\lambda')\ \sin^2(\theta'/2)$, $A(KB)$ and $I_0(KB)$ are optical functions [27], B is the diameter of the disc, λ' is the wavelength of the light in the suspension medium, θ' is the angle of observation (in this case 90°), $\mu = p/kT$ and $\delta = (\gamma_1 - \gamma_2)/T$, where p is the permanent dipole moment along the symmetry axis; γ_1 and γ_2 are electric polarizabilities along the symmetry and transverse axes, respectively, k is the Boltzmann constant, and T is the absolute temperature.

Measurements of electric light scattering effect in a.c. and d.c. electric fields allowed evaluation of the permanent dipole moment and electric polarizability, as can be seen from eq. (2).

The transient process of disorientation of the fragments after switching off the external electric field is given by [28]:

$$\alpha_t = \alpha_0 \exp(-t/\tau) \quad (3)$$

where α_t and α_0 are the electro-optical effects at time t after switching off the electric field and at steady state (full) orientation, respectively; $\tau = 1/6D_r$ is the relaxation time of disorientation related to the rotational diffusion constant (D_r). The diameter B of the thin circular disc was calculated from the relaxation time of disorientation according to [29]:

$$B = \sqrt[3]{\frac{9kT}{2\eta}}\,\tau \quad (4)$$

where η is the viscosity of the medium.

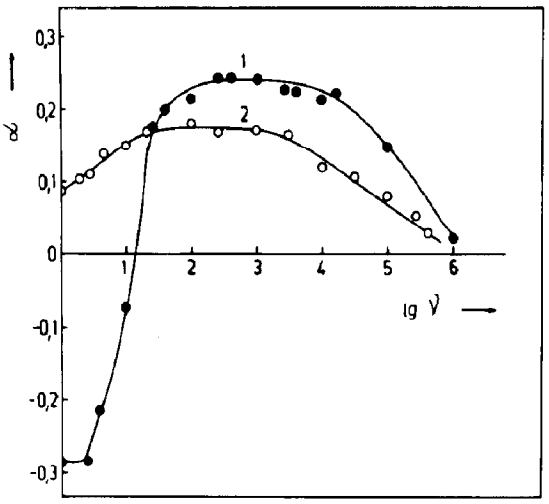

Fig. 1. Dispersion dependence of the relative electric light scattering effect, measured at a.c. electric field of strength 3.9 kV/m; curve 1–native PM (●), curve 2–DL-PM (○).

3. Results

It has been shown previously that PM fragments possess a permanent dipole moment (p) directed along the symmetry axis of the disc and an electric polarizability ($\gamma = \gamma_2 - \gamma_1 > 0$) whose component in the disk plane is much greater than that perpendicular to it [14,15,18].

The dependence of the electro-optic effect on the frequency of the applied electric field (at low electric field strengths from the linear part of the a.c. electric field dependence – i.e. low energy of orientation) gives information on both electric moments dependent on the frequency region (dispersion dependence Fig. 1). Three parts can be distinguished in the dispersion dependence – low frequency, plateau and high frequency regions. The positive values of the electro-optic effect α in the region 30 Hz–10 kHz (plateau region) indicate that the membrane fragments orient with symmetry axis perpendicular to the direction of the electric field and the orientation of the membrane fragments is due to the electric polarizability. The fact that in this region the electro-optic effect α is greater for nPM ($\alpha = 0.24$) than that for DL-PM ($\alpha = 0.15$) fragments proves that the interfacial electric polarizability is greater for nPM. At low frequencies (low frequency part of the dispersion curves – up to 100 Hz) the decrease of α with the decrease of the frequency and the change of the sign of the effect (about 30 Hz) for nPM are connected with the permanent dipole moment (Fig. 1 curve 1). It is noticed from the low frequency region of the dispersion dependence that the electro-optic effect for DL-PM does not change from a positive to a negative value with decrease in frequency (Fig. 1 curve 2). The dispersion dependence of deionized delipidated PM (dei-DL-PM) is similar to that of DL-PM where a positive electro-optic effect was observed at low frequencies (results not shown).

From the initial slope of the a.c. field dependence $\alpha(E^2)$ (Fig. 2) the electric polarizability γ (induced dipole moment) can be obtained, since the orientation is mainly due to the interaction of the induced dipole moment with the electric field (the electro-optic effect is positive). The frequency of the applied a.c. electric field was 1 kHz (corresponding to the plateau frequency region of the dispersion dependence) and is high enough to permit the relaxation of the permanent dipole moment. Saturation of the electro-optic effect (saturated value $\alpha_\infty = 1.7$) is reached at a higher field strength ($E = 47$ kV/m) for the three preparations.

The inset of Fig. 2 represents the extended part of $\alpha(E^2)$ dependence at low electric field

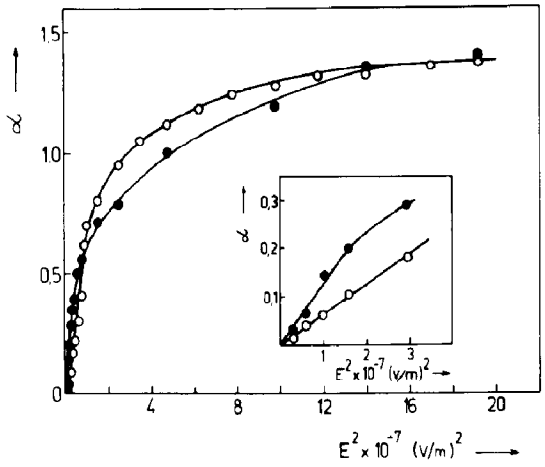

Fig. 2. Electric light scattering effect α vs. the square of the a.c. electric field strength E^2 with frequency 1 kHz; symbols as in Fig. 1. (Inset) For better comparison of the initial slopes the extended part of $\alpha(E^2)$ dependence is presented at low electric field strengths.

strengths (Fig. 2). The magnitude of the electro-optic effect α is larger for native PM than those for DL-PM which proves a greater interfacial polarizability. This result coincides with the dispersion dependence (plateau region, see Fig. 3). The reverse in the magnitudes of the effect α at intermediate field strengths might be connected with a change in the optical properties of the particles.

Figure 3 shows the dependence of the electro-optic effect α on the square of the d.c. electric field strength. While for nPM a negative electric

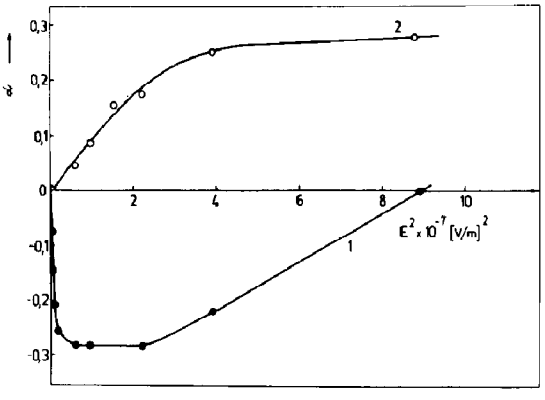

Fig. 3. Dependence of the electro-optic effect on the square of the d.c. electric field; symbols as in Fig. 1.

Table 1

Electric moments (electric polarizability γ, permanent dipole moment p) and electrophoretic mobility (u_e) of native, delipidated and deionized – delipidated purple membranes

Sample	$\gamma \times 10^{28}$ (F m^2)	$p \times 10^{24}$ (C m)	$u_e \times 10^8$ (m^{2}V^{-1}s^{-1})
PM	5.17 ± 0.07	6.10 ± 1.00	2.38 ± 0.02
DL-PM	2.33 ± 0.11	0.56 ± 0.33	1.99 ± 0.02
dei-DL-PM	1.64 ± 0.03	0.50 ± 0.11	2.09 ± 0.03

light scattering effect ($I_E < I_0$) is observed at low d.c. electric field strengths (Fig. 3 curve 1) which turns positive at field strength values higher than 10 kV/m (Fig. 3 curve 1), only positive effects were observed for DL-PM (Fig. 3 curve 2) and dei-DL-PM (results not shown) in the whole region of d.c. electric field applied (Fig. 3). The lack of a negative effect at low d.c. fields for the latter two cases (DL – PM and dei-DL-PM) indicates a reduced permanent dipole moment caused by both delipidation and deionization and suggests a domination of the induced dipole moment in the orientation of DL-PM and dei-DL-PM. Using the value of the electric polarizability γ (obtained from the $\alpha(E^2)$ dependence in a.c. electric field) and the initial slope of the $\alpha(E^2)$ dependence in d.c. field the permanent dipole moment was evaluated according to eq. (2).

The estimated values for the electric polarizability, permanent dipole moment and the electrophoretic mobility of the membrane fragments are presented in Table 1. The value of the permanent dipole moment of nPM ($p = 6 \times 10^{-24}$ C m) corresponds to previously reported data [14,15,18] and to the structural data for the asymmetric distribution of the membrane components [30]. Delipidation of PM and subsequent deionization of DL-PM affect the permanent dipole moment more than the electric polarizability of the PM fragments. The value of the permanent dipole moment of DL-PM ($p = 0.56 \times 10^{-24}$ C m) is about ten times smaller than that of native PM, while the electric polarizability is not reduced to the same extent upon delipidation. The decrease of the electric moments after delipidation of PM fragments corresponds to the observed decrease of the electrophoretic charge (Table 1).

Both delipidation of PM and deionization of DL-PM do not result in a change of the diameter of the membrane fragments, which was calculated according to eq. (4), to be ca. 700 nm for the three membrane preparations.

The salt concentration effect on the electric polarizability, permanent dipole moment and electrophoretic mobility for DL-PM is presented in Fig. 4. A correlation between the changes of the permanent dipole moment and the electrophoretic mobility upon the increase of NaCl concentration is observed. This suggests a connection of the permanent dipole moment with the translational electrokinetic behaviour of the delipidated PM fragments. The change of the electric polarizability of LD-PM with salt concentration is in an opposite direction than those of the electrophoretic mobility and permanent dipole moment (Fig. 4).

4. Discussion

In this study, we have shown that the extraction of the native purple membrane lipids is associated with a considerable reduction of the electric moments of purple membranes.

The reduction of the electric polarizability γ (connected with the movements of the diffuse double layer ions and charge dynamics) by delipidation of PM, as well as by deionization of LD-PM, indicates that the structure of the electric double layer is significantly changed. The subsequent decrease of the electric polarizability upon deionization of DL-PM suggests that ions associated with the membrane surface participate in the orienting torque. It is worthwile to mention that this can be supposed if the optical properties of the membrane fragments remain unchanged after delipidation.

It is likely that after treatment of PM with CHAPS lipids are removed from both sides of PM fragments, since: (i) the decrease of the lipid phosphorus has been shown to range from 9–10 to 3.3–3.4 P/bR and that of glycolipid content from 3 to 1 or 2 molecules per bR [10]; and (ii), as shown by Henderson et al. [31], glycolipids are entirely located on the external side of the PM, and phospholipids – though distributed on both external and cytoplasmic sides – occur predominantly on the cytoplasmic side [4].

The reduction of the value of the permanent dipole moment after delipidation (ca. ten times) is larger than that caused by the removal of the negatively charged amino acid residues from the C-terminal tail (reported to be ca. three times) [18,20]. This finding supports the idea that the charged lipid polar head groups contribute considerably to the charge asymmetry (permanent dipole moment) of PM fragments. On the other hand, comparison of the difference in the permanent dipole moment between native PM and DL-PM ($\Delta p_{DL} = p^{PM} - p^{DL-PM}$) and that between native PM and blue form of PM (dei-PM) ($\Delta p_{dei} = p^{PM} - p^{dei-PM}$) [22] shows that delipidations has even a stronger effect ($\Delta p_{DL} = 5.54 \times 10^{-24}$ C m) on the charge asymmetry than does the removal of bound metal cations ($\Delta p_{dei} = 3.1 \times 10^{-24}$ C m). This observation suggests that Δp_{DL} might involve also changes due to the removal of cations which balance the charges of the lipid polar head groups.

In spite of the widely accepted concept for the structural origin of the permanent dipole moment, several experimental evidences exist for a "nonstructural" component of the permanent dipole moment. The observed electrolyte dependence of the permanent dipole moment of DL-PM (Fig. 4) and the lower permanent dipole moment value of deionized DL-PM (Table 1) presented in this work confirms the existence of an "interfacial" permanent dipole moment connected with movements of charges in the close vicinity of the particle surfaces. In our previous work, intended to investigate the permanent dipole moment of the deionized blue form of PM, a convincing evidence for the existence of an "interfacial" component of the permanent dipole moment of PM was presented [22]. This was inferred from the considerable decrease (more than four times) of the permanent dipole moment upon deionization (i.e. a process of cation exchange by protons which takes place near the membrane surface) of PM.

The observed decrease of the electric polarizability of DL-PM with the increase of salt con-

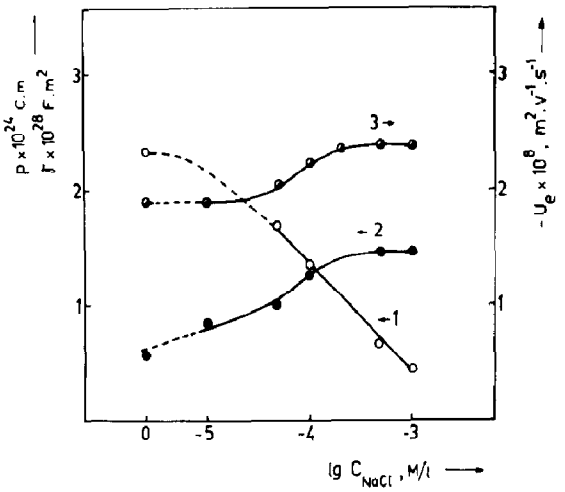

Fig. 4. Salt concentration effect on the electric polarizability (curve 1), permanent dipole moment (curve 2) and electrophoretic mobility (curve 3) of delipidated PM.

centration is connected with the decrease of the thickness of the diffuse part of the electric double layer. A decrease of the electric polarizability γ with the increase of the salt (NaCl) concentration has been already observed for many colloid particles [18,19,26]. The increase of both the electrophoretic mobility u_e and the permanent dipole moment p with the increase of the electrolyte concentration might be connected with a change in the ionic equilibrium leading to adsorption of coions [32]. The correlation between the changes of the permanent dipole moment and the electrophoretic mobility of DL-PM with the electrolyte concentration reveals the electrokinetic nature of the membrane charge asymmetry [19,26].

These data together with the evidences for permanent dipole moment component related to the asymmetry of the charges of the lipid polar head groups (DL-PM) and amino acid residues of the polypeptide chain [18,20] confirm the complex nature of the permanent dipole moment. Thus the "electro-optical" permanent dipole moment (p) can be considered as determined by: $p = p^{bR} + p^{L} + p^{EDL}$ (where p^{bR} is the dipole moment due to the asymmetric distribution of the charged amino acid residues, p^{L} results from the asymmetric distribution of the charged lipids and p^{EDL} is the component due to the ions in the electric double layer).

In agreement with the results presented in this work, the removal of both the natural lipids and the bound metal cations have been shown to affect the quenching efficiency of the tryptophan fluorescence, and to have a control role on the protein conformation [33]. The change of the values of the electric moments follows the same order as the change of the quenching amplitude of tryptophan fluorescence during the photocycle of DL-PM and nPM as reported in [34], i.e. delipidated PM, nPM.

The large decrease of the membrane charge asymmetry (permanent dipole moment) might be the reason for the reported change of the mechanism of proton transfer [11] and the reduction of the proton pump efficiency [35] after lipid extraction. A control role of the lipid composition and its surface charges on the protein conformational changes has been suggested by Jang et al. [36]. All these data confirm the complex nature of the permanent dipole moment (electro-optically determined). The charged amino acid residues of the polypeptide chain (C-terminal tail), the charged lipid head groups and the bound cations contribute to the permanent dipole moment. The large decrease of the permanent dipole moment value after delipidation and deionization of PM is compatible with the reduction [11,13] and the lack of M_{412}-intermediate formation [37,38] and proton pumping [10,37,38] in delipidated and deionized PM, respectively. These results suggest that the membrane charge asymmetry (permanent dipole moment) would be related to the functional activity of the membrane protein bR.

5. Conclusions

The charged lipid polar head groups contribute considerably to the charge asymmetry of purple membrane.

The electro-optically obtained charge asymmetry of PM and delipidated PM fragments is connected not only with the structural asymmetry but with the structure of the electric double layer as well.

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