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Induced dipole moment of purple membranes

Theoretical analysis

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The induced dipole moment of a purple membrane parallel to the membrane surface was analysed based on the two-dimensional fluctuation theory of counterions. The observed polarizability previously measured by electric dichroism and its dependence on the membrane size were derived from theory for the case where the interaction between counterions was screened. The screening effect of salts on the induced dipole moment was estimated from the theory on diffuse double layers.

1. Introduction

In the purple membrane of *Halobacterium halobium*, bacteriorhodopsin, a specific protein with retinal as chromophore, is arranged with several lipids in a two-dimensional crystalline array. The membrane is flat and rigid. The densities of positive and negative charges on both sides of the membrane can be estimated from the known arrangement of proteins and lipids. Bacteriorhodopsin is a light-driven proton pump which transports protons from the inside of the cell to the outside via a photoreaction cycle. To understand the molecular mechanism of the light-driven pump, we have studied the charge distribution on and across the membrane by measurement of the electric dichroism of a suspension of the membrane. The permanent dipole moment perpendicular to the membrane and the induced dipole moment parallel to the membrane have been established [1,2]. The induced dipole moment and its dispersion under an alternating field are related to the number of bound counterions and their movements on the membrane. The moment depends on the interaction of fixed charges of the membrane and counterions in the solution. Thus, the purple membrane is a typical model for the theoretical analysis of behaviors of counterions on the membrane. In this paper, we perform calculations on the induced dipole moment based on the two-dimensional fluctuation theory of counterions. The dependence of the induced dipole moment or the polarizability and its relaxation on the size of the membrane theoretically derived show good agreement with the experimental data. The effect of the ionic strength on the induced dipole moment is also discussed.

2. The induced dipole moment and counterion fluctuation

Thermal fluctuation in the concentration of counterions bound to a rod-like polyion has been

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analysed previously by Oosawa [3]. In this section, we apply the same method to counterions on a two-dimensional membrane.

The polarizability α at low electric fields is proportional to the square average of the instantaneous dipole moment M induced by thermal fluctuation of counterions in the absence of the field, $\langle M^2 \rangle$. The membrane is assumed to be a square of side length L and to have a uniform negative charge density. Positive counterions with charge $+e_0$ are bound on one side of the membrane. They are mobile along the surface of the membrane and distributed with an average concentration $C = N_+/L^2$, where N_+ is the average number of bound counterions. The concentration fluctuation of bound counterions deviating from the average by $\delta C(x,y)$ is expanded in a Fourier series on the surface as follows;

$$\delta c(x,y) = \sum_{k,k'} c_{k,k'} \exp(2\pi i(kx/L) + 2\pi i(k'y/L)) \qquad (1)$$

where x and y are spatial coordinates on the membrane, $0 < x < L$ and $0 < y < L$, and k and k' are integers, giving wave numbers.

The excess free energy δf due to the concentration fluctuation is given by the sum of the excess interaction energy and the negative entropy as;

$$\begin{aligned}\delta f = {} & (1/4)\int_0^L\int_0^L\int_0^L\int_0^L \delta C(x,y)\,\delta C(x',y') \\ & \times (2f(x-x',y-y') \\ & + g(x-x',y-y'))\,\mathrm{d}x\,\mathrm{d}x'\,\mathrm{d}y\,\mathrm{d}y' \\ & + (k_bT/2C)\int_0^L\int_0^L (\delta C(x,y))^2\,\mathrm{d}x\,\mathrm{d}y \qquad (2)\end{aligned}$$

where k_b is Boltzmann's constant, T the absolute temperature, and $f(x-x',y-y')$ and $g(x-x',y-y')$ the interaction energies of counterions on the same side of the membrane and between opposite sides of the membrane, respectively. By using the Fourier components of these interaction energies, we find

$$\delta f = (k_bT/2) \sum_{k,k'} c_{k,k'}^2 \big(L^4(2f_{k,k'} + g_{k,k'}) /4k_bT + L^2/C\big) \qquad (3)$$

where

$$\begin{aligned} f_{k,k'} = {} & (1/L^2)\int_0^L\int_0^L f(x'',y'')\exp(2\pi i(kx''/L) \\ & + 2\pi i(k'y''/L))\,\mathrm{d}x''\,\mathrm{d}y'' \\ g_{k,k'} = {} & (1/L^2)\int_0^L\int_0^L g(x'',y'')\exp(2\pi i(kx''/L) \\ & + 2\pi i(k'y''/L))\,\mathrm{d}x''\mathrm{d}y'' \end{aligned}$$

where the end effect has been neglected.

Since the probability of fluctuation $\delta C(x,y)$ is proportional to $\exp(-\delta f/k_bT)$, the mean square of the fluctuation of each component $c_{k,k'}$ is given by

$$\langle c_{k,k'}^2 \rangle = 1/\big((L^2/C) + \big(L^4(2f_{k,k'} + g_{k,k'})/4k_bT\big)\big) \qquad (4)$$

The electric dipole along the membrane surface produced by the fluctuation $\delta C(x,y)$ is given by

$$M = 2e_0\int_0^L\int_0^L ((x-x_0)\boldsymbol{I}_x + (y-y_0)\boldsymbol{I}_y) \times \delta C(x,y)\,\mathrm{d}x\mathrm{d}y \qquad (5)$$

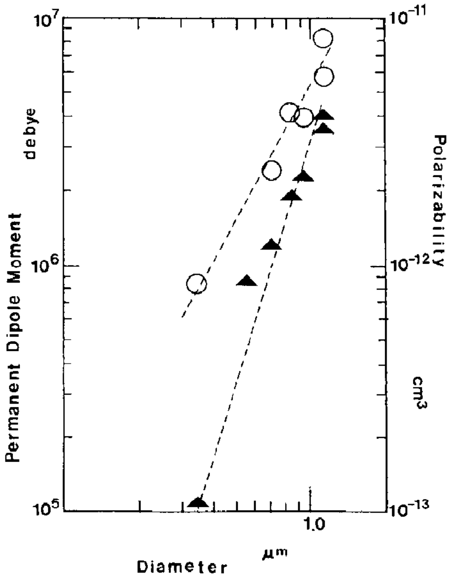

Fig. 1. Permanent dipole moment (○) and polarizability (▲) of the purple membrane plotted vs the diameter of the membrane L; data are from ref. 1; the experimental methods and conditions have been described previously [1]. Broken lines represent the proportionality of the permanent dipole moment to L^2 and the polarizability to L^4, respectively.

where (x_0, y_0) represents the centre of the square and $\boldsymbol{I}_x$ and $\boldsymbol{I}_y$ are the unit vectors in the x and y directions. The factor 2 is derived from the case of two sides of the membrane. Then,

$$\begin{aligned} M^2 = 4e_0^2 \int_0^L \int_0^L & ((x-x_0)(x'-x_0) \\ & + (y-y_0)(y'-y_0)) \\ & \times \delta C(x,y)\ \delta C(x',y')\ \mathrm{d}x\mathrm{d}y\mathrm{d}x'\mathrm{d}y' \end{aligned} \tag{6}$$

After integration, fluctuations in the x and y directions are separated. The polarizability α is related to the square average of the dipole moment as $\alpha = \langle M^2 \rangle / k_\mathrm{b}T$. Only the Fourier components $c_{k,0}$ and $c_{0,k}$ make contributions to $\langle M^2 \rangle$. Using eq. 4 we find

$$\begin{aligned} \alpha &= \langle M^2 \rangle / k_\mathrm{b}T \\ &= 4e_0^2 L^4 \sum_k (L/2\pi k)^2 \langle c_{k,0}^2 \rangle / k_\mathrm{b}T \\ &= (2e_0^2/\pi^2)(CL^4/k_\mathrm{b}T) \\ &\quad \times \sum_k (1/(1 + N_+(2f_{k,0} + g_{k,0})/4k_\mathrm{b}T)/k^2 \end{aligned} \tag{7}$$

This result shows that the contribution to the polarizability decreases with increasing k. The main contribution comes from the fluctuation of $k = 1$.

The purple membrane has a length L of 5000–10000 Å and a thickness of about 45 Å. The number of fixed charges per unit area of protein of about 1150 Å^2 is about 10. If the number of counterions which contribute to the polarizability N_+ is set equal to the total number of charges N_+^0 of the membrane, the value of α calculated from eq. 7 becomes of the order of 10^{-10} cm^3 if f and g are zero. If, in the other limiting case, Coulombic interaction is assumed to estimate f and g, α becomes of the order of 10^{-14} cm^3. The experimental value of α obtained in the presence of salts of 10^{-3}–10^{-2} M was 10^{-11}–10^{-12} cm^3, as shown in fig. 1. The interaction among counterions must be screened. If the Debye-Hückel interaction is assumed, for large values of L and small values of k, e.g., $k = 1$;

$$\begin{aligned} N_+ f_{1,0} &= (N_+/L^2) \int e_0^2 \exp(-\kappa r)/\epsilon r (2\pi r)\ \mathrm{d}r \\ &= (N_+/L^2)(2\pi)(e_0^2/\epsilon k_\mathrm{b}T)(1/\kappa) \end{aligned} \tag{8}$$

where ϵ is the dielectric constant and κ the Debye-Hückel parameter, $(1/\kappa)$ is the radius of the ionic atmosphere. Then, the value of α calculated approaches the experimental value. $N_+ f_{1,0}$ and $N_+ g_{1,0}$ are not dependent on the size of the membrane. Therefore, the polarizability given by eq. 7 is proportional to L^4.

As shown in fig. 1, the observed value of α is nearly proportional to L^4. This fact strongly supports the idea that the polarization is due to the counterion fluctuation on the membrane surface and that the interaction between counterions is screened in the presence of salt ions.

On the other hand, the permanent dipole moment perpendicular to the membrane surface is given by the sum of permanent dipole moments of individual protein molecules and lipid molecules in the membrane. Therefore, it is reasonably understood that the observed permanent dipole is proportional to L^2, as shown in fig. 1.

3. Relaxation of the induced dipole

Fluctuations in the counterion distribution of different modes have different relaxation times. According to Oosawa's theory [3], the diffusion equation of the counterion fluctuation of $\delta C(x,y,t)$ is given by

$$\begin{aligned} \zeta(\partial/\partial t)\ \delta C(x,y,t) &= (\partial^2/\partial x^2 + \partial^2/\partial y^2) k_\mathrm{b}T\, \delta C(x,y,t) \\ &\quad + (\partial^2/\partial x^2 + \partial^2/\partial y^2) C \int_0^L \int_0^L \delta C(x',y',t) \\ &\quad \times (2f(x-x', y-y', t) \\ &\quad + g(x-x', y-y', t))\ \mathrm{d}x'\mathrm{d}y' \end{aligned} \tag{9}$$

where ζ is the frictional coefficient of counter-ions. The equation for the Fourier component $c_{k,0}$ is obtained in the following form;

$$\begin{aligned} \mathrm{d}c_{k,0}(t)/\mathrm{d}t &= -(k_\mathrm{b}T/\zeta)(2\pi k/L)^2 \\ &\quad \times (1 + N_+(2f_{k,0} + g_{k,0})/4k_\mathrm{b}T) c_{k,0}(t) \end{aligned} \tag{10}$$

The relaxation time of the mode $(k,0)$ is given by

$$\tau_{k,0} = (\zeta/k_bT)(L/2\pi k)^2 \\ /(1 + N_+(2f_{k,0} + g_{k,0})/4k_bT) \qquad (11)$$

The relaxation time $\tau_{k,0}$ is proportional to the square of the length L, as in the one-dimensional case. In the Debye-Hückel approximation, using the same estimation of $f_{k,0}$ as before, the relaxation time $\tau_{1,0}$ is estimated to be of the order of 10^{-6}–10^{-7} s, provided the frictional coefficient ζ is assumed to be the same as in bulk water. The dispersion of the polarizability observed in the high-frequency region of electric field gave a value around 10^{-6} s for the relaxation time. This value of the relaxation time indicates that the relaxation is due to the long-distance diffusion of counterions on the membrane, although the proportionality of the relaxation time to L^2 has not yet been confirmed experimentally. Two samples of purple membranes which had a ratio of rotational relaxation times of (101/57) were examined. A difference in the relaxation time $\tau_{1,0}$ could not be detected between the two samples. Since the rotational relaxation time is proportional to L^3, the ratio of the relaxation times of the dielectric dispersion is expected to be $(101/57)^{2/3} = 1.4$, which is not very different from unity.

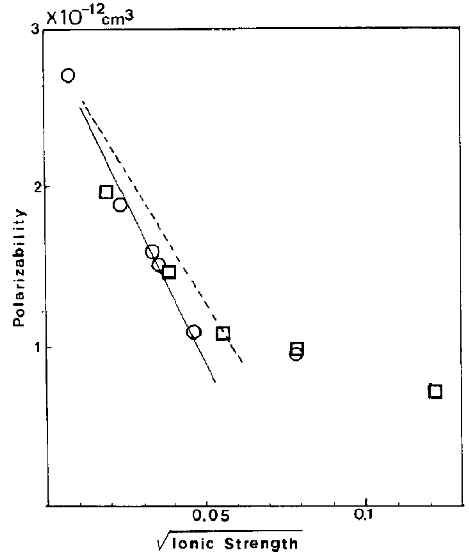

Fig. 2. Relation between the polarizability and the ionic strength of the purple membrane in the presence of monovalent salt ions (○) and divalent salt ions (□); data are from ref. 1; the experimental methods and conditions have been described previously [1]. The solid line and the broken line show calculated values for monovalent and divalent salts, respectively.

4. Effects of salts on the induced dipole

The polarizability of the purple membrane decreases with increasing ionic strength, as shown in fig. 2. According to the theoretical result in section 3, the polarizability is proportional to the number of counterions N_+ near the membrane if the interaction between counterions is weak. The number of counterions N_+ which contribute to the polarizability may decrease with increasing ionic strength. The relation between the number N_+ and the salt concentration can be analysed using the electric double layer theory [4].

The average charge density $e_0\rho(z)$ and the electric potential $\psi(z)$ at distance z from the membrane are related by the Poisson equation, and if a Boltzmann distribution is assumed;

$$\begin{aligned} d^2\psi(z)/dz^2 \\ &= -4\pi e_0\rho(z)/\epsilon \\ &= -(4\pi/\epsilon)e_0\sum_i \nu_i n_i \exp(-\nu_i e_0\psi(z)/k_bT) \end{aligned} \qquad (12)$$

where ν_i is the valence of the i-th ion and n_i its density far from the membrane. The negative charge density $-e_0N_+^0/L^2$ on the membrane surface is equal to the total spatial charge from $z = 0$ to $z = \infty$;

$$N_+^0/L^2 = \sigma = \int_0^\infty \rho(z)\,dz \qquad (13)$$

The counterions which contribute to the polarization are assumed to be those ions retained within the distance $z = Z$ from the membrane surface by the negative potential; i.e.,

$$N_+/L^2 = C = \int_0^Z \rho(z)\,dz \qquad (14)$$

where the distance Z is determined by assigning a certain critical value to $-e_0\psi(Z)/k_bT$ ($= Y(Z)$). The quantity $Y(z)$ is the ratio of the potential energy to the thermal energy of counterions. This assumption means that the potential energy of counterions which contribute to the polarization is very much larger than the thermal energy. The concentration of counterions in this region is very much higher than that far from the membrane and the concentration of co-ions is negligible.

Then, the calculated result is

$$\begin{aligned} C &= (\epsilon/4\pi e_0)\int_0^Z (\mathrm{d}^2\psi(z)/\mathrm{d}z^2)\,\mathrm{d}z \\ &= (\epsilon/4\pi e_0)\,|\mathrm{d}\psi(z)/\mathrm{d}z\,|_{z=0}^{z=Z} \\ &= \sigma - \left(2\epsilon k_bTn/\pi e_0^2\right)^{1/2}\sinh(Y(Z)/2) \qquad (15) \end{aligned}$$

If we apply the numerical values of the purple membrane,

$$\begin{aligned} C &= \sigma\Big(1 - \left(L^2/N_+^0\right)\left(2\epsilon k_bTn/\pi e_0^2\right)^{1/2} \\ &\quad \times \sinh(Y(Z)/2) \\ &= \sigma\left(1 - 0.74I^{1/2}\sinh(Y(Z)/2)\right) \end{aligned}$$

where n is the concentration of counterions far from the membrane and I the ionic strength. The value of C decreases in proportion to the square root of the ionic strength, if a constant value is given to $Y(Z)$ or $-e_0\psi(Z)/k_bT$. The solid and broken lines in fig. 2 are the results of calculation for the ratio of the polarizability in the presence of salts to that in the absence of salts in the case of $Y(Z) = 6$, which gave the best fit to the experimental data over a certain range of the ionic strength. The results of calculation, however, do not explain the data at high concentrations of salts. We have to consider the changes of both the number of bound counterions near the membrane and the effect of screening by other salt ions with the ionic strength. A more consistent theoretical treatment is necessary.

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