DIELECTRIC PROPERTIES OF THE POLAR HEAD GROUP REGION OF ZWITTERIONIC LIPID BILAYERS

ANTONIO RAUDINO AND DAVID MAUZERALL
The Rockefeller University, New York, New York 10021

ABSTRACT A theoretical model describing the dielectric properties of the lipid membrane-water interface region was developed. The rotating polar head groups (e.g. phosphatidylcholine) were simulated as a collection of interacting dipoles imbedded in a nonhomogeneous dielectric. The interactions between the nearest neighborhood were explicitly taken into account, while the other interactions were evaluated by means of the continuum theories. The values of the dielectric constant, its anisotropy and the spontaneous polarization of the interface were evaluated. As an application, we calculated the energy of interaction between an ion and the membrane polar head group region. The results indicate a small spontaneous polarization of the interface (1–1.7 Debyes per lipid molecule) due to the tilting angle of the choline residue with respect to the membrane surface. This dipolar field partially compensates that of opposite orientation originating from the ester group region, giving calculated overall dipolar potentials in better agreement with the experimental data. Our model suggests also a very strong dielectric anisotropy of the interface region, the component of the dielectric constant perpendicular to the membrane plane being much smaller than the parallel component.

INTRODUCTION

The knowledge of the dielectric properties of the membrane-water interfacial region plays a key role in understanding several phenomena such as cell-cell adhesion, chemical reactivity on the membrane surface and lipid-water solute exchange rates. Owing to the complexity of the molecular structure of the interface, a calculation of the dielectric properties can be accomplished only by using simplified models based on mean field or continuum theories (1). Until now, an evaluation of the dielectric properties of the membrane-water interface region has not been performed, chiefly because of lack of suitable theoretical models for this nonhomogeneous medium: the dielectric constant changes from ~2 to 78 in a range of 10–15 Å (2).

There is some experimental information available on the dielectric properties of the polar region of lipid membranes. For example, capacitance measurements on planar lipid bilayers (3) indicate that only a quite low (10–30) interfacial dielectric constant can fit the data without requiring excessively large values of the membrane thickness. Spectroscopic and kinetic studies of probes dissolved in the lipid membrane polar region suggest similar values (4, 5).

On the other hand, a recent series of papers devoted to the study of the dielectric relaxation properties of phospholipid vescicles, clearly shows much larger values of the dielectric constant arising from the rotation of the polar head groups in a plane parallel to the membrane surface (6, 7). This last model seems to agree very well with the one used to rationalize nuclear magnetic resonance (NMR) data of the choline group (8).

Finally, measurements on lipid monolayers show a polarization of the lipid membrane, the hydrocarbon interior being generally more positive (9). This polarization has been ascribed to the ester group orientation in phospholipid membranes; however, theoretical values obtained by this previous model are three times as large as the experimental values (3). The reaction of charged electron acceptors with photoexcited porphyrins across the lecithin bilayer-water interface indicates that the negatively charged acceptors approach the interface more easily than do acceptors with positive charge (10).

In this paper we will rationalize these experimental findings on the basis of a theoretical model that relates the dielectric properties of the interfacial region with its molecular structure.

As an application of the present formalism and of the calculated dielectric properties, we will estimate the electrostatic solvation energy for an ion moving across the membrane polar region.

CALCULATIONS

The basic equations for calculating the dielectric properties of a system are (11)

$$\mathbf{P} = \frac{\overline{\epsilon} - 1}{4\pi} \cdot \mathbf{E}_i \tag{1a}$$

$$\mathbf{P} = N \langle \mu \rangle_E \cdot \mathbf{e}\,, \tag{1b}$$

where **P** is the polarization vector calculated at the site of

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Dr. Raudino's present address is Department of Chemistry, University of Catania, V. A. Doria 8, 95125, Catania, Italy.

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interest, $\overline{\epsilon}$ is the dielectric constant tensor, $\langle\mu\rangle_E$ the mean induced dipole per molecule, $\hat{e}$ a unit vector and $\mathbf{E}_i$ the internal field, i.e. the field acting on the molecule of interest screened by the other surrounding molecules. N is the number of molecules per unit volume. Eliminating $\mathbf{P}$ from Eq. 1, the two basic ingredients for the evaluation of $\overline{\epsilon}$ are the internal field $\mathbf{E}_i$ and the mean induced dipole moment $\langle\mu\rangle_E$. We will start from the calculation of $\mathbf{E}_i$ in the case of a nonhomogeneous system. For a system without freely moving charges, the Maxwell's equation div $\epsilon\cdot\mathbf{E} = 0$, and $\mathbf{E} = -$ grad Φ (12) give

$$\mathrm{div}\,(\overline{\epsilon}\,\mathrm{grad}\,\Phi) = 0. \tag{2}$$

For symmetry reasons, we have at the membrane surface: $\epsilon_x = \epsilon_y$ (x and y being parallel to the surface). Considering a lipid membrane, the variation of the dielectric constant will occur only along the z axes (perpendicular to the membrane surface). Neglecting dielectric anisotropy effects (the role of the anisotropy is discussed in Appendix I), the dielectric constant tensor can be written in matrix notation as: $\epsilon = \epsilon(z)I$, I being a unit matrix. Making the change of variable: $\Phi = \epsilon^{-1/2}\,S$, we can rewrite Eq. 2 as

$$\nabla^2 S - \left[\left(\frac{\nabla\epsilon}{2\epsilon}\right)^2 - \frac{\nabla^2\epsilon}{2\epsilon}\right]S = 0. \tag{3}$$

Eq. 3 can be solved analytically only if the term in square brackets is zero or constant. The first condition is fulfilled by ϵ = constant, and, in this case, we obtain the well-known Laplace's equation: $\nabla^2 S = 0$. The second condition is fulfilled by the exponential function, i.e. : $\epsilon = \epsilon_0 e^{hz}$. An exponential profile is a good trial function for describing the thermally averaged variation of the membrane polarity on going from the hydrocarbon region to the aqueous solution.

A possible criticism to the exponential profile is that $\epsilon(z)$ tends to infinity for large values of Z. However, for large values of ϵ, the numerical values of the polarization effects tend asymtotically to the same limit. To check the validity of this approximation we used also a more refined stepwise model, i.e. : $\epsilon = \epsilon_0 e^{hz}$ when $\epsilon \leq \epsilon_{\mathrm{water}}$ and $\epsilon = \epsilon_{\mathrm{water}}$ otherwise. As we will discuss later, this latter model does not change the result obtained by the simple exponential model. Inserting $\epsilon = \epsilon_0 e^{hz}$ into Eq. 3, we obtain

$$\nabla^2 S - \frac{h^2}{4}S = 0, \tag{4}$$

which is the well-known Poisson's equation. The calculation of the dielectric properties of a system of interacting molecules can be reduced to the evaluation of the electric field and intermolecular forces acting upon a single molecule of the system. Then, assuming a spherical shape of the examined molecules, it is useful to express the solution of Eq. 4 in polar coordinates r, θ and ϕ, i.e. (13)

$$S = \sum_{n=0}^{\infty}\sum_{m=-n}^{+n} C_n^m \left(\frac{1}{\frac{hr}{2}}\right)^{1/2} K_{n+1/2}\left(\frac{hr}{2}\right) P_n^m(\cos\theta)\,e^{im\phi}. \tag{5}$$

$K_{n+1/2}(x)$ is a modified Bessel function of the second kind of half-integer order (14) and P_n^m ($\cos\theta$) is theAssociated Legendre function. The coefficients C_n^m are to be determined by the boundary conditions. Since Eq. 5 is expressed in polar coordinates, it is useful to express $\epsilon(z)$ in the same coordinate system. Let: $z = z_0 = r\cos\theta$, where z_0 is the coordinate of the center of mass of the inducing charge distribution and $r\cos\theta$ is the coordinate of a generic point calculated with the origin at z_0. We obtain $\epsilon(z) = \epsilon_0 \exp\cdot h(z_0 + r\cos\theta) = \epsilon(z_0)\exp h\,r\cos\theta$. Combining the previous equations with $\Phi = \epsilon^{-1/2}S$, one obtains the following expression for the potential outside the cavity containing the studied molecule

$$\Phi_{\mathrm{ex}} = \sum_{n=0}^{\infty}\sum_{m=-n}^{+n} C_n^m f_n(r)\,e^{-}h/2^{r\cos\theta}\,P_n^m(\cos\theta)\,e^{im\phi}, \tag{6}$$

where $f_n(r) \equiv [1/(h\,r/2)]^{1/2}K_{n+1/2}\,(h\,r/2)$ is the radial component of the Poisson's equation solution (Eq. 5).

Inside the cavity the dielectric can be considered homogeneous, so the potential obeys Laplace's equation $\nabla^2\Phi_i = 0$, the solution of which is

$$\Phi_i = \sum_{n=0}^{\infty}\sum_{m=-n}^{+n} B_n^m r^n P_n^m(\cos\theta)e^{im\phi}. \tag{7}$$

Internal Field

Let us apply the previous equations to the calculation of the internal field. When we apply an electric field to a dielectric medium containing a cavity, the electrostatic potential inside the medium is the sum of the applied potential plus that arising from the polarization of the cavity surface (Eq. 6). The potential inside a nonhomogeneous medium due to an applied electric field can readily be calculated by integrating the basic relationship: $\mathbf{E} = -\mathrm{grad}\,\Phi$. In the case of a field applied along the z axis and assuming an exponential variation of ϵ with z, one obtains

$$\Phi_{\mathrm{ex}}(E) = -\int_z E\,\mathrm{d}z = -\int_z \frac{D}{\epsilon(z)}\,\mathrm{d}z$$

$$= -E(z_0)\frac{1}{h}(1 - e^{-hz}) \xrightarrow[h\to 0]{} -Ez, \tag{8}$$

where $z = r\cos\theta$. Putting: $\Phi_{\mathrm{ex}}^{\mathrm{TOT}} = \Phi_{\mathrm{ex}}(E) + \Phi_{\mathrm{ex}}$ and applying the usual boundary conditions (15):

$$(\Phi_i)_{r=a} = (\Phi_{\mathrm{ex}}^{\mathrm{TOT}})_{r=a} \tag{9a}$$

$$\epsilon_i\left(\frac{\partial\Phi_i}{\partial r}\right)_{r=a} = \left[\epsilon(z)\left(\frac{\partial\Phi_{\mathrm{ex}}^{\mathrm{TOT}}}{\partial r}\right)\right]_{r=a}, \tag{9b}$$

one can calculate the coefficients C_n^m and B_n^m (Eqs. 6 and

7). To apply the previous boundary conditions, we must eliminate the complex relationship between Φ_{ex}^{TOT} and $\cos\theta$ and express Φ_{ex}^{TOT} as a linear combination of Legendre polynomials $P_n^m(\cos\theta)$. This task can be accomplished by a Fourier-Bessel expansion of $(\Phi_{ex}^{TOT})_{r=a}$ and $[\epsilon(z)(\partial\Phi_{ex}^{TOT}/\partial r)]_{r=a}$ i.e. (16)

$$(\Phi_{ex}^{TOT})_{r=a} = \sum_{n,\nu=0}^{\infty} A_{\nu}^{(1)}(n)\, P_{\nu}(\cos\theta) \qquad (10a)$$

$$\left[\epsilon(z)\left(\frac{\partial\Phi_{ex}^{TOT}}{\partial r}\right)\right]_{r=a} = \sum_{n,\nu=0}^{\infty} A_{\nu}^{(2)}(n)\, P\nu(\cos\theta), \qquad (10b)$$

where we have omitted the ϕ-dependent terms because of the cylindrical symmetry of the system. Combining Eqs. 6 through 10, we obtain, after some algebra, the following set of coupled algebric equations:

$$B_n a^n + H_n E(z_0) = \sum_{\nu=0}^{\infty} C_{\nu} f_{\nu}(a) A_n(\nu) \qquad (11a)$$

$$nB_n a^{n-1} + \epsilon(z_0)E(z_0)\delta_{n1} = \frac{\epsilon(z_0)}{a}\sum_{\nu=0}^{\infty} C_{\nu} f_{\nu}(a) B_n(\nu), \qquad (11b)$$

where

$$H_n \equiv \frac{2n+1}{2}\int_{-1}^{+1}\frac{1}{h}(1 - e^{-2\lambda x})\, P_n(x)\,dx. \qquad (11c)$$

$$A_n(\nu) \equiv \frac{2n+1}{2}\int_{-1}^{+1} e^{-\lambda x}\, P_n(x)P_{\nu}(x)\,dx. \qquad (11d)$$

$$B_n(\nu) \equiv \frac{2n+1}{2}\int_{-1}^{+1} e^{\lambda x}(Q_n(a) - \lambda x)P_n(x)P_{\nu}(x)\,dx.$$

$$Q_n(a) \equiv \frac{a}{fn(a)}\frac{\partial fn(a)}{\partial a}; \qquad x \equiv \cos\theta; \qquad \lambda \equiv \frac{ha}{2}, \qquad (11e)$$

and δ_{n1} is the Kronecker delta. Unfortunately, the equations system (11) cannot be solved analytically. However, a close inspection of Eqs. 11c, d, e shows that the Fourier-Bessel coefficients $A_n(\nu)$ and$B_n(\nu)$ are large only when $n = \nu$. Then, assuming as zero-th order approximation: $A_n(\nu) = A_n(\nu)\delta_{n\nu}$ and $B_n(\nu) = B_n(\nu)\delta_{n\nu}$, we can easily solve the equations system (11). The process can be iterated and the results reported here refer to a two-cyclediteration solution.

The knowledge of the B_n coefficients allows one to calculate the internal field

$$E_i = -\frac{\partial}{\partial z}\Phi_i = -\frac{\partial}{\partial z}\sum_{n=0}^{\infty} B_n a^n P_n(\cos\theta).$$

In contrast to the case of homogeneous dielectrics, the internal field is not uniform inside the spherical cavity. However, since we are not interested in the details of E_i but mainly on its mean value, we can average over all the orientations of the studied molecule inside the cavity. The details of this calculation are reported in Appendix 2; here we quote the final result

$$\langle E_i\rangle_{\perp} \simeq -\frac{3\epsilon(z_0)}{2\epsilon(z_0)+1}\{1 + f[\epsilon(z_0)]\lambda^2 + O(\lambda^4)\}\cdot E_0, \qquad (12a)$$

where $f[\epsilon(z_0)]$ is a function only of $\epsilon(z_0)$, while λ ($\lambda \equiv ha/2$) is a measure of the dielectric inhomogeneity. The explicit expression of $f[\epsilon(z_0)]$ is

$$f[\epsilon(z_0)] \simeq 2 - \frac{2.5}{\epsilon(z_0)} + O(\epsilon(z_0)^{-2}).$$

In the limit of λ tending to zero we obtain, as expected, the well-known formula valid for homogeneous media (15)

$$E_i = -\frac{3\epsilon}{2\epsilon+1}E_0.$$

A similar procedure can be used for the case of a field applied along the x or y axes (parallel to the membrane surface). Some algebra gives

$$\langle E_i\rangle_{\parallel} \simeq -\frac{3\epsilon(z_0)}{2\epsilon(z_0)+1}\{1 + g[\epsilon(z_0)]\lambda^2 + O(\lambda^4)\}\cdot E_0, \qquad (12b)$$

where

$$g[\epsilon(z_0) \simeq -\frac{1}{6} + \frac{g}{20\epsilon(z_0)} + O[\epsilon(z_0)^{-2}].$$

As we can see from Eq 12, the dielectric inhomogeneity causes an increasing of the internal field (with respect to the homogenous case) when we apply a field perpendicular to the membrane surface, while a smaller negative variation is obtained when we consider a parallel field.

Finally, preliminary calculations performed using a more sophisticated profile of the dielectric constant ($\epsilon = \epsilon_0 e^{hz}$ when $\leq \epsilon/\epsilon_{water}$ and $\epsilon = \epsilon_{water}$ otherwise) did not change the previous trend, giving only a very small [$\propto 1/\epsilon(z_0)(2a)^3 \cdot [\epsilon_w - \epsilon(z_0)]/[\epsilon_w + \epsilon(z_0)]$ additional contribution to the internal field.

Induced Dipole Moment

The calculation of the projection of the mean dipole moment $\langle\mu\rangle_E$ along the external field direction is the second basic ingredient in calculating the dielectric properties of a system. This calculation is very easy in the approximation of dipolar noninteracting molecules (11). In our system this approximation is poor for two reasons: (*a*) the motion of the polar head groups take place in a region with a sharp dielectric gradient; and (*b*) the polar head groups strongly interact with each other because of their zwitterionic structure and the relatively small interlipid distances. The inclusion of these effects in a model of surface dielectric constant will unravel new phenomena such as spontaneous polarization of the membrane surface and the anisotropy of the dielectric constant.

Let us write the induced dipole moment along a generic axis as

$$\langle \mu \rangle_E = \mu_i \langle \cos\theta \rangle_E$$
$$= \mu_i \left(\langle \cos\theta \rangle_{E=0} + \frac{\partial \langle \cos\theta \rangle}{\partial E_i} \frac{\partial E_i}{\partial E} \bigg|_{E=0} \cdot E + \cdots \right), \quad (13)$$

where μ_i is the internal dipole moment, i.e. the effective dipole moment inside the cavity. In writing Eq. 13 we have neglected higher order terms that are responsible for nonlinear dielectric effects not considered here. Simple algebra gives

$$\frac{\partial \langle \cos\theta \rangle}{\partial E_i} \bigg|_{E=0} = \frac{\mu_i}{kT} \frac{\int_v \cos^2\theta \exp\left(-\frac{U(\theta, x)}{kT} \right) \mathrm{d}V}{\int_v \exp\left(-\frac{U(\theta, x)}{kT} \right) \mathrm{d}V}. \quad (14)$$

The potential $U(\theta, x)$ takes into account all the interactions between the central dipole and the neighboring molecules (x being the set of their coordinates). Evaluation of the integrals appearing in Eq. 14 can be performed only by adopting a reasonable approximation. The following picture seems quite realistic. Because of the strong dipolar interactions, the membrane surface can be represented as an array of randomly oriented domains. Inside each domain the dipoles will mostly be oriented in such a way as to minimize the energy of the system.

Assuming from x-ray data that the phospholipids pack themselves in hexagonal face-centered structures (17), the most stable configuration is that depicted in Fig. 1. When we apply an electric field parallel to the membrane surface, an angle between the field direction and the preferred orientation of the central dipole will be determined. Then, assuming a crystal-like structure of the polar head group, we would expect a dielectric constant value lower than that obtained using a freely-rotating-dipoles model. On the other hand, if we assume that the cage of surrounding molecules follows the motion of the central dipole (fluid-like model), larger values of dielectric constant would be obtained. The relaxation properties of the membrane surface have little effect on the perpendicular component of the dielectric constant, and, in any case, the strong intermolecular interactions lead to a decrease of the polar head groups mobility.

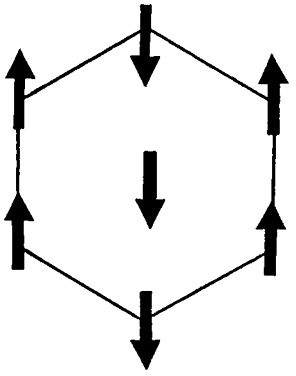

FIGURE 1 Schematic drawing of the minimum energy conformation of the phosphatidylcholine unit cell in the lipid bilayer. The arrows represent the rotating dipoles.

Let us derive a simple expression for the energy of interaction between a dipole and its environment. The calculation is easier if we consider separately the perpendicular and parallel components to the membrane plane of the dipole moment. The main forces acting on the perpendicular component are: (*a*) the polarization energy, which tends to rotate the moving end of the polar head group toward the aqueous phase, and (*b*) the electrostatic repulsion between the perpendicular components of the polar groups dipole moments, which tends to orientate the moving ends parallel to the membrane surface.

The calculation of the first contribution can be performed following the same approach used in calculating the internal field, the only difference being that the applied field must be replaced by the field of the dipole inside the cavity. Remembering that the polarization energy W_p is related to the internal potential Φ_i and to the inducing charge distribution e(**r**) by reference 15

$$W_p = \frac{1}{2} \int_v \mathrm{e}(\mathbf{r}) \Phi_i(\mathbf{r}) \mathrm{d}V, \quad (15)$$

we obtain after some algebra

$$W_p \simeq -\frac{3\mu_i \mu}{a^3 \epsilon(z_0)} \cdot$$
$$\lambda \left\{ \frac{\mu}{qa} \cos\alpha - \lambda \left[\frac{3}{5} + \frac{1}{8} \left(\frac{\mu}{qa} \right)^2 \right] \cos^2\alpha \right\} + \mathrm{O}[\epsilon(z_0)^{-2}], \quad (16)$$

where α is the angle between the dipole orientation and the z axis, a is the cavity radius and q is the unit charge. μ and μ_i are the bare dipole moment and the effective dipole moment inside the cavity, respectively. In deriving Eq. 16 we have also taken into account the displacement of the center of mass of the dipole as the result of the rotation around the fixed end (generally the PO_4^- group).

The second energy contribution describes the interaction between the perpendicular components of the surface dipoles

$$W_R = \sum_i \frac{\mu_\perp \langle \mu_j \perp \rangle_\perp}{L^3} \simeq 6 \frac{\mu \langle \mu \rangle_\perp}{L^3} \cos\alpha, \quad (17)$$

where L represents the interlipid distance. In Eq. 17 we have limited the sum to the nearest neighborhood ($N = 6$) and used the mean field approximation: $\langle \mu_j \rangle_\perp \simeq \langle \mu \rangle_\perp$. The energy of interaction between the component parallel to the membrane plane of the dipole moment and its environment depends on the relaxation properties of the cage formed by the surrounding molecules. Considering the cage depicted in Fig. 1, we obtain

$$W_D = \begin{cases} -\dfrac{2\mu^2}{L^3}\sin^2\alpha\cos\gamma & \text{Unrelaxed cage} \\ -\dfrac{2\mu^2}{L^3}\sin^2\alpha\left(1-\dfrac{\sqrt{3}}{2}\right)\cos^2 3\gamma & \text{Relaxed cage,} \end{cases} \tag{18}$$

where γ is the rotation angle of the central dipole parallel to the membrane plane. Combining together Eqs. 16 through 18 we have

$$W_{\mathrm{TOT}} = A - B\cos\alpha - C(\gamma)\sin^2\alpha, \tag{19}$$

where

$$A \equiv \frac{3\mu_i\mu}{a^3}\frac{\lambda^2}{\epsilon(z_0)}\left[\frac{3}{5}+\frac{1}{8}\left(\frac{\mu}{qa}\right)^2\right]$$

$$B \equiv \frac{3\mu_i\mu^2}{4a^4}\frac{\lambda}{q\epsilon(z_0)} - 6\frac{\mu\langle\mu\rangle_\perp}{L^3}$$

$$C \equiv \frac{2\mu^2}{L^3}\begin{pmatrix}\cos\gamma \\ \left(1-\dfrac{\sqrt{3}}{2}\right)\cos^2 3\gamma\end{pmatrix} + A.$$

The knowledge of the forces acting on the rotating dipole allows us to calculate the dielectric properties of the membrane interface. Combining Eqs. 1, 13, and 19, we obtain the following equations for the permanent dipole moment (or spontaneous polarization) and dielectric constant component perpendicular to the membrane plane

$$\langle\mu\rangle_\perp = \mu_i\langle\cos\alpha\rangle_{E=0} \tag{20a}$$

$$\epsilon_\perp(z_0) - 1 = 4\pi N\frac{\mu_i^2}{kT}\langle\cos^2\alpha\rangle_{E=0}\cdot\frac{E_i}{E_o}, \tag{20b}$$

where $\langle\cos\alpha\rangle_{E=0}$ and $\langle\cos^2_\alpha\rangle_{E=0}$ are given by

$$\langle\cos\alpha\rangle_{E=0} \simeq \frac{B}{2\langle C\rangle_\gamma}\left(1+\frac{3}{\langle C\rangle_\gamma}kT\right) \tag{20c}$$

$$\langle\cos^2\alpha\rangle_{E=0} \simeq \frac{kT}{2\langle C\rangle_\gamma}\left(1+6B\sqrt{\frac{kT}{\pi\langle C\rangle_\gamma^3}}\right). \tag{20d}$$

The symbols A, B, and $C(\gamma)$ have been defined in Eq. 19. The ratio E_i/E_0 between the internal and applied field has been calculated for the case of a nonhomogeneous dielectric (Eq. 12a). Finally, the internal dipole moment μ_i is related to the dielectric properties of the medium through the relationship: $\mu_i = \mu g$, μ being the bare dipole moment and g a function of the electronic polarization of the system given by (11): $g = (\epsilon_\infty + 2)/3(\epsilon_\infty$ is the electronic component of the dielectric constant).

The nonlinear equation system (20) can be solved numerically, yielding $\langle\mu\rangle_\perp$ and $\epsilon(z_0)$ as a function of the molecular structure of the interface. The numerical results will be given in the next section. A similar procedure can be utilized to calculate the component parallel to the membrane surface of the dielectric constant. The results are

$$\langle\mu\rangle_{|} = 0 \tag{21a}$$

$$\epsilon_{|}(z_0) - 1 = 4\pi N\frac{\mu_i^2}{kT}\langle\cos^2\alpha\rangle E = 0\cdot\frac{E_i}{E_0}, \tag{21b}$$

where

$$\langle\cos^2\gamma\rangle_{E=0} = \begin{cases} \dfrac{1}{2}\langle\sin^2\alpha\rangle & (21c) \\ \dfrac{1}{2}\left[1-\left(\dfrac{I_\perp(y)}{I_0(y)}\right)^2\right]. & (21d) \end{cases}$$

$I\perp(y)$ and $I_0(y)$ being Bessel functions (14) and $y \equiv 1/kT\cdot 2\mu^2/L^3\,\langle(\sin^2\alpha\rangle$. The result reported in Eq. 21a has been obtained assuming a random distribution of the crystalline-like domains over the membrane surface.

Ion Membrane Interaction

The solution of the potential equation for a nonhomogeneous dielectric together with the calculation of the membrane surface dielectric constant, allows us to evaluate the electrostatic solvation energy of an ion crossing the membrane interface. This model avoids the disturbing singularities in the energy values arising when the ion is near the boundary between two dielectric media, as well as it better describes the progressive increasing of the membrane polarity on going from the hydrocarbon region to the aqueous phase.

The calculation can be performed along the same lines used for calculating the internal field. The coefficients C_n and B_n of the electrostatic potential (see Eqs. 6 and 7) can be obtained by solving the system

$$B_n a^n + \frac{Q}{a} = \sum_{\nu=0}^{\infty} C_\nu f_\nu(a)A_n(\nu) \tag{22a}$$

$$nB_n a^{n-1} - \frac{Q}{a^2} = \frac{\epsilon(z_0)}{a}\sum_{\nu=0}^{\infty} C_\nu f_\nu(a)B_n(\nu). \tag{22b}$$

Q is the ion net charge; the other symbols have been previously defined. (see Eqs. 11). The knowledge of the internal potential Φ_i allows us to calculate the polarization energy through Eq. 15. After some algebra we find

$$W \simeq -\frac{Q^2}{2a}\left\{1-\frac{1}{\epsilon(z_0)}\left[1-\lambda + \left(-\frac{1}{3}+\frac{\epsilon(z_0)-1}{2\epsilon(z_o)+1}\right)\lambda^2 + O(\lambda^3)\right]\right\} \tag{23}$$

from which, correctly, we re-obtain the well-known Born charging formula (15) in the limit of λ tending to zero.

RESULTS AND DISCUSSION

The calculation of the membrane surface dielectric properties requires the knowledge of some physical parameters concerning the structure of the lipid polar region. They are:

(*a*) the number of lipids per unit volume N; this parameter can be determined from the area per lipid molecule (usually 50–80 $Å^2$; (see reference 18) and from the thickness of the rotating dipoles region (~6 Å). (*b*) The internal field E_i is given by Eq. 12. It depends on the dielectric constant $\epsilon(z_0)$ (to be determined) and on the λ parameter defined as $\lambda \equiv ha/2$; h measures the variation of the dielectric constant across the interface: $\epsilon = \epsilon_0 e^{hz_0}$. If we assume a reasonable value of the polar region thickness z_0 (~10 Å) and of the hydrocarbon region dielectric constant ϵ_0 (~2.4; see reference 2), we can relate h to $\epsilon(z_0)$. (*c*) The internal dipole moment μ_i is defined as: $\mu_i = \mu(\epsilon_\infty + 2)/3$; ϵ_∞ is almost constant for the organic non aromatic compounds (~2; see reference 19) while μ strongly depends on the molecular conformation of the polar head group. NMR measurements indicate that the conformation of the phosphatidylcholine, the most common phospholipid, is the gauche one (20). The same conclusion is reached if we consider the geometrical constraints determined by the interlipid distances, lipid packing and van der Waals radii of the polar head groups. Then, assuming a gauche conformation, the dipole moment is: $\mu = qD\eta$, where q is the net charge of the ionic ends, D their distance and η a correction term depending on the polarizability of the ends (15). A value of ~20 Debyes is obtained for the phosphatidylcholine residue. (*d*) The cavity radius a is obtained from the polar head group molecular weight (density ~1).

Spontaneous Polarization

A first result is the evidence of a spontaneous polarization of the polar head group region. This effect is caused by the balance between polarization energy, which tends to rotate the moving end of the dipole toward the aqueous phase, and the interdipole interactions, which tend to align the dipoles parallel to the membrane surface. We have calculated a permanent dipole moment per lipid molecule of ~1.3 Debyes,[1] which means a tilting angle[2] of ~4°, in agreement with NMR data (8). On the other hand, the ester groups, lying just under the rotating dipoles region, show a molecular conformation having the carbonyl group nearly perpendicular to the membrane surface (22). Since the dipole moment of the ester group is ~1.8 Debyes (23) and there are two ester groups per lipid molecule, a permanent dipole moment of ~3.6 Debyes is to be expected. The direction of the spontaneous polarization of the choline residue and that of the ester groups are opposite. If we take into account only the ester groups and integrate over the membrane surface, we obtain a dipolar potential of ~1,200 mV (a similar calculation reported in the literature gave 1,500 mV; see reference 3). The inclusion of the spontaneous polarization of the rotating zwitterions reduces this potential to ~760 mV, in better agreement with the experimental value (~440 mV; see reference 3). It is often claimed that bound water affects the properties of the bilayer-water interface. We note that this would decrease the effective dielectric constant $\epsilon(z_0)$ and thus the parameter h in our calculation. The decreasing of $\epsilon(z_0)$ leads to an increasing of the dipolar potential which now better counteracts that of the ester groups region. However, a quantitative evaluation of these dielectric saturation effects is difficult and will not be further discussed. The spontaneous polarization of the rotating dipoles region not only decreases the macroscopic potential of the lipid membrane, but also suggests a more complex profile at the microscopic level, where the field reverses itself twice. This could be important in understanding chemical reactions occurring at the membrane-water interface (10).

Dielectric Constant of the Interface Region

Despite the large dipole moment of the phosphatidylcholine, the dielectric constant component perpendicular to the membrane surface appears to be quite low. Using Eq. 20 and including the relatively small electron polarization contribution, we calculated a dielectric constant value of ~23–29 (see Table I) in good agreement with previous values inferred from eperimental data (4, 5, 24, 25). This value is almost insensitive to the magnitude of the polar head group dipole moment. In fact, on the one hand, an increase of the magnitude of the dipole moment increases the polarization of the membrane surface, but, on the other hand, it also reduces the out-of-plane mobility of the polar head groups because of their increased interactions.

Another consequence of the strong dipolar interactions is the anomalous dependence of the dielectric constant on the temperature. In fact, while the classical theories give: $\epsilon \propto 1/T$ (11), our model predicts a weak increase of $\epsilon_\perp$ with T (Eq. 20). Experimental observations seem to confirm this trend (26). The numerical values of the parallel component of the dielectric constant depend on the model. If we assume a crystalline-like model we have: $\epsilon_\parallel$ = 13–16 (see Table I). This value agrees well with the librational

TABLE I

CALCULATED DIELECTRIC CONSTANT COMPONENTS OF THE ROTATING DIPOLES REGION FOR THE PHOSPHATIDYLCHOLINE LIPID BILAYER

L*	8 Å	9 Å	10 Å	
ϵ_1	23	26	29	Eq 20
$\epsilon_\parallel$	13	15	16	crystalline model (Eqs. 21b, c)
	535	423	343	fluid model (Eqs. 21b, c)
$\epsilon_1 - \epsilon_\parallel$	297	235	191	freely rotating dipoles model (11)

*L is the interlipid distance.

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[1] This value has been obtained assuming the interlipid distance to be 9 Å. 1.0 and 1.7 Debyes have been obtained using 8 and 10 Å.

[2] This value must not be confused with the greater tilting angle of the choline residue with respect to the hydrocarbon chains (21), which are themselves tilted (~14°) with respect to the plane of the membrane.

dielectric constant measured by Kaatze et al. ($\epsilon^c - \epsilon^c_\infty = 10.7$; see reference 6), but is very far from the values measured at low frequencies (6,7). This fact clearly indicates that a disordered crystalline-like picture of the polar head groups region is not the correct one. An alternative fluid-like model, which allows the relaxation of the 'cage' surrounding the central dipole, gives better results. In fact, using dielectric data on liposomes (6, 7), remembering that they contain both the perpendicular and parallel component of the dielectric constant, and assuming our calculated value for $\epsilon_\perp$ (~25), the 'experimental' zero-frequency parallel component of $\epsilon_\parallel$ ranges from ~450 to 560.[3] Our model predicts a value of ~420 (for an interlipid distance equal to 9 Å). In calculating the parallel component of the dielectric constant in the crystalline-like model, we have neglected the dielectric anisotropy. This approximation is correct because of the small differencies between the perpendicular and parallel components of ϵ (see Table I and the Appendix). However, when we consider the fluid-like model, this approximation is no longer valid, so we used another model for calculating the internal field. Accordingly, the cavity is placed in a highly nonisotropic dielectric (the polar head group region) squeezed between two isotropic media with different dielectric constants (the aqueous and the hydrocarbon region, respectively). The formulas for calculating the internal field in nonisotropic media were taken from the literature (12) and the effect of the two adjacent media was considered by the image charge approach.

Finally, the barrier to the rotation of the choline residue calculated by the present model (see Eq. 17) was of the same order of magnitude as the experimental one (ΔH = 2.1 Kcal/mol fluid model, 15 Kcal/mol crystal model). Unfortunately, the experimental values are quite scattered (2.6–12.5 Kcal/mol (7, 26–28); however, once again, they lie between the calculated upper and lower limits.

A 'micropolarity' can be measured by optical probes. These techniques suggest a quite low (10–30) value of the dielectric constant at the membrane-water interface (4, 5). If the probe is located inside the membrane polar region with its ground and excited dipole moment perpendicular to the surface, it will measure only the perpendicular component of the dielectric constant. The calculated and experimental values of ϵ then agree. More complex is the situation when the dipole moment of the probe lies parallel to the membrane surface. Here we would expect to measure a very large dielectric constant, which is in contrast with the experimental findings. However, we must bear in mind that the theoretical relationships between frequency shifts and medium polarity have been derived in a model that completely neglects the solvent-solvent interactions (29). This is a good model for three-dimensional slightly interacting fluids. In our case the strong dipolar interactions and the lower two dimensionality of the membrane surface favor the cooperativity of the system (as suggested also by the better numerical values of $\epsilon_\parallel$ calculated using the fluid model). The cooperative effects cause a better response of the system to a delocalized perturbation (e.g. the electromagnetic field in capacitance and NMR measurements) than to a localized perturbation (e.g. the dipole moment variation of the optical probe during the electronic transition), explaining, at least qualitatively, the observed anomalies. A carefully analysis of these effects will appear in a forthcoming paper.

Solvation Energy at the Membrane-Water Interface

In this subsection we will report the calculated energies of solvation (Eq. 23) for an ion moving across the membrane polar region. The numerical values are compared with those obtained by the simple Born formula assuming the same variation of ϵ along the z axis. Putting $a = 2$ Å, $Q = 1$ and 18.7 the dielectric constant of the polar head group region (this value is the weighted average of the perpendicular and parallel components of ϵ in the crystalline-like model, see the previous sub-section) and 12 Å the thickness of the polar region, we obtain the following values: $W \simeq 15$ Kcal/mole and: $W_{\mathrm{Born}} \simeq 18$ Kcal/mole. These results indicate that a smaller amount of energy is required to cross the polar region than that predicted by the Born model.

APPENDIX I

In this appendix we will discuss the effect of the dielectric anisotropy on the value of the internal electric field.

If the dielectric constant component parallel to the membrane surface is different from the perpendicular one, we can write: $\epsilon_z = \epsilon_y = \epsilon_0 e^{hz}$ and: $\epsilon_z = \epsilon_0 e^{HZ}$. Inserting these equation into Eq. 2 and expanding it in power series of H-h, we can rewrite Eq. 2 as

$$\kappa^{(0)}\,\Phi + \kappa^{(1)}\,\Phi = 0, \tag{A1}$$

where the symbol $\kappa^{(0)}\,\Phi$ means the isotropic component of Eq. 2 (i.e. $\epsilon_x = \epsilon_y = \epsilon_z$), while the operator $\kappa^{(1)}$ is defined as

$$\kappa^{(1)} = (H-h)\left(\frac{\partial}{\partial z} + h\frac{\partial}{\partial z} + z\frac{\partial^2}{\partial z^2}\right) + \mathrm{O}(H-h)^2 \tag{A2}$$

Let us write the general solution of Eq. A1 as

$$\Phi = \Phi^{(0)} + \Phi^{(1)}, \tag{A3}$$

where $\Phi^{(0)}$ is the solution of the isotropic Eq. (Eq. 2) and $\Phi^{(1)}$ is a correction term taking into account the anisotropy effects. Inserting Eq. 3A1 into 1A1 and disregarding second order terms, we obtain

$$\Phi^{(1)} \simeq \int_{v_0} G_0\,(\mathbf{r}|\mathbf{r}_0)\,f(r_0)\,\mathrm{d}V_0, \tag{A4}$$

where $f(\mathbf{r}_0) = \kappa^{(1)}\Phi^{(0)}$ and $G_0\,(\mathbf{r}|\mathbf{r}_0)$ is the Green's function relative to Eq.

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[3] Slightly different values can be obtained making different assumptions about the size and shape of the liposomes.

4 (isotropic case). It can be written as

$$G_0(\mathbf{r}|\mathbf{r}_0) = \frac{1}{|\mathbf{r} - \mathbf{r}_0|} + \mathrm{O}(h). \tag{A5}$$

In the same way, the function $f(\mathbf{r}_0)$ is evaluated as

$$f(\mathbf{r}_0) \equiv \kappa^{(1)}\, \Phi^{(0)} \propto \frac{1}{r_0^3} + \mathrm{O}(h)\,. \tag{A6}$$

Inserting Eqs. 5A1 and 6A1 into Eq. 4A1 and expanding Eq. 5A1 as[4] (16)

$$\frac{1}{|r - r_0|} = \frac{1}{r_0} \sum_{n=1}^{\infty} \left(\frac{r_0}{r}\right)^n P_n(\cos\theta)\, e^{im\phi}. \tag{A7}$$

Integrating over the volume element: $\mathrm{d}V_0 = r_0^2\, \mathrm{d}r_0\, \sin\theta_0 \mathrm{d}\theta_0 \mathrm{d}\phi_0$ and remembering that (16)

$$\int_0^{\pi}\int_0^{2\pi} P_n(\cos\theta_0) e^{im\phi 0} \sin\theta_0\, \mathrm{d}\theta_0 \mathrm{d}\phi_0 = 0 \quad \text{for} \quad n \geq 1, \tag{A8}$$

eventually we obtain $\Phi^{(1)} = \mathrm{O}[h(H - h)]$, which is a small contribution with respect to $\Phi^{(0)}$.

APPENDIX II

The equations system (11) can be solved iteratively yielding the B_n and C_n coefficients. However, since we are interested in the internal field, we need only the B_1 coefficient; in fact

$$\langle E_i \rangle = \left\langle -\frac{\partial}{\partial z} \sum_{n=0}^{\infty} B_n a^n P_n(\cos\theta) \right\rangle = -nB_n a^{n-1} \cdot \delta_{n1}. \tag{A9}$$

Let $B_n = B_n^{(0)} + B_n^{(1)} + B_n^{(2)}$ and $C_n = C_n^{(0)} + C_n^{(1)} + C_n^{(2)}$; assuming in the zero-th order approximation: $A_n(\nu) = A_n(\nu)\, \delta_{n\nu}$ and $B_n(\nu) = B_n(\nu)\delta_{n\nu}$ and inserting these formulas into Eq. 11, we obtain after standard algebra

$$B_1^{(0)} = \frac{\epsilon}{a} \cdot \frac{\gamma_1 H_1 - a}{1 - \epsilon\gamma_1} E_0. \tag{A10a}$$

$$B_1^{(1)} = 0. \tag{A10b}$$

$$B_1^{(2)} = \frac{\epsilon}{\mathrm{a}} \frac{H_1 - \epsilon a}{A_1(1)} \frac{1}{(1 - \epsilon\gamma_1)^2} \sum_{m \neq 1} \cdot \frac{\epsilon B_m(1) - mA_m(1)}{m - \epsilon\gamma_m} \frac{\mathrm{E}_0}{A_m(m) \cdot f_m(a)}, \tag{A10c}$$

where $\gamma_m = B_m(m)/A_m(m)$ and all the other symbols have been previously defined (see Eqs. 11d, e, f). It is easy to show that the sum appearing in Eq. 2A2c converges rapidly, since its generic m-th term is proportional to λ^m, so very few terms are necessary in order to obtain a good evaluation of Eq. 2A2c. Finally, the integrals $A_n(\nu)$, $B_n(\nu)$ and H_n can be reduced to well-known integrals by expanding the exponential function in power series of λ. Disregarding higher order terms than λ^3, eventually we obtain the results reported in Eq.12.

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[4] Notice that in Eq. 7A1 the summation starts from $n = 1$ because there are no monopole contributions in the case of an applied electric field.

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