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Dipole Potentials of Monolayers of Phosphatidylcholine, Phosphatidylserine, and Phosphatidic Acid on Mercury

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A novel procedure for the measurement of the absolute value of the surface dipole potential χ of self-assembled lipid monolayers, which makes use of a phospholipid monolayer supported on mercury, is described. It consists of increasing the tilt angle of the lipid molecules with respect to the monolayer normal by expanding progressively the supporting mercury drop and in measuring the charge on the mercury surface that accompanies the drop expansion. Dipole potential values of $+145 \pm 10$ mV for neutral dioleoylphosphatidylcoline (DOPC) and dioleoylphosphatidylserine (DOPS) and of $+30 \pm 3$ mV for neutral dioleoylphosphatidic acid (DOPA) were determined. A gradual increase in the negative charge of the DOPS and DOPA headgroups due to an increase in pH causes an increase in χ. This suggests that the dipole potential of about +145 mV in neutral dioleoylphospholipids stems from the ester linkages to the glycerol backbone (which in DOPA monolayers are screened by the water molecules) and possibly, to a minor extent, from the orientation of the hydration water molecules. The latter contribution is small in neutral phospholipid monolayers, but becomes progressively more positive with an increase in the negative charge on the polar heads of the lipids.

Introduction

The transmembrane potential $\Delta\phi$, namely the potential difference between the bulk aqueous phases bathing the two sides of a membrane, is thermodynamically significant, and hence is experimentally accessible without the need of modelistic assumptions. The effect of $\Delta\phi$ on the functioning of integral proteins can therefore be investigated, say, by placing electrodes in the two solutions phases. $\Delta\phi$ can be regarded as consisting of three different contributions,[1,2] which may modulate the functions of proteins to different extents. They are (i) the surface ionic potential ψ_d, namely the potential difference across the diffuse layer adjacent to the membrane surface; (ii) the surface dipole potential χ, which is mainly located in the polar head region and stems from the orientation of the polar heads and of the adjacent water molecules; and (iii) the potential difference across the hydrocarbon tail region, which is normally considered to vary linearly with distance. All three contributions are potential differences between regions of different composition, and therefore have no thermodynamic significance and cannot be estimated without making extrathermodynamic assumptions. The surface ionic potential ψ_d depends critically upon the electrolyte concentration in the aqueous phase and is estimated to a first approximation on the basis of the Gouy−Chapman theory. The potential difference across the hydrocarbon tail region is normally estimated on the basis of the expression for a parallel plate capacitor, with a dielectric constant close to 2. The most difficult quantity both to control and to measure is the surface dipole potential. This is the reason why it has so far received relatively little attention, in spite of the fact that it could play an important role in modulating membrane functions. χ is known to affect the permeability of membranes to lipophilic ions and the binding of these ions to the membranes.[3,4] To a smaller extent, it can also affect the conductance properties of ion channels in membranes[5,6] and the interactions of proteins with membranes.[7]

The extrathermodynamic assumptions underlying the estimate of the surface dipole potential may be more or less crude. Two main procedures have been so far employed to estimate χ in lipid films, namely measurements of the surface potential (Volta potential difference) of lipid monolayers spread on an aqueous subphase and measurements of ratios of the translocation rates of oppositely charged lipophilic probe ions of similar structure across lipid bilayers. In principle, the surface potential is the difference in electric potential between a point in vacuo just outside the lipid monolayer and a point in vacuo just outside an ionizing or vibrating condenser placed at a very short distance from the monolayer. By "just outside" we mean a distance ranging from 10^{-5} to 10^{-3} cm, far enough from the surface for a test charge not to experience the various potentials arising from short-range interactions, but close enough to experience the whole Coulombic potential created by any charges distributed on the surface.[8] Being a potential difference between two points in the same phase (the vacuum), the surface potential is thermodynamically significant, and hence experimentally accessible. In practice, however, what is measured is the difference ΔV between the surface potential of a monolayer-covered subphase and that of the clean surface of the aqueous phase. Consequently, ΔV includes the difference between the surface dipole potential contribution from the water molecules adjacent to the lipid monolayer

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in the subphase and that from the water molecules at the clean surface of the aqueous phase; this difference can be far from negligible. Smaby and Brockman[9] measured the ΔV value of expanded monolayers of several neutral and charged lipids and found that it increases linearly with the number density N of the lipid molecules, with a nonzero intercept ΔV_0 on the $N = 0$ axis. On assuming that the lipid molecules maintain the same dipole moment normal component μ_{dip} with varying N, they set the slope of the ΔV vs N plot equal to $4\pi\mu_{dip}/\epsilon$, according to the Helmholtz equation with a dielectric constant $\epsilon = 1$, thus determining μ_{dip}. Having observed that the ΔV_0 value depends on the nature of the lipid, they proposed that this N-independent contribution may come from a reorganization of the water structure by the lipid headgroups. As a matter of fact, ΔV_0 measures the difference in the surface dipole potential of the water molecules in passing from the subphase to the clean surface. The ΔV_0 values reported by Smaby and Brockman[9] are positive toward the vapor phase and range from 90 to 130 mV for almost all uncharged lipids. If the water dipoles at the clean surface are oriented with the positive end toward the aqueous phase more than those in the subphase, then ΔV_0 will indeed be positive. Even though the surface dipole potential χ_w of the water liquid/vapor interface is not experimentally accessible, several pieces of experimental evidence combined with modelistic assumptions point to a value positive toward the liquid phase. Thus, values of $+150 \pm 50$ mV,[10] $+80 \pm 60$ mV,[11] $+50$ mV,[12] and $+25 \pm 10$ mV[13] have been reported. Molecular dynamics simulations of the water liquid/vapor interface by Matsumoto and Kataoka[14] also predict a χ_w value of $+160$ mV. It seems therefore reasonable to conclude that the ΔV_0 values reported by Smaby and Brockman[9] for neutral lipid monolayers are due primarily to χ_w, and hence that the orientational contribution to ΔV_0 from the water molecules adjacent to the polar headgroups of these lipids is relatively small. Clarke[15] estimated the surface dipole potential χ of a number of lipids by multiplying the slope, $4\pi\mu_{dip}$, of the ΔV vs N plots estimated by Smaby and Brockman[9] by the number density N of the lipid molecules expected from X-ray crystallographic data. The roughly linear correlation between these χ values and the fluorescence response of two lipophilic fluorescent molecular probes allowed him to calibrate this response and to provide a set of χ values for several phosphatidylcholines of different chain length and degree of unsaturation. These χ values have large errors, due to the scatter of the literature values of χ used for the calibration.

The estimate of χ from the ratio of the translocation rates across lipid bilayers of oppositely charged lipophilic probe ions of similar structure (usually the tetraphenylborate anion on the one hand and the tetraphenylphosphonium or tetraphenylarsonium cation on the other) relies on the assumption that the conductance of these ions is proportional to their concentration inside the lipid bilayer, which is considered to depend on χ according to a Boltzmann distribution law. The ratio G_-/G_+ of the conductance of the lipophilic anion to that of the cation is therefore set equal to $\exp(2e\chi/kT)$.[3,4] This implies that the mobilities and the nonelectrostatic contributions to the partition coefficients of the two oppositely charged ions are identical. While the mobilities are probably similar, the chemical contributions to the partition coefficients are likely to be definitely different.[16,17]

As a rule, the surface dipole potential measurements for bilayers are 100−150 mV lower than for monolayers.[18,19] This lends further support to the position that the positive ΔV_0 values reported by Smaby and Brockman[9] for neutral lipids are mainly to be ascribed to the surface dipole potential of water molecules at the clean surface of the aqueous phase; this contribution is expected to affect the χ values obtained from surface potential measurements on monolayers, with a resulting overestimate of these values.

Adsorption of molecules on top of the polar heads of the membrane or their incorporation into the polar head region alters χ; if these molecules are charged, they also affect ψ_d. The above procedures have also been employed to measure the changes, $\Delta\chi$, in χ that accompany the adsorption or incorporation of neutral molecules. Thus, the translocation rates of lipophilic cation and anion spin labels in unilamellar vesicles were used to measure the changes $\Delta\chi$ produced by the incorporation of phloretin and 6-ketocholestanol molecules.[20] The negative $\Delta\chi$ shift produced by phloretin incorporation into BLMs was also measured from the change in the translocation rate of ion carriers and lipophilic ions[21] or by a current relaxation method.[22] Changes in χ were also measured by the potentiodynamic technique known as the "capacitance minimization method."[23,24] Some of the errors involved in the use of extrathermodynamic assumptions for the estimate of χ values may be partially canceled when measuring changes $\Delta\chi$ in the surface dipole potential.

This work describes a novel procedure for the measurement of the absolute value of the surface dipole potential χ of lipid monolayers, which makes use of a phospholipid self-assembled monolayer supported on mercury, with the hydrocarbon tails directed toward the hydrophobic mercury surface and the polar heads directed toward the solution.[2,25−27] This film has a high mechanical stability, a high resistance to electric fields and a notable reproducibility. Over the potential range from −0.2 to −0.8 V/SCE it behaves like a half-membrane. Thus, it is impermeable to inorganic ions and its differential capacitance is twice that of a black lipid membrane (BLM). This novel procedure relies on mild extrathermodynamic assumptions, especially in the case of neutral lipids. In the latter case the main assumption consists in assuming that an expansion in the area of the mercury drop that supports the lipid monolayer causes the lipid molecules to tilt with

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respect to the monolayer normal, so as to continue covering the drop surface: the estimate of χ from the dependence of the charge on the mercury surface upon the tilt angle is then straightforward. This assumption is supported by the experimental evidence that no water molecules permeate the lipid monolayer to a detectable extent during the drop expansion.

Experimental Section

The water used was obtained from light mineral water by distilling it once, and by then distilling the water so obtained from alkaline permanganate, while discarding the heads. Merck suprapur grade KCl was baked at 500 °C before use to remove any organic impurities. Dioleoylphosphatidylcholine (DOPC) was obtained from Lipid Products (South Nutfield, Surrey, England), and dioleoylphosphatidylserine (DOPS) and dioleoylphosphatidic acid (DOPA) were from Avanti Polar Lipids (Alabaster, AL). All measurements were carried out at 25 ± 0.1 °C in aqueous solutions of 0.1 M KCl previously deaerated with argon. The desired pH values were realized with HCl over the pH range from 2 to 5, with a 1×10^{-3} M $HPO_4^{2-}/H_2PO_4^-$ buffer over the pH range from 6.5 to 7.5 and with a 1×10^{-3} M H_3BO_3/NaOH buffer over the pH range from 8.5 to 9.

Capacitive charge measurements were carried out by a computerized chronocoulometric apparatus described elsewhere.[28] The microprocessor used to control the operations was a Model NOVA 4X from Data General (Westboro, MA), whereas an Amel Model 559 (Milano, Italy) potentiostat was employed for the potentiostatic control of the three-electrode system. The reference electrode was a saturated calomel electrode (SCE), the counter electrode a platinum wire. The detailed scheme of the homemade electronic current integrator working under microprocessor control is described elsewhere.[29] Use was made of a homemade hanging mercury drop electrode (HMDE) described elsewhere,[30] which allows the resolution of changes in the drop area of as little as 0.04 mm^2 and highly reproducible drops to be obtained throughout the piston movement. The capillary and the mercury reservoir were thermostated at 25 ± 0.1° C by the use of a water-jacketed box to avoid any changes in drop area due to a change in temperature. A homemade glass capillary with a finely tapered tip, about 1 mm in outer diameter, was employed.[31] Differential capacitance measurements were carried out using a Metrohm Polarecord E506 (Herisau, Switzerland). The ac signal had a 10 mV amplitude and a 75 Hz frequency. The system was calibrated using a precision capacitor.

Two different procedures were adopted for measuring the capacitive charge. The first procedure (procedure 1) consists of spreading a solution of the lipid in pentane on the surface of an aqueous electrolyte, allowing the solvent to evaporate, and immersing a hanging mercury drop electrode in the solution across the lipid film, about 4 monolayers thick. The resulting lipid-coated mercury electrode is then expanded by consecutive steps, operating manually the micrometric head that moves the piston, and the charge that accompanies each expansion step is measured by the chronocoulometric apparatus. The charge Q involved in the overall drop expansion is obtained by summing the last charge measurement to all preceding measurements on the same drop.

The second procedure (procedure 2) consists of contracting a lipid-coated mercury drop while keeping its neck in contact with the lipid reservoir spread at the water/argon interface.[31] This procedure ensures that the thickness and all other properties of the lipid monolayer remain unaltered during the contraction. The charge following the contraction divided by the decrease in drop area yields directly the charge density σ_M on the mercury surface. With this procedure, the tip of the capillary is positioned just above the surface of the electrolyte solution, covered with a lipid film about 7 monolayers thick. The contact between the mercury drop and the solution is then realized by simply extruding a mercury drop manually from the capillary while taking care to maintain the contact of the drop neck with the lipid reservoir on the solution surface by avoiding wetting the glass tip. This allows a free exchange of lipid material between the lipid monolayer that coats the mercury drop and the lipid film spread on the solution. This procedure ensures that the monolayer maintains its properties, including its thickness, as the drop is contracted.

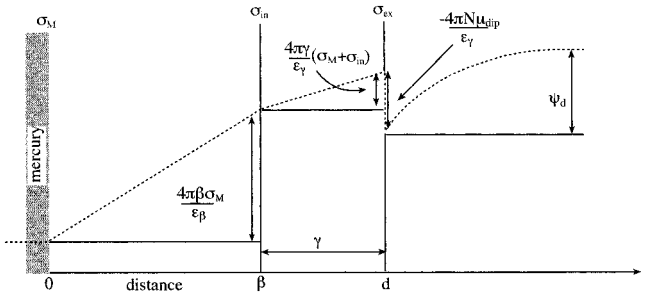

Figure 1. Schematic picture of the model for a lipid monolayer deposited on mercury. The dashed curve represents the profile of the average potential against the distance from the mercury surface. The diffuse-layer thickness has been compressed with respect to the monolayer thickness, for ease of representation.

The Model

The potential difference $\Delta\phi$ across the interphase of the self-assembled lipid monolayer can be expressed on the basis of a model of the membrane/solution interphase that acconts for the presence of any charged ionizable groups buried well inside the polar head region and is schematically depicted in Figure 1.[2] In this model the potential difference $\Delta\phi$ consists of the sum of the potential differences across the hydrocarbon tail region, the polar head region and the diffuse layer region. It can be written as

$$\Delta\phi = 4\pi\frac{\beta}{\epsilon_\beta}\sigma_M + 4\pi\frac{\gamma}{\epsilon_\gamma}(\sigma_M + \sigma_{in}) - 4\pi\frac{N\mu_{dip}}{\epsilon_\gamma} + \psi_d(c, \sigma_{tot}) \quad (1)$$

with $\sigma_{tot} \equiv \sigma_M + \sigma_{in} + \sigma_{ex}$. Here the first term is the potential difference across the hydrocarbon tail region, of thickness β and dielectric constant $\epsilon_\beta \sim 2$, which depends on the charge density σ_M on the metal. The second term is the potential difference across the polar head region, of thickness γ and dielectric constant ϵ_γ, which depends upon the sum of σ_M and of the charge density σ_{in} of any charged groups buried in the polar head region. The third term is the dipole potential due to the polar heads, where μ_{dip} is the dipole moment normal component of the polar heads and N is their number per unit surface; this term also includes any contribution to the dipole potential from the orientation of the water molecules in direct contact with the polar heads. Finally, ψ_d is the potential difference across the diffuse layer, which according to the Gouy−Chapman theory is a function of the electrolyte concentration c and of the whole charge density σ_{tot} experienced by the diffuse layer ions, namely the sum of σ_M, σ_{in}, and the charge density σ_{ex} of any ionized groups directly exposed to the aqueous phase.

Strictly speaking, the dielectric constant is a continuum property, and its extension down to a molecular scale, albeit widely adopted in the literature, can be regarded as a gross approximation. However, several molecular

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models of the interphase relate the dielectric constant ϵ of the "inner layer" to the molecular polarizability α. Thus, application of the mean field approximation to a simple monolayer of solvent molecules at an electrode yields $\epsilon = (\alpha c/d^3 + 1)$, where c is the number of nearest neighbors and d is the distance between them.[32] Application of the mean spherical approximation to an ensemble of hard-sphere ions and dipoles extending from the electrode surface up to the bulk yields an interfacial dielectric constant that is closely approximated by a parameter λ, which is related to the bulk dielectric constant ϵ_b of the solvent by the implicit equation $\lambda^2(1 + \lambda)^4 = 16\epsilon_b$.[33] This provides a justification for the use of an interfacial dielectric constant, although its value is often estimated by comparison of a model with the experimental behavior.

From eq 1 it follows that the reciprocal of the differential capacitance of a neutral phospholipid, where ψ_d equals zero, is given by

$$\frac{1}{C_{sam}} \equiv \frac{d\Delta\phi}{d\sigma_M} = 4\pi\left(\frac{\beta}{\epsilon_\beta} + \frac{\gamma}{\epsilon_\gamma}\right) \qquad (2)$$

This differential capacitance is exclusively ascribed to the lipid SAM, and amounts to about 1.7 μF cm^{-2} for all phospholipid monolayers investigated.[2] Equation 1 can then be written in the more compact form:

$$\Delta\phi = \frac{\sigma_M}{C_{sam}} + \frac{4\pi\gamma}{\epsilon_\gamma}\sigma_{in} - \frac{4\pi N\mu_{dip}}{\epsilon_\gamma} + \psi_d(c,\sigma_{tot}) \qquad (3)$$

To estimate the whole surface dipole potential, a property of the phospholipid monolayers that are in the liquid-crystalline state at room temperature is exploited: the initial area A of these monolayers can be more than doubled by expanding the mercury drop completely immersed in the aqueous electrolyte. As already reported by Nelson and Benton,[25] the curve of the quadrature component of the electrode admittance against the applied potential E exhibits a broad flat minimum that is directly proportional to the drop area A upon expansion. Over the potential range of the minimum and at the frequency adopted herein ($\nu = 75$ Hz), the in-phase component of the admittance is entirely negligible with respect to the quadrature component, which can therefore be identified with $2\pi\nu C$, where C is the differential capacitance. Figure 2 shows C vs E plots corresponding to different drop expansions for the case of DOPA; an analogous behavior is shown by DOPC and DOPS. It is apparent that these plots are practically flat at the lower expansions, while their curvature increases with an increase in drop expansion. The potential of the capacity minimum does not change to an appreciable extent by drop expansion. Conversely, some of the peaks on the negative side of the flat minimum change position due to drop expansion, as may be expected in view of the fact that they stem from a reorientation of the lipid molecules. As pointed out in ref 2, a potential-dependent reorientation of the polar heads of the lipid molecules along the flat minimum is to be excluded, since it would involve a notable increase in C. Thus, e.g., the passage of the polar head of the DOPS molecule from a horizontal to a vertical orientation would cause an increase in C as high as 18 μF cm^{-2}.[2]

Figure 3 shows the proportionality of the minimum capacity to the drop area. If we consider that, with the mercury drop completely immersed in the solution, the amount of lipid material on the drop surface remains constant during the expansion, the behavior in Figure 3 may find a straightforward explanation only by assuming that the drop expansion causes a progressive tilt of the lipid molecules, without incorporation of water into the lipid monolayer. In fact, the volume of the lipid material is given by the product of the film thickness, $d = \beta + \gamma$, and the drop area A. Since the film expansion maintains the lipid volume constant, A is inversely proportional to d. For the film capacitance C_{sam} to be directly proportional to A, and hence inversely proportional to d as expressed by eq 2, the dielectric constant of the film must remain constant during the expansion. More precisely, since β is $> \gamma$ and ϵ_β is $\ll \epsilon_\gamma$, γ/ϵ_γ can be disregarded with respect to β/ϵ_β as a first approximation; hence, the above considerations apply substantially to the thickness β of the hydrocarbon tail region and to its dielectric constant ϵ_β. In this respect, we cannot completely exclude the possibility of a few water molecules penetrating into the polar head region during the expansion, thus increasing the ϵ_γ value. At any rate, penetration of water molecules into the hydrocarbon tail region during the expansion is to be excluded, since it would cause a notable increase in the dielectric constant there, and hence in C. An abrupt increase in C (not reported in Figure 2) is indeed observed when the expansion of the mercury drop exceeds a certain limit. We must conclude that, during the gradual drop

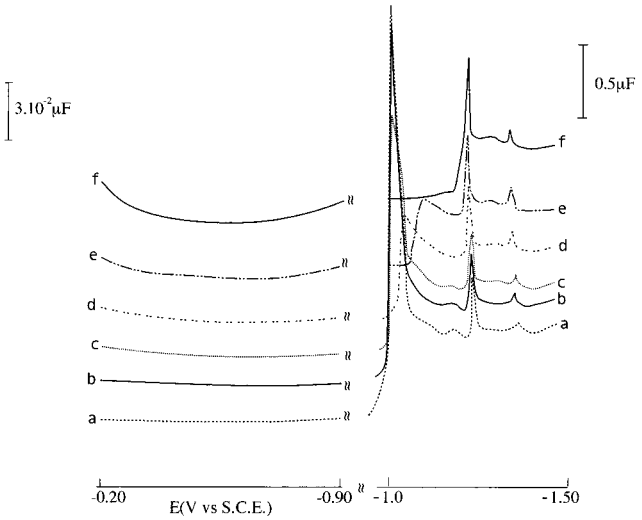

Figure 2. Differential capacitance against potential for a DOPA monolayer at pH 6.2 at different drop expansions. Initial drop area: 2.21 × 10^{-2} cm^2. Surface area relative to the initial drop area: (a) 1; (b) 1.31; (c) 1.59; (d) 1.84; (e) 2.08; (f) 2. 31.

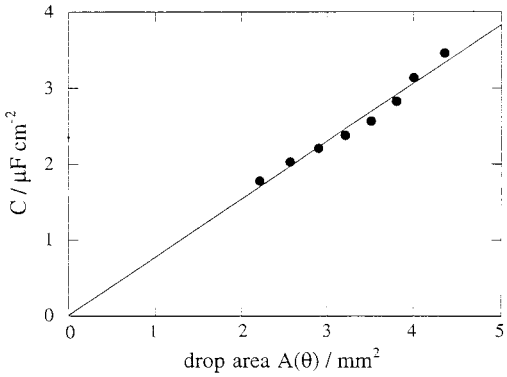

Figure 3. Differential capacitance for a DOPA monolayer at pH 7.5 and $E = -0.55$ V/SCE against the expanded drop area $A(\theta)$; the initial unexpanded area is 2.21 × 10^{-2} cm^2.

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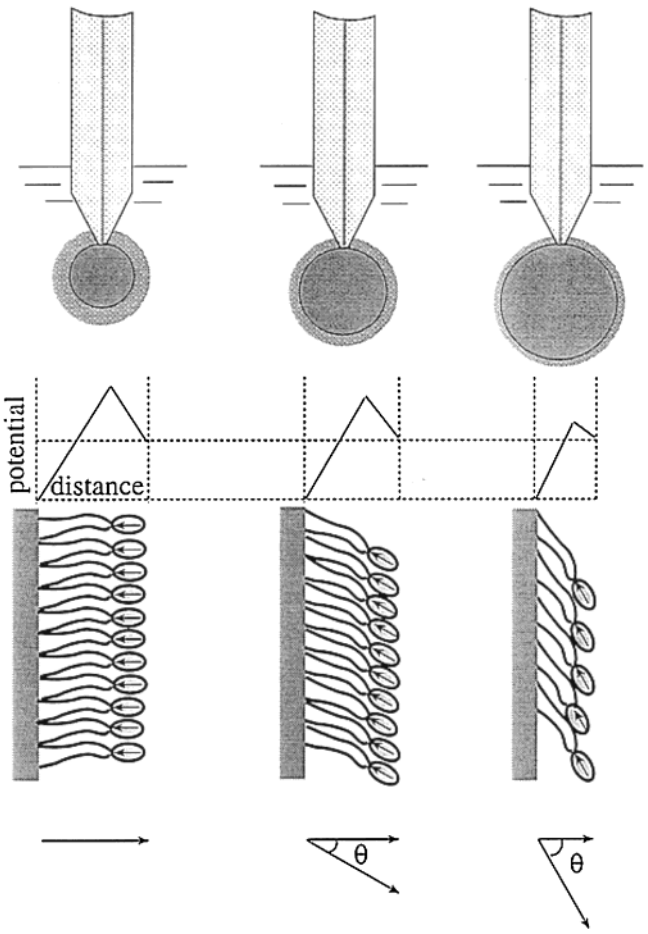

Figure 4. Schematic picture of the effect of the drop expansion on the tilt of the lipid molecules and on the potential profile across the lipid monolayer at constant applied potential.

expansion, the lipid molecules progressively increase their tilt and decrease their number N per unit surface, so as to continue covering the whole drop surface, as depicted schematically in Figure 4.

Denoting by θ the tilt angle of the lipid molecules with respect to the monolayer normal, its cosine is just equal to the ratio $A/A(\theta)$ of the initial unexpanded area A of the film to the expanded one, $A(\theta)$. Moreover, after the drop expansion, the number N of lipid molecules per unit surface, the dipole moment normal component μ_{dip} of their polar heads, the charge densities σ_{in} and σ_{ex} of the ionized groups of the polar heads, and the thickness β and γ of the hydrocarbon tail and polar head regions will all be equal to their initial values times cos θ:

$$N(\theta) = N \cos\theta; \quad \mu_{dip}(\theta) = \mu_{dip} \cos\theta; \quad \sigma_{in}(\theta) = \sigma_{in} \cos\theta$$

$$\sigma_{ex}(\theta) = \sigma_{ex} \cos\theta; \quad \beta(\theta) = \beta \cos\theta; \quad \gamma(\theta) = \gamma \cos\theta$$

$$\frac{1}{C_{sam}(\theta)} = 4\pi\left(\frac{\beta(\theta)}{\epsilon_\beta} + \frac{\gamma(\theta)}{\epsilon_\gamma}\right) = \frac{\cos\theta}{C_{sam}} \qquad (4)$$

Here, the quantities whose dependence on θ is not specified refer to the unexpanded area.

The potential difference across the whole interphase after the drop expansion is obtained by replacing the various initial quantities, i.e., those for $\theta = 0$, by those for $\theta = \theta$ in eq 3. After rearrangement we obtain

$$\frac{\sigma_M(\theta)\cos\theta}{C_{sam}} + \psi_d(c,\sigma_{tot}\cos\theta) \equiv \xi(\theta) = \left(\frac{4\pi N\mu_{dip}}{\epsilon_\gamma} - \frac{4\pi\gamma}{\epsilon_\gamma}\sigma_{in}\right)\cos^2\theta - \Delta\phi \qquad (5)$$

The applied potential E differs from $\Delta\phi$ by a constant depending on the choice of the reference electrode. Hence, a plot of the left-hand side of eq 5, $\xi(\theta)$, against $\cos^2\theta$ at constant applied potential is expected to yield a linear segment whose slope is equal to the quantity between round brackets on the right-hand side of this equation. This is just the surface dipole potential $\chi = 4\pi N\mu_{dip}/\epsilon_\gamma$, created by the polar heads of the lipid monolayer, minus the potential difference across the polar head region created by any charged groups buried well inside this region.

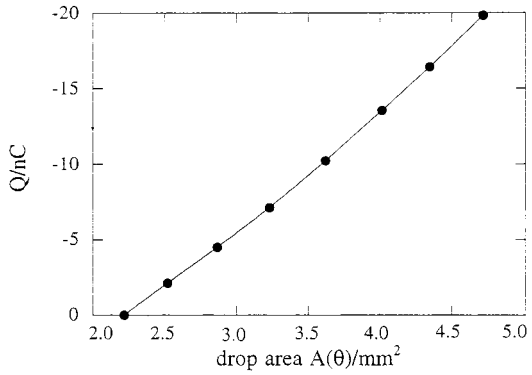

Figure 5. Charge $Q(\theta)$ on the mercury surface against the expanded drop area $A(\theta)$ for a DOPS monolayer at pH 4.3 and $E = -0.500$ V/SCE. The initial unexpanded area is 2.21×10^{-2} cm^2.

Results

The capacitive charge $Q(\theta)$ that accompanies the expansion of a lipid-coated mercury drop completely immersed in the aqueous electrolyte is clearly equal to

$$Q(\theta) = \sigma_M(\theta)A(\theta) - \sigma_M A \qquad (6)$$

A typical plot of $Q(\theta)$, measured by procedure 1 in the Experimental Section, is plotted against $A(\theta)$ in Figure 5. The charge density σ_M on the unexpanded film is obtained by measuring the charge that accompanies the contraction of a lipid-coated mercury drop while keeping its neck in contact with the lipid film spread on the surface of the aqueous electrolyte, according to procedure 2 in the Experimental Section. Hence, eq 6 allows $\sigma_M(\theta)$ to be estimated. By calculating the potential difference ψ_d across the diffuse layer as a function of θ on the basis of the Gouy−Chapman theory, the whole left-hand side of eq 5 is therefore measured as a function of θ.

Figure 6 shows $\xi(\theta)$ vs $\cos^2\theta$ plots for DOPC, DOPS, and DOPA monolayers at pH values at which these monolayers are neutral, and hence the diffuse-layer potential is zero. Incidentally, DOPC is neutral at pH > 4,[26] DOPS at pH ∼ 5.5, and DOPA at pH ∼ 2.[2] Moreover, at pH ∼ 5.5 the phosphate group of DOPS, which is buried inside the polar head region, is almost completely protonated, and hence its charge density σ_{in} is practically equal to zero. The slopes of the plots for DOPC and DOPS are similar, and yield dipole potentials of +140 and +145 mV, positive toward the interior of the film. Conversely,

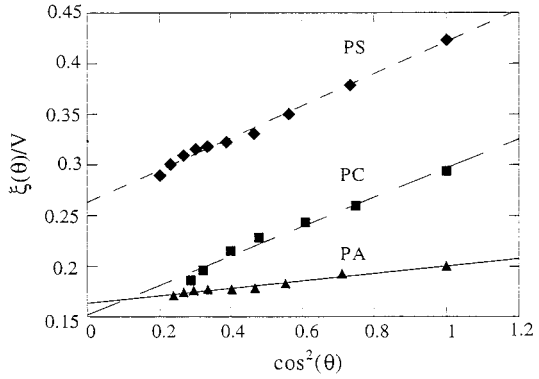

Figure 6. $\xi(\theta)$ vs $\cos^2\theta$ plots at −0.500 V/SCE for DOPC at pH 4, DOPS at pH 5.5, and DOPA at pH 2.

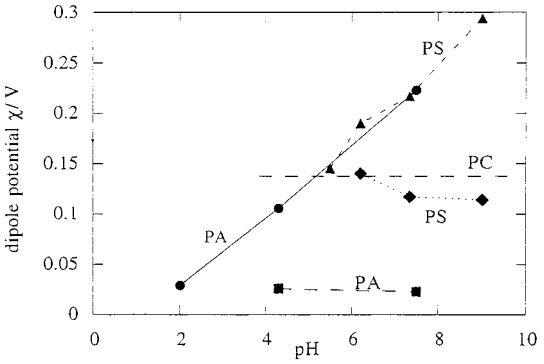

Figure 7. pH dependence of the χ values for DOPA (●) and DOPS (▲), and of the $\chi - \Delta\chi$ values for DOPA (■) and DOPS (◆). For comparison, the horizontal line marks the χ value for DOPC.

the dipole potential for DOPA is much smaller, and amounts to about +30 mV. The standard errors in the dipole potential measurements were less than ±8%. The χ value for DOPC is relatively close to that, +171 ± 89 mV, estimated by Clarke[15] using potential-sensitive fluorescent styryl dyes. Conversely, it is appreciably smaller that the value, +244 mV, estimated by Clarke[15] from the μ_{dip} value of Smaby and Brockman[9] upon introducing in the Helmholtz equation the number density, $N = 1.22 \times 10^{14}$ mol cm^{-2}, of the DOPC molecules extracted from X-ray crystallographic data. The same value of +244 mV was also estimated by Pickar and Benz[3] from the translocation rates across lipid bilayers of oppositely charged lipophilic probe ions. By the same procedure, Flewelling and Hubbell[34] obtained a value of +240 ± 20 mV for egg PC. Albeit measured on a monolayer, the χ value for DOPC herein obtained cannot be compared with the much higher values obtained from surface potential measurements on monolayers spread at the air/water interphase, because the latter include the difference between the surface dipole potentials of water molecules in the subphase and at a clean water surface.

Values of the surface dipole potential χ of DOPS at pH 6.2, 7.5, and 9.0 are shown in Figure 7. At these pH values the phosphate group buried in the polar head region is appreciably deprotonated, imparting a negative charge density σ_{in} to the lipid. Moreover, the positive charge of the ammonium group and the negative one of the carboxyl group almost compensate each other, so that $\sigma_{tot} \sim \sigma_{in}$. Values of σ_{in} for the phosphate group were obtained from its intrinsic protonation constant $K_1 = 5 \times 10^6$ M^{-1} [2] and are reported in Table 1 together with the corresponding

(34) Flewelling, R. F.; Hubbell, W. L. *Biophys. J.* **1986**, *49*, 531−540.

Table 1. Values of σ_M, σ_{in}, σ_{ex}, and $\Delta\chi$ for DOPS and DOPA at Different pH Values, Calculated As Described in the Text

lipid	pH	σ_M (μC cm^{-2})	σ_{in} (μC cm^{-2})	σ_{ex} (μCcm^{-2})	$\Delta\chi$ (V)
DOPS	6.2	−0.5	−3.3	+2.8	+0.07
DOPS	7.3	−0.5	−5.8	+1.6	+0.15
DOPS	9.0	−0.45	−13	+0.6	+0.21
DOPA	4.3	−0.35	0	−2.1	+0.10
DOPA	7.5	−0.45	0	−23	+0.24

σ_M and σ_{ex} values. These values were used both to estimate the $\psi_d(c,\sigma_{tot}\cos\theta)$ potential in eq 5 and to correct the slopes of the resulting $\xi(\theta)$ vs $\cos^2\theta$ plots for the $-4\pi\gamma\sigma_{in}/\epsilon_\gamma$ term, so as to extract the χ values. To this end, the γ/ϵ_γ ratio was set equal to 0.1×10^{-8} cm, as in ref 2. The ψ_d values were obtained from the Gouy−Chapman equation as applied to a 0.1 M aqueous 1,1-valent electrolyte at 25 °C:

$$\psi_d = 0.0514 \ln[x + (x^2 + 1)^{1/2}] \quad \text{with } x \equiv \frac{\sigma_{tot}}{3.706}$$

with ψ_d in V and σ_{tot} in μC cm^{-2}. From Figure 7 it is apparent that the surface dipole potential for the DOPS monolayer increases notably with an increase in pH. This figure also shows the χ values for DOPA at pH 4.3 and 7.5, obtained from the corresponding $\xi(\theta)$ vs $\cos^2\theta$ plots by accounting for the negative charge of the phosphate group according to the same procedure. To this end, the intrinsic protonation constants for the phosphate group of DOPA were given the values 1×10^8 and 1×10^5 M^{-1} and γ/ϵ_γ was set equal to 0.2×10^{-8} cm, as in ref 2. The σ_M, σ_{in}, and σ_{ex} values for DOPA are summarized in Table 1. Even the surface dipole potential for DOPA increases notably with an increase of pH.

Discussion

DOPS has three ionizable groups: an ammonium group, a carboxyl group, and a phosphate group. The latter group is closer to the hydrocarbon tails than the other two and is therefore buried deep inside the polar head region; it is almost completely protonated at pH 5.5, where the lipid film is neutral. DOPC has two ionizable groups, a tetraalkylammonium group and a phosphate group, which are both charged of opposite sign at pH values >4. DOPA is a synthetic phospholipid that has only a phosphate group, with the glycerol backbone practically exposed to the aqueous solution. The fact that neutral monolayers of DOPS and DOPC have very similar surface dipole potentials in spite of their different headgroup structure indicates that their dipole potential is not to be ascribed to the serine or choline group, but rather to a group common to these two lipids and buried deeper inside the polar head region. This can be primarily identified with the ester linkages to the glycerol backbone, although a contribution from oriented water molecules cannot be excluded. This also explains the low value of the dipole potential of neutral DOPA, whose glycerol backbone is exposed to the aqueous phase and is therefore effectively screened by the water molecules.

The increase in the dipole potential of DOPS and DOPA monolayers with an increase in pH shown in Figure 7 is due to the progressive alignment of the water dipoles adjacent to the polar heads along the direction of the electric field created by their negative charge, with the hydrogens turned toward the polar heads. Recently, Moncelli et al.[2] used the present interfacial model to estimate the change $\Delta\chi$ in the surface dipole potential following a change in the overall charge density ($\sigma_{in} + \sigma_{ex}$) of the polar heads of both DOPS and DOPA, apart from

an additive constant. To this end they measured the charge density σ_M and the differential capacitance on DOPS and DOPA "unexpanded" monolayers supported on mercury at different pH values and KCl concentrations, so as to vary the charge density ($\sigma_{in} + \sigma_{ex}$) and to exploit the concentration dependence of the diffuse-layer capacitance expressed by the Gouy−Chapman theory. At an unexpanded monolayer the tilt angle of the lipid molecules relative to the monolayer normal (if any) is kept constant, and any change in χ is to be ascribed to a reorientation of the water molecules with a change in ($\sigma_{in} + \sigma_{ex}$) (The absolute value of σ_M is practically negligible with respect to the charge density on the polar heads.). The plot of $\Delta\chi$ vs ($\sigma_{in} + \sigma_{ex}$) in Figure 7 of ref 2 is sigmoidal in shape and shows the maximum slope for a zero value of the total charge. In this respect it resembles, both qualitatively and semiquantitatively, the plot of the surface dipole potential due to the gradual alignment of the water molecules adjacent to the surface of a bare metal along the direction of the electric field created by the charge density σ_M on the metal;[35] in this case the maximum slope, which denotes the maximum availability of the water molecules to undergo a change in orientation, lies at $\sigma_M = 0$ and corresponds to the lack of a preferential orientation of the water molecules, i.e., to $\chi = 0$. This strongly suggests that the change $\Delta\chi$ in the surface dipole potential following a change in the charge of the polar heads of the lipid is also due predominantly to a gradual reorientation of the water molecules adjacent to the film. Moreover, the maximum slope of the $\Delta\chi$ vs $\sigma_{in} + \sigma_{ex}$ plot lying at $\sigma_{in} + \sigma_{ex} = 0$ seems to denote the absence of a net preferential orientation of the water molecules at an uncharged lipid monolayer. This agrees with the experimental observation that neutral DOPC and DOPS monolayers have practically the same surface dipole potential, in spite of the appreciable difference in their polar heads. However, the presence of hydrating water molecules deep inside the polar head region, with an orientation practically unaffected by the charge of the ionizable groups, cannot be completely excluded (see further).

If we subtract from the χ values for the negatively charged DOPS and DOPA films in Figure 7 the $\Delta\chi$ values due to water reorientation, as estimated from Figure 7 of ref 2 for different $\sigma_{in} + \sigma_{ex}$ values and summarized in Table 1, the χ values so corrected exhibit a much lower pH dependence and approach the value for the corresponding neutral lipid. We may therefore conclude that the dipole potential of dioleoylphospholipids consists of two main contributions: a contribution from the ester linkages to the glycerol backbone (possibly including a small contribution from hydrating water molecules with a charge independent orientation), which amounts to about 145 ± 10 mV and is positive toward the hydrocarbon tails, and a further contribution from the orientation of the adsorbed water molecules. This becomes progressively more positive with an increase in the negative charge on the polar heads of the lipid.

It is worth mentioning that the latter conclusions differ somewhat from those drawn by Zheng and Vanderkooi,[36] who estimated the dipole potential of PC bilayers by solving the finite difference linearized Poisson−Boltzmann equation. Their calculations yield a positive contribution to χ from the acyl ester groups which is smaller than the negative contribution from the phosphocholine headgroup, so that the total potential in the central part of the bilayer turns out to be negative with respect to the solution. As an indirect consequence of this result, these authors postulated a positive contribution from the water of hydration, not included in their calculations, in order to justify the much higher translocation rate and partition coefficient of lipophilic anions with respect to cations of similar structure. A positive contribution from the dipole moment of the water molecules surrounding the headgroup of dipalmitoylphosphatidylcholine (DPPC) was also postulated by Gawrisch et al.[4] on the basis of the measured difference, 227 − 109 = 118 mV, between the dipole potentials of DPPC and dihexadecylphosphatidylcholine (DHPC). Under the assumption that the normal component of the headgroup dipole moment is the same for both these lipids, they ascribed this positive difference to the acyl ester groups of DPPC; the further positive contribution present in both lipids was ascribed to a dipole moment of the hydration water molecules positive enough to overcompensate the negative contribution from the P^-N^+ vector, assumed to be slightly turned toward the aqueous phase.

Our data were obtained at 25 °C, and therefore are not directly comparable with those by Gawrisch et al.,[4] which were obtained at 50 °C where DHPC and DPPC are in the liquid-crystalline state; moreover, DPPC and DHPC are likely to have a different packing density to DOPC. Nonetheless, some comments on the discrepancy between our results and those by Gawrisch et al.[4] are appropriate. As pointed out in the Introduction, the estimate of χ from the ratio of the translocation rates across lipid bilayers of oppositely charged probe ions of similar structure relies on the assumption that the hydration energies of the two probe ions are identical. This assumption is not entirely justified[16,17] and may introduce a spurious additive constant in the χ values obtained by this procedure. However, such a constant would cancel in calculating differences between the dipole potentials of different lipids estimated by using the same probe ions. Such differences must therefore be regarded as much more accurate than the absolute values, as correctly pointed out by Gawrisch et al.[4] If we therefore regard the contribution of +118 mV to χ from the acyl ester groups of DPPC, as estimated by Gawrisch et al.,[4] as correct and we compare it with our χ values for DOPC and DOPS, i.e., +140 and +145 mV, we may reasonably ascribe a value of about +25 mV to the water contribution to the dipole potential of neutral phopsholipids. If this is true, then the slope of the $\Delta\chi$ vs $\sigma_{in} + \sigma_{ex}$ plot for DOPS and DOPA in Figure 7 of ref 2 attaining a maximum value at $\sigma_M = 0$ may be tentatively explained by assuming that the lipid film contains two classes of hydrating water molecules: (1) those hydrating the charged headgroups of DOPS and DOPA, and (2) some with an unchanging orientation deeper within the lipid monolayer. The latter water molecules should be responsible for the small water contribution of about +25 mV which persists even when the lipid monolayer is neutral. At any rate, a water contribution to χ greater than +100 mV, as postulated by Gawrisch et al.,[4] seems definitely too large in the light of our measurements. On the other hand, the attempts made by these authors to confirm such a high water contribution to χ from the expected quadrupolar splitting of deuterated water and from the expected correlation between dipole potentials and hydration forces were unsuccessful.[4]

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