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A Test of Discreteness-of-Charge Effects in Phospholipid Vesicles: Measurements Using Paramagnetic Amphiphiles†

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ABSTRACT: A new series of negatively charged, paramagnetic alkylsulfonate probes was synthesized and can be used to measure both the internal and the external surface potentials of model membrane systems. We tested for discreteness-of-charge effects in lipid membranes by comparing the surface potentials, estimated by use of these negatively charged amphiphiles, with that of a series of positively charged alkylammonium nitroxides in charged membranes. From the partitioning of these probes, the membrane surface potential was estimated in phosphatidylcholine membranes containing either phosphatidylserine or didodecyldimethylammonium bromide. The surface potentials, estimated with either positive or negative probes, were identical, within experimental error, in either positive or negative membranes, and they were well accounted for by a simple Gouy-Chapman-Stern theory. This symmetry, with respect to the sign of the charge, indicates that discreteness-of-charge effects are not significant in determining the potential-sensitive phase partitioning of these probes in model membranes. Thus, despite the fact that charge on membranes is discrete, models that assume a uniform density of charge in the plane of the membrane adequately account for the potentials measured by these amphiphilic probes.

Molecular probe techniques provide a powerful methodology for the determination of membrane electrical properties; for example, a number of procedures using fluorescence, EPR,[1] or NMR can be used to estimate the potential at the membrane surface (Castle & Hubbell, 1976; Eisenberg et al., 1979; McDaniel et al., 1984; McLaughlin, 1982; Cafiso & Hubbell, 1981; Cafiso et al., 1986). The surface potential is extremely important in biological systems, affecting processes such as ion conduction, the gating of channels, and the pH or ion concentrations at the membrane-solution interface. This potential is a consequence of fixed-charge density associated with lipid or protein at the membrane-solution interface (McLaughlin, 1977). By use of fluorescent or EPR probes that exhibit a potential-dependent phase partitioning, the surface potential in biological and model membrane vesicles can be estimated. To relate this potential to a charge density at the membrane-solution interface, a simple Gouy-Chapman-Stern model is often employed. This model assumes that charge is uniformly distributed over the surface of the membrane and includes a correction for the absorption of ions to the membrane-solution interface. While this theory clearly ignores the discrete nature of charge on the membrane surface, it nonetheless appears to account for the behavior of certain paramagnetic probes. For example, the phase partitioning of positive alkylammonium nitroxides in negatively charged membranes is well accounted for by this simple theory (Castle & Hubbell, 1976; Gaffney & Mich, 1976; McDaniel et al., 1986).[2] On the other hand, for certain negatively charged

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[1] Abbreviations: EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance; egg PC, egg phosphatidylcholine; PS, phosphatidylserine; HTAC, hexadecyltrimethylammonium chloride; DDDA, didodecyldimethylammonium; TLC, thin-layer chromatography; MOPS, 3-(*N*-morpholino)propanesulfonate.

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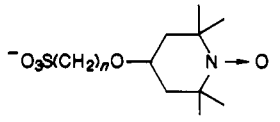
amphiphiles, such as doxylalkyl sulfate and doxylalkylcarboxylate spin probes, the magnitudes of the measured surface potentials in negatively charged membranes are dramatically underestimated with this theory (Gaffney & Mich, 1976; McDaniel et al. 1986). The reasons for this "nonideal" behavior are not clear, but they could be the result of discreteness-of-charge effects.

As described in the accompanying paper by Winiski et al. (1986), significant discreteness effects might be expected for molecular probes of surface potential. By use of a simple model that accounts for discreteness (Nelson & McQuarrie, 1975), it is predicted that fluorescent, NMR, or EPR probe techniques will significantly overestimate potentials when the membrane and probe charge have a dissimilar sign and underestimate the potential when they have the same sign. In some cases, for example, the adsorption of hydrophobic ions to membranes, discreteness effects are important (Andersen et al., 1978; Wang & Bruner, 1978). While many studies have tested the general utility of the Gouy–Chapman theory for quantitating membrane surface potentials, critical tests for discreteness-of-charge effects have not been made.

In this paper, we describe experiments designed to determine the importance of the discreteness of charge in surface potential determinations. We specifically addressed the question of discreteness by examining the behavior of voltage-sensitive EPR probes on well-characterized, positively and negatively charged phospholipid vesicle surfaces. Both cationic and anionic paramagnetic probes of surface potential, which work by identical mechanisms, were used in these studies. For an anionic probe, we synthesized and characterized a new series of alkylsulfonate nitroxides, I(n). For a cationic probe, we



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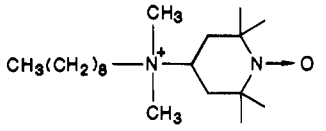

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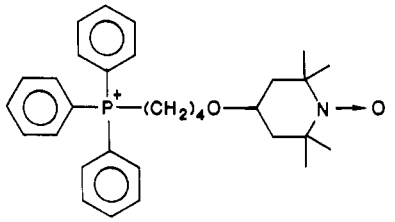

III

used the alkylammonium nitroxide II. The alkylphosphonium nitroxide III was used in conjunction with label I(n) to investigate the asymmetry in the surface potential across the vesicle membrane.

The measurements described in this paper were planned and carried out in conjunction with work described in the accompanying paper by Winiski et al. (1986). In that paper, discreteness-of-charge effects were examined by NMR and fluorescence probes. The ζ potential measurements described by Winiski et al. are particularly important for the interpretation of our results, since they provide a measure of the average surface potentials for the systems we studied. Taken together, the experiments presented in these two papers represent a comprehensive test of discreteness-of-charge effects for bilayers containing charged lipids.

Materials and Methods

Egg phosphatidylcholine (egg PC) was prepared according to the procedure of Singleton et al. (1965) and stored in chloroform under argon at −20 °C at a concentration of 100 mg/mL. Bovine brain phosphatidylserine (PS) was obtained from Avanti Biochemicals (Birmingham, AL). Hexadecyltrimethylammonium chloride (HTAC) and didodecyldimethylammonium bromide were purchased from Eastman Kodak (Rochester, NY). DDDA was purified by the procedure of Angel et al. (1983). The alkylammonium nitroxide II was synthesized as previously described (Castle & Hubbell, 1976).

Preparation of Phospholipid Vesicles. To aliquots of egg PC in chloroform, charged lipids or amphiphiles were added. The chloroform solution was dried under a stream of nitrogen and vacuum desiccated for approximately 15 h. The dried lipids were suspended an aqueous solution containing either 10 or 100 mM NaCl and 0.1 or 5 mM MOPS, pH 7.3–7.5. These dispersions were sonicated to form vesicles as previously described (Castle & Hubbell, 1976). The pH of these solutions was checked following sonication. The sample was then centrifuged at approximately 30000g for 20 min to remove titanium dust originating from the sonicator tip. The concentration of phospholipid in these samples was determined by phosphate analysis as previously described (Bartlett, 1959).

Synthesis of Alkylsulfonate Spin-Labels. To 750 mg (4.4 mmol) of 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-1-oxy, dissolved in 7 mL of dry 2-methyl-2-propanol, was added a slight excess (4.5 mmol) of freshly prepared potassium *tert*-butoxide. The solution was then warmed to 40–50 °C for 2–3 h. To this solution containing the spin-labeled alkoxide a 1.4-fold excess (6.2 mmol) of the appropriate 1,n-dibromoalkane was added rapidly with stirring. The solution was again heated to 45–50 °C for approximately 10 h. The formation of the bromoalkyl nitroxide was followed by TLC on silica gel G plates developed in hexane–acetone, 1:1 ($R_f \approx 0.40$ for n = 8 or 10), and visualized with a fluorescein spray reagent (Stahl, 1969). The KBr precipitate was removed by centrifugation followed by removal of the solvent with rotary evaporation. The bromoalkyl nitroxide was purified by silica gel chromatography (silica gel 60, E. Merck Reagents) in hexane–acetone (5:1). The product eluted rapidly, just behind the unreacted dibromoalkane.

Sulfonation of the bromoalkyl nitroxide was carried out by a procedure similar to that previously described (Marvel & Sparberg, 1943). The bromoalkyl nitroxide (300 mg) was dissolved in 25.5 mL of water–ethanol, 1:3, and heated to 70–80 °C with stirring. A 1.1-fold excess (1.1 mmol) of Na_2SO_3 in 6.75 mL of water was added slowly over a 1-h period. After addition, the mixture was held at 70–80 °C for approximately 2 h. The solvent was then removed by rotary evaporation, and the residue was further dried under vacuum. The product was extracted from the dry residue with 100 mL of hot ethanol. The crude product was then chromatographed on flash-grade silica with chloroform–methanol–acetone, 3:2:1, where the unreacted bromoalkyl nitroxide eluted rapidly

[2] From the phase partitioning of these probes, a value for the membrane surface potential is readily estimated. By use of the Gouy–Chapman theory, the surface charge density can be determined from these surface potential measurements. The accuracy of this surface charge determination is not only dependent on the validity of the Gouy–Chapman theory but dependent on uncertainty in the values for the area per lipid head group and the adsorption of inorganic ions to the membrane–solution interface.

followed by the product. On TLC in chloroform–methanol–acetone, 3:1:2, the product showed a single spot with sulfuric acid charring ($R_f \approx 0.39$, $n = 10$; $R_f \approx 0.5$, $n = 8$). The product was obtained in an overall yield of ~20%, on the basis of the starting nitroxide. From the expected product masses, each product yielded the expected spin concentration as determined EPR. Secondary ion mass spectrometry yielded the expected primary molecular ions as well as the undissociated sodium salts: [M], m/z 391 ($n = 10$), 363 ($n = 8$); [M + 23], m/z 414 ($n = 10$), 386 ($n = 8$). Also abundant were the characteristic [M − 15] peaks of piperidinyl nitroxides (Morrison & Davies, 1970).

EPR Measurements. EPR spectroscopy was carried out on either a modified Varian V-4500 series or a Varian E-line Century Series X-band spectrometer. Spectra were taken on 100-μL samples in quartz flat cells with a modulation amplitude of 1.0 G peak-to-peak and a power of 10 mW. In all cases, labels I(n), II, and III were used at a concentration of 20 μM. For measurements of time-dependent processes, a pneumatic rapid-mixing device similar to that described previously was used (Cafiso & Hubbell, 1982).

Surface potentials were determined from the phase partitioning of the nitroxide probes I(n) or II as previously described (Castle & Hubbell, 1976). In each case, the ratio of membrane bound to aqueous probe λ was determined from the amplitude of the high-field nitroxide resonance, A, and the amplitude of the high-field resonance in the absence of vesicles, A_f^0, as follows (Cafiso & Hubbell, 1982):

$$\lambda = (A_f^0 - A)/[A - (\beta/\alpha)A_f^0] \quad (1)$$

The parameter β/α is a constant that reflects the contributions aqueous and bound spin make to the high-field resonance amplitude. This parameter is usually small; for label I(8), we set $\beta/\alpha = 0$. Using this procedure we can determined λ from a single spectral parameter, A.

The transmembrane migration of probe I or III was followed by monitoring time-dependent changes in A following mixing of the label with vesicle suspensions as previously described (Cafiso & Hubbell, 1982; Sundberg & Hubbell, 1986). Following mixing, the amplitude of A decays in an exponential fashion given by

$$A_f(t) - A_f(\infty) = [A_f(0) - A_f(\infty)] \exp(-\gamma t) \quad (2)$$

Here, $A_f(t)$, $A_f(0)$, and $A_f(\infty)$ are the amplitudes due to aqueous (free) spin at $t = t$, at $t = 0$, and at equilibrium, respectively. Here, $A_f(t)$ is determined from the high-field resonance amplitude $A(t)$, where $A_f(t) = [A(t) - (\beta/\alpha)A_f^0]/(1 - \beta/\alpha)$ and γ is the relaxation time-constant. The equilibration of the probe with the external membrane–solution interface is extremely rapid on the time scale of our measurements, and only the slower transmembrane migration of the probe is revealed by changes in $A_f(t)$. The amplitude of the relaxation for the high-field resonance can be explicitly written in terms of probe binding constants and intrinsic quantities describing the vesicle suspension (Sundberg & Hubbell, 1986). Here, we will use the values of λ at $t = 0$ and $t = \infty$ and eq 3 to

$$K_i/K_o = (V_{mo}/V_{mi})[(V_i/V_o + 1)[\lambda(\infty)/\lambda(0)] - 1] \quad (3)$$

determine the internal and external probe binding constants K_i and K_o in the absence of a surface potential (Cafiso & Hubbell, 1982; Cafiso, 1986). In eq 3, V_o and V_i are the external and internal aqueous vesicle volumes, respectively, and V_{mo}/V_{mi} is the ratio of volumes occupied by probes I or III when bound to the membrane (approximately the ratio of surface areas). In the presence of surface potentials, the binding constants are modified so that $K_i' = K_i \exp[zF\psi_i/(RT)]$ and $K_o' = K_o \exp[zF\psi_o/(RT)]$. In this case, the apparent ratio of binding constants K_i'/K_o' becomes

$$K_i'/K_o' = (K_i/K_o) \exp[zF(\psi_o - \psi_i)/(RT)] \quad (4)$$

where ψ_o and ψ_i are the external and internal surface potentials, respectively. Although the phosphonium probe III strictly measures an interfacial boundary potential, this potential is the sum of the surface potential and an interfacial potential component. We assume that this interfacial potential component is independent of ionic strength and can be effectively included in the binding constants K_i and K_o. Therefore, we can use changes in K_i' and K_o' for label III to assess surface potential differences.

External surface potentials were determined from the phase partitioning at $t = 0$, λ(0), with eq 5 as previously described

$$\psi_o = -[RT/(zF)] \ln [\lambda(0)/\lambda_o] \quad (5)$$

[see Castle & Hubbell (1976)]. In eq 5, λ_o is the partitioning of probe to neutral egg PC vesicles. In some cases, λ was measured as a function of the mass of lipid per unit volume (m_l/V_t). A plot of λ^{-1} vs. V_t/m_l yielded a straight line of slope α, and the potential was calculated according to

$$\psi_o = -[RT/(zF)] \ln (\alpha/\alpha_o) \quad (6)$$

In this case, α_o is the slope of the binding curve for neutral egg PC vesicles. Taken together, eq 3–5 allow a determination of both the internal and external surface potentials from the time-dependent phase partitioning of paramagnetic amphiphiles.

Results

Measurements of ψ_o Using Positive Alkylammonium Nitroxides (II). The phase partitioning of label II was measured in vesicles containing either PS, HTAC, or DDDA under conditions of varied ionic strength and surface charge density. The external membrane surface potential ψ_o was calculated from the phase partitioning with eq 5 or 6. Shown in Figure 1 are plots of the voltages determined with the alkylammonium label II in membranes as a function of the mole percent of positive (DDDA) or negative (PS) charge. Figure 1A shows the magnitudes of the potentials estimated with 100 mM NaCl, and Figure 1B is a plot of the potentials measured at 10 mM NaCl. As seen in Figure 1, probe II measures potentials that approximate those expected from a simple Gouy–Chapman–Stern theory. This behavior was previously observed for II in negatively charged vesicles containing phosphatidic acid (PA) (Castle & Hubbell, 1976) and PS (McDaniel et al., 1986). Potentials estimated from II in vesicles containing positive charge, HTAC or DDDA, are also very close to those expected. These probes do appear to slightly underestimate potentials in positively charged vesicles at low ionic strength, see Figure 1B.

Measurements of ψ_o Using Negative Alkylsulfonate Nitroxides (I). The alkylsulfonate labels I(8) and I(10) were used to measure surface potentials in sonicated vesicles containing either negative or positive charge. Because of the rapid transmembrane migration rate of the alkylsulfonates, relative to the alkylammonium nitroxides (see below), the partitioning, λ, was measured immediately after mixing I(8) or I(10) with vesicle suspensions. Shown in Figure 2 are the potentials, determined from λ with eq 5 or 6, in positive vesicles containing DDDA or negative vesicles containing PS. Panels A and B of Figure 2 are the potentials as a function of surface charge density for sonicated vesicles in 100 and 10 mM NaCl, respectively. The potentials determined with the negative probes I(8) or I(10) are reasonably close to the potentials expected on the basis of a simple Gouy–Chapman–Stern theory, given

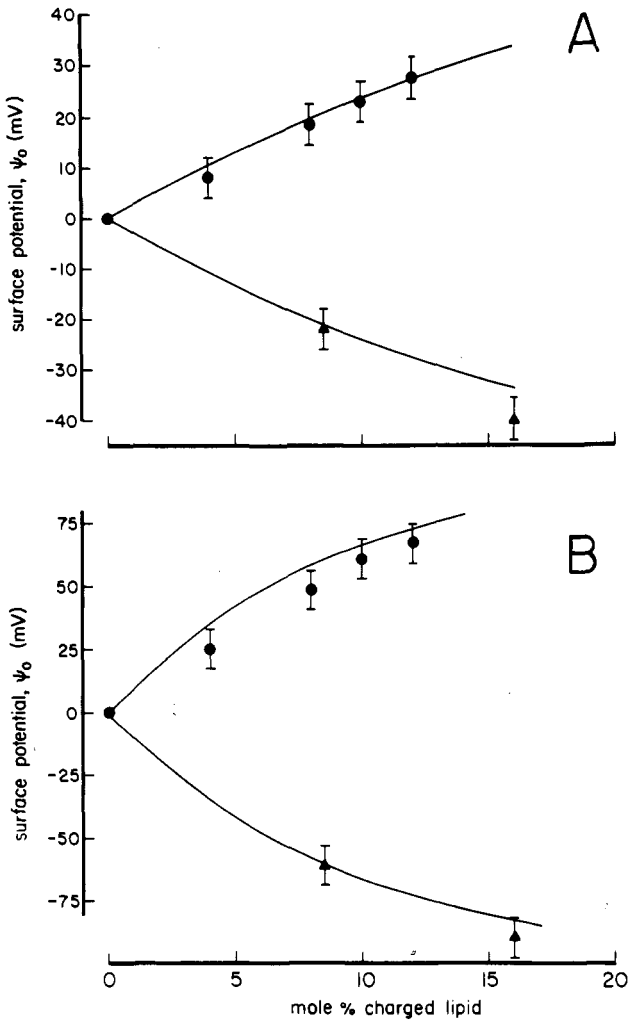

FIGURE 1: Surface potentials measured with the alkylammonium nitroxide II in egg PC vesicles as a function of the mole percent of charged lipid: (A) surface potentials for vesicles in 100 mM NaCl containing DDDA (●) or PS (▲); (B) surface potentials of vesicles in 10 mM NaCl composed of DDDA (●) or PS (▲). The total lipid concentration was approximately 15–20 mM in all cases, and probe II was used at a concentration of 20 μM. The solid lines (—) are plots of the potentials expected from the Gouy–Chapman–Stern theory [see McLaughlin (1977)] assuming an intrinsic association constant for Cl^- to DDDA and Na^+ to PS of 1 M^{-1}.

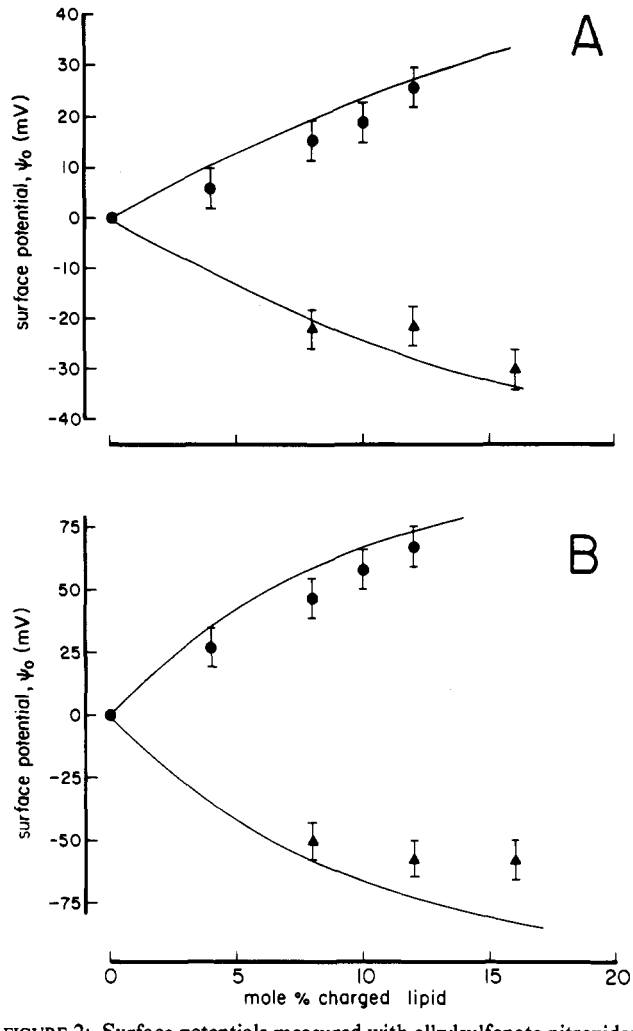

FIGURE 2: Surface potentials measured with alkylsulfonate nitroxides I in egg PC vesicles as a function of the mole percent of charged lipid. Potentials in DDDA-containing vesicles were estimated with probe I(8) (●). Potentials in PS-containing vesicles were calculated with probe I(10) (▲). Probe concentrations were 20 μM, and total lipid concentrations were between 15 and 20 mM. Surface potentials were determined in (A) 100 and (B) 10 mM NaCl. The solid lines are plots determined from the Gouy–Chapman–Stern theory assuming intrinsic binding constants for Cl^- to DDDA and Na^+ to PS of 1 M^{-1}.

the range of experimental error. Significant, but small differences do appear in positively charged DDDA membranes, where measured potentials are low at both 10 and 100 mM by approximately 15%. Potentials measured in negative PS-containing membranes are generally within experimental error of the expected values except at 10 mm NaCl, where probe I(10) appears to underestimate ψ_o at higher charge densities.

Binding and Transmembrane Migration of Alkylsulfonate and Alkylphosphonium Nitroxides. As described above, the transmembrane migration of amphiphilic nitroxides can be followed by measuring time-dependent changes in their phase partitioning. We measured the transmembrane migration of the alkylsulfonate probes I(n) and find that they transit egg PC vesicle bilayers more rapidly than the positively charged alkylammonium probe II. Shown in Figure 3A is a tracing of the high-field resonance amplitude for the alkylsulfonate I(8) when mixed with phospholipid vesicles containing 10 mol % DDDA in 100 mM NaCl. Shown in Figure 3B are log plots of the fractional change in free-signal intensity when sulfonates are mixed with DDDA or egg PC vesicles. The I(10) and I(8) alkylsulfonates have a $t_{1/2}$ for migration of 37 and 90 min, respectively, in sonicated egg PC vesicles. As seen in Figure 3B, the transmembrane migration rate is significantly faster in vesicles containing DDDA; a $t_{1/2}$ of $\approx$6 min is found in 12 mol % DDDA vesicles. This contrasts with the much longer $t_{1/2}$ of $\approx$10 h for migration of the alkylammonium II(8) across egg PC vesicles (Castle & Hubbell, 1976). From these amplitude changes, we calculated the phase partitioning for the probe at $t = 0$ and $t = \infty$ [$\lambda(0)$ and $\lambda(\infty)$, respectively]. From this initial and equilibrium value of λ, the ratio of the internal/external binding constants, K_i/K_o, for the sulfonates I(n) in egg PC vesicles is found to be $\sim$1.5. In vesicles containing 10 mol % DDDA, at 100 mM NaCl, K_i/K_o has a value of 3.5 for the sulfonate I(10). This surprisingly large asymmetry in binding constants could result from a structural asymmetry produced by the addition of DDDA (i.e., a change in K_o/K_i) or an asymmetry in the surface potential of about 22 mV.

A tracing of the high-field nitroxide resonance for the phosphonium probe III in DDDA-containing vesicles is also shown in Figure 3A. The relaxation amplitude is much smaller than that for the positive sulfonates. Here, the value of K_i/K_o estimated for the phosphonium is 0.48. In egg PC vesicles, the ratio K_i/K_o for this probe has a value of approximately 1.2. This decrease in K_i/K_o is also consistent with an asymmetry in the surface potential of about 22 mV, with the in-

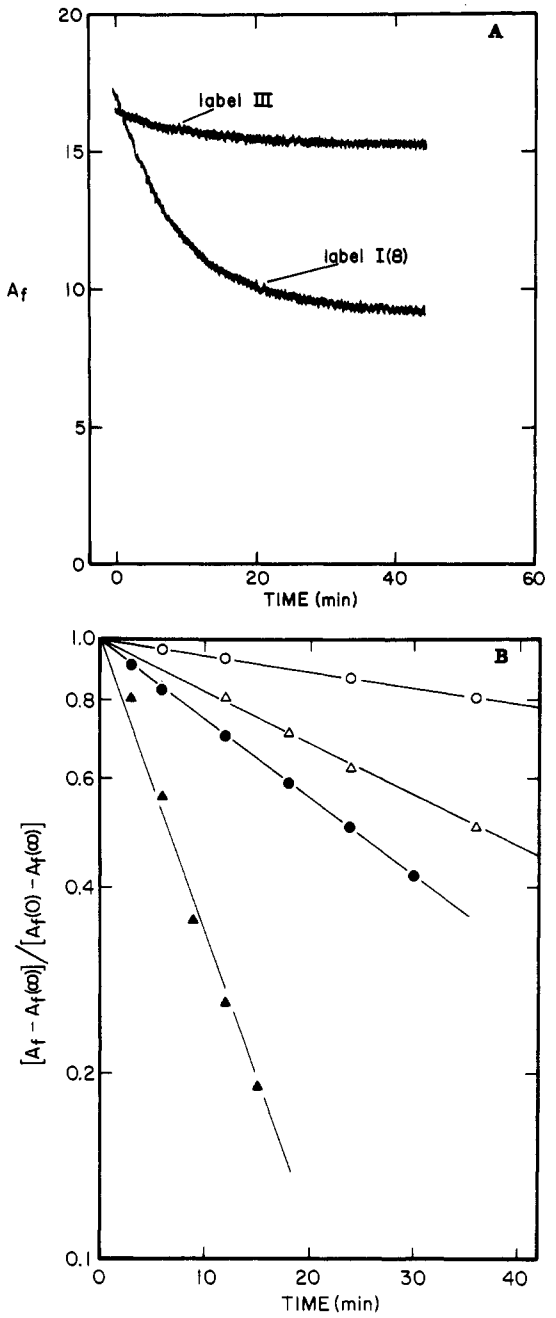

FIGURE 3: (A) Time dependence of high-field nitroxide resonance for label I and III as a function of time following mixing with phospholipid vesicles containing 10 mol % DDDA in 100 mM NaCl. This time dependence reflects the transmembrane migration of probe. (B) Plot of $[A_f(t) - A_f(\infty)]/[A_f(0) - A_f(\infty)]$ vs. time for probe I(8) in vesicles composed of egg PC (○), egg PC + 4 mol % DDDA (●), and egg PC + 12 mol % DDDA (▲) and for probe I(10) in egg PC (△).

ternal surface being more positive than the external surface. The complementary behavior of both positive and negative probes provides strong evidence that the change in binding asymmetry between surfaces in DDDA vesicles is electrostatic in origin.

Measurements of λ *in Neutral Vesicles Containing DDDA and PS.* As an additional check for discreteness-of-charge effects, the phase partitioning of label I(8) and label II was measured in egg PC vesicles and compared with that in egg PC containing a 1:1 mixture of DDDA and PS. Mixtures were examined that contained 5 mol % DDDA and PS or 10 mol % DDDA and PS. Within experimental error the phase partitioning, λ, was identical in neutral vesicles with and without charged amphiphiles. There was no ionic strength dependence on the phase partitioning of either label in these vesicles. Thus, the surface potentials were zero (±3 mV) with label I(8) or II in egg PC or egg PC + DDDA/PS vesicles. From a simple model for discrete charge [see Nelson and McQuarrie (1975)], we calculated the potentials expected at a distance of 2 Å from a square lattice of alternating positive and negative charges. For 5 and 10 mol % positive and negative charge, at 10 mM monovalent salt, we expect potentials of about ±27 and ±38 mV, respectively [(+) for label I(8), (−) for label II]. The parameters and procedure for calculating the potentials expected from these probes are given in the accompanying paper by Winiski et al. (1986).

Discussion

The quantitative application of potential-sensitive probes in complex biological membranes will require at least an understanding of their behavior in model membrane systems. Here, we tested the ability of the Gouy–Chapman–Stern theory to quantitatively account for the voltage-dependent phase partitioning of both positive and negatively charged probes to charged membrane vesicles. This theory assumes that surface charge is uniformly smeared in the plane of the membrane and ignores the discreteness of charge. If the Gouy–Chapman–Stern theory is used to estimate membrane potentials, discreteness-of-charge effects should be manifested as an overestimate of the surface potential when the probe and surface charge have opposite signs. When the probe and surface charge have the same sign, the membrane surface potential would be underestimated with this theory.

The experiments carried out here clearly demonstrate that discreteness effects are not large. While small differences between the predicted and experimental potentials are observed, the systematic changes that are expected from discreteness effects are not seen. Measurements made on neutral vesicles containing both PS and DDDA should be quite sensitive to the effects of discreteness.[3] For these measurements, the discrepancy between the Gouy–Chapman and discrete-charge calculations is large. Essentially no evidence for discreteness is found on these membrane surfaces within the resolution of our techniques. Thus, the probes used here report surface potentials in close agreement with the simple smeared-charge theory. These results are consistent with the results presented in the accompanying paper by Winiski et al. (1986). Electrophoretic mobility and nonactin conductance provide estimates of the average membrane surface potential. In PS- and DDDA-containing membranes, the results of measurements using electrophoretic mobility and nonactin conductance are generally in quantitative agreement with the data presented here. In addition, NMR and fluorescence measurements on these membranes do not show any evidence for discreteness effects [see the accompanying paper by Winiski et al. (1986)]. Taken together, these results demonstrate that a range of potential-sensitive probes, which partition to membrane surfaces, are adequately accounted for by models that assume a uniform density of charge.

The Gouy–Chapman–Stern theory has generally been successful in describing the potentials at planar aqueous in-

[3] We are assuming here that there is no lateral phase separation of the charged components DDDA and PS. While we have not carried out experiments to explicitly eliminate this possibility, we see no evidence that such a phase separation occurs. There is no change in the partitioning of labels I or II to pure egg PC vesicles when compared to egg PC vesicles with DDDA and PS. There are also no changes in the molecular order indicated by either label. DDDA and PS also have significantly different chain lengths.

terfaces. In the present case, the reasons for this agreement are not clear. The lateral diffusion of membrane-bound charges might account for the lack of discreteness we observe. However, measurements using bound fluorescence probe in gel-state bilayers, where lateral diffusion should be very slow, also show no evidence for discreteness effects (Winiski et al., 1986). These measurements argue against the role of lateral diffusion. Another explanation for the absence of discreteness effects could be the finite size of the lipid and probe. Charged lipid and probe will not interact as true point charges, and their close approach will be limited by their finite size. As pointed out by Winiski et al. (1986), when this finite size is taken into account, the discreteness effects predicted by a simple model are greatly reduced.

At present, we do not understand the basis for the anomalous behavior of the negatively charged chain-labeled doxyl probes previously examined (McDaniel et al., 1986). As shown here, their behavior is unlikely to be due to discreteness, and other factors must account for their diminished response to surface potentials.

The time-dependent changes in free-signal amplitude for probes I and III, shown in Figure 3, provide information both on the rate of probe migration and on the asymmetry of the membrane surface potentials. Measurements of this asymmetry were recently shown to be quantitatively feasible with the alkylammonium label II (Sundberg & Hubbell, 1986). In this case, a small quantity of tetraphenylboron, Ph_4B^-, was added to the lipid vesicles to promote the transmembrane migration of probe. The relatively rapid transmembrane migration of the alkylsulfonates described here, I(n), makes them ideally suited for this asymmetry measurement, without the addition of any carrier. The relaxation amplitudes for I and III are both consistent with the internal surface of 10 mol % DDDA-containing vesicles being approximately 20 mV more positive than the external surface. This asymmetry is likely due to two factors. First, in small vesicles the packing density of the internal vesicle surface is expected to be higher than on the external surface [see, for example, Israelachvili and Mitchell (1975) and Huang and Mason (1978)]. Huang and Mason (1978) estimate that the packing density for phospholipid on the internal vesicle surface should be ~20% higher than on the external surface. This asymmetry in 100 mM NaCl should yield a surface potential that is ≈10 mV more positive on the internal surface, for membranes containing 10 mol % charge. Sundberg and Hubbell (1986) in fact report an asymmetry in vesicles containing PS of −10 mV under similar ionic conditions. By assuming an ≈20% asymmetry in packng (area/phospholipid), we can only account for a fraction of the asymmetry measured here in the DDDA-containing sample. A second source of this asymmetry could of course be a preferential distribution of the positively charged amphiphile DDDA to the internal vesicle surface. An asymmetry in the distribution of positively charged DDDA that diminished the expected external surface charge density by ≈12% could account for the remainder of the observed probe binding asymmetry. This modest reduction in the surface potential could well be the source of the underestimation in positive external surface potential we see in Figures 1 and 2.[4]

[4] A number of factors could account for the small differences seen between expected and measured surface potentials. For example, the Gouy–Chapman theory overestimates potentials on spherical surfaces (Ohshima et al., 1982). In small vesicles containing 10 mol % charged lipid, this overestimate is small, approximately 10%. In addition, small changes in the intrinsic binding constants of labels I and II in DDDA vesicles could contribute to an underestimate in potential.

In summary, we estimated surface potentials by using anionic and cationic paramagnetic probes in membranes containing both positive and negative charge density. The measured potentials are close to those predicted from a simple Gouy–Chapman–Stern model. Systematic variations in the potentials due to discreteness-of-charge effects are not seen. Thus, by assuming a uniform density of charge, we can adequately account for the potential dependence of these probes. This does not rule out the importance of discreteness effects under different circumstances, for example, with protein-bound charges, low charge densities, or multivalent bound charges. A new series of alkylsulfonate probes was synthesized here. Unlike previously examined anionic probes, their behavior is well accounted for in both positive and negative membranes. They also undergo a more rapid transmembrane migration than positively charged alkylammoniums probes, a feature that makes them useful probes for the determination of membrane surface charge asymmetry.

Registry No. I (n = 8), 104778-49-6; I (n = 10), 104778-50-9; II, 61165-80-8; III, 100791-13-7; DDDA, 3282-73-3.

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