Stress-Induced Leakage from Phospholipid Vesicles: Effect of Membrane Composition

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When subjected to large hypoosmotic gradients, lipid vesicles swell and rupture, expelling contents in an attempt to reach mechanical equilibrium. To date, the influence of the lipid composition on this leakage process has not been studied. In this paper, we present osmotic leakage data for vesicles made of a series of phosphatidylcholine lipids and show that the lipid composition is an important control parameter in osmotically forced leakage. We also show that current models for the leakage phenomenon fail to predict experimental results and offer an improved model that allows for the possibility of a mixed population of unilamellar and multilamellar vesicles. Finally, we demonstrate that mechanical properties found using the micropipet aspiration method might not be applicable to osmotic swelling experiments utilizing nanometer-scale vesicles.

Introduction

A chief function of cellular membranes is to provide the cell with a physical barrier against the extracellular environment. Cellular membranes are normally considered impermeable to solutes such as salts and carbohydrates but readily pass water, thus permitting the formation of osmotic gradients.[1] These gradients can effect many alterations in membrane characteristics, including the activation of membrane-bound mechanosensitive ion channels[2] and enhancement of membrane fusion.[3] The application of large gradients can result in membrane rupture and gross leakage,[4] which can result in cell death. In addition to offering insight into biological processes, studies of membrane response to osmotic stress may assist in the improved engineering of biomimetic structures such as drug delivery vehicles.

Many of the material properties of cellular membranes, such as their response to osmotic gradients, are derived primarily from the properties of the membrane's lipid matrix. Vesicles, synthetic bilayers made up entirely of lipid, have therefore been frequently used as model systems in the study of membrane mechanics. The response of vesicles to hydrostatic,[5] shear,[6] thermal,[7] and osmotic stresses[4,8−11] has thus been studied to gain insight into the material properties of cell membranes.

Vesicles subjected to hypoosmotic gradients undergo a series of substantial structural modifications. Light scattering studies have shown that vesicles initially exhibit an elastic response as they swell in an attempt to balance the applied osmotic pressure.[4,8,9,11] Once a critical gradient is reached, however, the membrane fails and transient defects result in rapid leakage of vesicle lumen. Interestingly, once sufficient lumen has been expelled, the vesicle reseals into a mechanically stable structure.[8,10] A convenient method to monitor leakage of the interior solution relies on fluorescent probes such as carboxyfluorescein (CF) that become self-quenched at high concentrations. Leakage can be observed through an increase in the signal as the probe escapes the high-concentration vesicle interior and moves into the low-concentration exterior. This assay thus allows observation of both elastic deformation (denoted by the period of swelling prior to leakage) and critical failure of lipid membranes.

The lipid composition of membranes has been shown to affect the material properties of membranes including membrane elasticity, permeability, and critical failure.[12,13] In fact, in nature cells demonstrate the ability to control membrane characteristics through modulation of the lipid composition.[14] However, there has yet to be completed a systematic study of osmotic stress-induced leakage as a function of the membrane lipid composition. In this work, we use fluorescence assays to report osmotically induced leakage of vesicles made of a series of related phospholipids and show that indeed the lipid composition represents an important tuning parameter. We then attempt to correlate leakage with lipid mechanical properties found using the micropipet aspiration method. We show that when these mechanical properties are used, an existing model for osmotic stress fails to qualitatively predict vesicle leakage. In response, we offer an improved model that might account for the discrepancy between the predicted and observed leakage amounts. Finally, we discuss the possible inapplicability of mechanical properties found using the micropipet method to nanometer-sized vesicles.

Experimental Section

Palmitoyloleoylphosphatidylcholine (POPC), stearoyloleoylphosphatidylcholine (SOPC), dioleoylphosphatidylcholine (DOPC), dilinoleoylphosphatidylcholine (DL_2-PC), and dilinolenoylphosphatidylcholine (DL_3PC) were obtained from Avanti Polar Lipids (Alabaster, AL). 5(6)-Carboxyfluorescein (CF) was received from Molecular Probes (Eugene, OR). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was obtained from Boehringer Manheim (Indianapolis, IN). Water used was produced by a Milli-Q UF unit (Millipore, Bedford, MA) having a resistivity of 18.2 MΩ. All other chemicals were from Sigma (St. Louis, MO). All chemicals were of the highest purity available and were used as received.

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Manufacture of Nanovesicles. Vesicles used in osmotic leakage studies were formed using the high-pressure extrusion method.[15] Briefly, for each lipid, a 50 mg/mL $CHCl_3$ solution was dried under vacuum for at least 3 h. A CF solution for the vesicle interior was added (unless otherwise stated, a 1550 mOsm solution consisting of 700 mM NaCl, 100 mM CF, and 50 mM HEPES, titrated to pH 7.4 with 1550 mM NaOH, and filtered), and multilamellar vesicles were created using a process of vortexing, freezing in liquid nitrogen, and thawing in warm (~40 °C) water. After this freeze/thaw cycle was repeated a total of five times, the vesicles were passed 10 times through two stacked 100 nm polycarbonate filters (Whatman, Clifton, NJ) in a high-pressure extruder (Lipex Biomembranes, Vancouver, British Columbia, Canada). To remove extravesicular CF, the vesicle solution was then passed through a gel chromatography column filled with Sephadex G-50 (Piscataway, NJ) that had been equilibrated with an isoosmolar CF-free solution (unless otherwise stated, consisting of 750 mM NaCl and 50 mM HEPES and titrated to pH 7.4 with 1550 mM NaOH). The final phospholipid concentration was found using the spectrophotometric assay supplied by Avanti Polar Lipids. Vesicles were used within 8 h of preparation.

Dynamic light scattering measurements for POPC vesicles were performed using a Lexel model 85 ion laser (Fremont, CA) operating at a wavelength of 514 nm. Data were analyzed by an ALV-5000 autocorrelator (ALV Laser, Langen, Germany) and were fit using a cumulant analysis with second-order residuals. All scattering measurements were performed at 25.0 °C, and the results were averaged over five runs. Results for samples in equiosmolar solutions (i.e., no swelling) were consistent with a monodisperse vesicle population with radii of 54.4 ± 0.4 nm (standard deviation).

Osmotically Induced Leakage Studies. The 100 nm vesicles created above were exposed to solutions of differing osmotic concentrations made by mixing the isoosmotic CF-free solution and a 50 mOsm dilution solution (50 mM HEPES, titrated to pH 7.4 with 50 mM NaOH). CF is self-quenched when present at concentrations above ~0.1 mM, so leakage could be monitored via the signal increase when CF was expelled from the self-quenched vesicle interior into a CF-free external solution. It was found that even a small amount of agitation drastically increased vesicle leakage, so mixing was accomplished simply through the addition of 2 mL of the iso/hypoosmotic solution to ~30 μL of the vesicle solution. Samples were allowed 5 min to equilibrate, and the fluorescence signal at 519 nm was measured using an F-4500 fluorescence spectrophotometer (Hitachi Instruments, Clifton, NJ) while exciting at 490 nm. After readings were taken, Triton X-100 was added to a final concentration of 0.1 wt % to destroy vesicles and entirely liberate the fluorescent tag. The samples were carefully shaken and allowed 5 min to equilibrate, and fluorescence signals were again taken. Leakage was calculated by monitoring S, the fraction of solutes remaining in the vesicle. S was defined $1 - (F_{signal} - F_{baseline})/(F_{100\%} - F_{baseline})$, where F_{signal} is the fluorescence intensity of the sample, $F_{baseline}$ is the intensity of vesicles in the isoosmotic solution, and $F_{100\%}$ is the intensity after disruption by the Triton surfactant. This calculation implicitly assumes that all solutes, including the fluorescent marker, are expelled indiscriminately, an assumption that was upheld by leakage studies performed with various solutes (see the Results and Discussion section). Vesicle concentrations were kept constant in each sample (total phosphorus concentration of 6 μM) to reduce scattering effects. Leakage measurements for each lipid tested were averaged over at least five runs using two separate batches of vesicles. A circulating bath maintained sample temperatures at 25.0 ± 0.1 °C.

Manufacture of Microvesicles. Vesicles for use in the micropipet technique were made by electroformation based on the method of Angelova et al.[16] as modified by Longo et al.[17] A POPC lipid solution (0.5 mg/mL in $CHCl_3$) was spread onto 1 mm diameter platinum electrodes held in a Teflon/glass chamber at a spacing of 5 mm. Films were dried under vacuum for at least 3 h. Because the electroformation technique does not form micron-sized vesicles when high ionic strength solutions are used (unpublished results), vesicles were formed in sugar solutions. The chamber was filled with a 150 mM sucrose solution to be used as the vesicle lumen. Vesicles were formed by applying a 3 V sine wave across the two electrodes. The frequency was held at 10 Hz for 30 min, 3 Hz for 15 min, 1 Hz for 7 min, and 0.5 Hz for 7 min. Vesicles were withdrawn from the Teflon chamber and mixed with an equal volume of a 170 mM glucose solution. Using different interior and exterior solutes allows for greater optical contrast and forces the vesicles to settle under gravity, simplifying vesicle collection. Using a slightly hyperosmotic solution also partially deflates the vesicles, which allows easier micromanipulation. Vesicles used in the micropipet technique typically had diameters between 30 and 50 μm and were used within 8 h of preparation.

Micropipet Technique. The micropipet technique was used to measure the elasticity and critical areal strain of POPC vesicles (for a review of the technique, see ref 5). Borosilicate capillaries of 1 mm diameter (Freidrich and Dimmock, Millville, NJ) were pulled to a fine point using a Kopf model 730 puller (Tujunga, CA). Pipets were then forged using a Narishige MF 830 microforge (Micron Optics, Cedar Knoll, NJ) to an inner diameter of ~7−9 μm. Final tip diameters were measured optically. To reduce vesicle adhesion, pipets and the glass observation chamber were treated with 2 wt % bovine serum albumin for 30 min, rinsed with Millipore water, and dried under nitrogen just prior to use. Pipet manipulation was done with a Narishige micromanipulator (MHW-3, Micron Optics, Cedar Knoll, NJ) attached to a Nikon TE 200 inverted microscope outfitted with phase and DIC optics (Micron Optics, Cedar Knoll, NJ). Images were captured using a Kodak ES 310 CCD camera and a PIXCI D imaging board (EPIX, Buffalo Grove, IL). During manipulations, a constant temperature of 25.0 ± 0.1 °C was maintained using a circulating bath. Suction pressures were applied by water displacement and were measured by in-line pressure transducers (Advanced Controls, Warminster, PA). In a common experiment, vesicles were individually selected with a micropipet by aspirating at very low pressures. The suction pressure applied to the vesicle was increased in steps of ~0.3 in. of water (maintaining for 10 s), and the resulting vesicle deformations were measured. Vesicle elasticity was calculated as the slope of the resulting stress/strain plot (found in the linear regime, at stresses >0.5 mN/m), and the critical areal strain was defined as the strain at which the vesicle failed. This elasticity thus represents the direct area

Table 1. Structural, Thermodynamic, and Mechanical Data for Lipid Series

lipid	chain structure	T_c (°C)[a]	α_c[b]	K[b] (mN/m)
DL_3PC	18:3, 18:3	−60	0.019 ± 0.004[c]	159 ± 19[d]
POPC	16:0, 18:1	−2	0.037 ± 0.006	178 ± 7
DL_2PC	18:2, 18:2	−53	0.027 ± 0.003[c]	190 ± 18[d]
SOPC	18:0, 18:1	6	0.043 ± 0.006[c]	208 ± 10[d]
DOPC	18:1, 18:1	−20	0.042 ± 0.008[c]	237 ± 16[d]

[a] Chain transition temperature, taken from ref 27. [b] Errors reported as standard deviations. [c] Taken from refs 12 and 13, assuming $\alpha_c = \tau_c/K$. [d] Taken from ref 13.

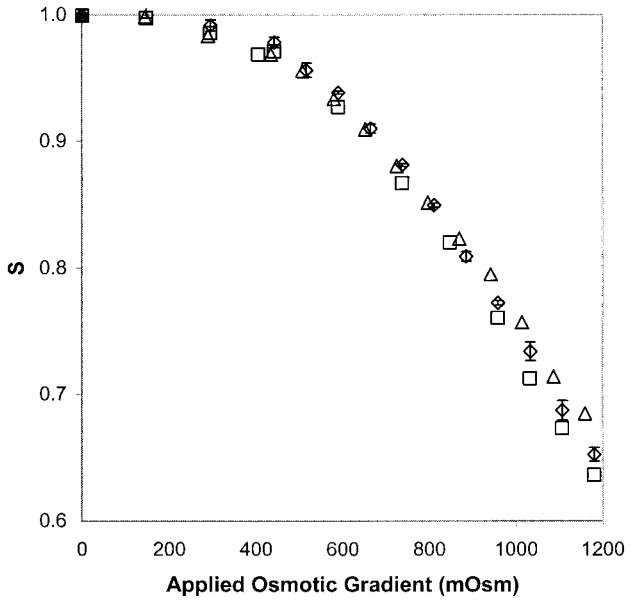

Figure 1. POPC vesicle leakage due to application of hypoosmotic gradients. Vesicles are made with internal solutions of NaCl (squares), Na_2SO_4 (triangles), or 50% glucose/50% NaCl (diamonds). Error bars shown for glucose/NaCl are the measured standard deviation and are representative of errors for all solutes.

expansion modulus for the material which, because membranes may be regarded as two-dimensional materials, appears in units of mN/m. The areal strain is defined as the change in membrane area scaled by the initial area ($\Delta A/A_0$) and is dimensionless. Measurements reported herein for POPC vesicles are the average of 22 individual vesicles.

Results and Discussion

We have examined the effect of alterations in the lipid structure on the behavior of osmotically stressed vesicles. The five lipids chosen for this study, listed in Table 1, all possess choline headgroups and differ only in the structure of their hydrophobic tails. These phosphocholine lipids are all biologically relevant lipids that allow us to modulate disorder and cohesion in the oily bilayer interior through minor variations in tail length and bond unsaturation (lipid tail structures are also indicated in Table 1).

The leakage behavior of all vesicles examined, regardless of composition, qualitatively agreed with a model in which membranes may support small osmotic gradients but fail and leak internal contents at sufficiently large ones. Figure 1 shows the leakage behavior of POPC vesicles plotted as S, the fraction of solutes remaining in vesicle interiors, versus the applied osmotic gradient. The general shape of the curve agrees with previous work by others[18] and with the scheme for leakage just outlined. When subjected to gradients of less than ~300 mOsm, vesicles are in the elastic regime where negligible leakage is seen. At larger gradients, however, a substantial increase in the fluorescent signal is found, which indicates leakage of up to 55% of the original vesicle contents (maximum gradient data not shown).

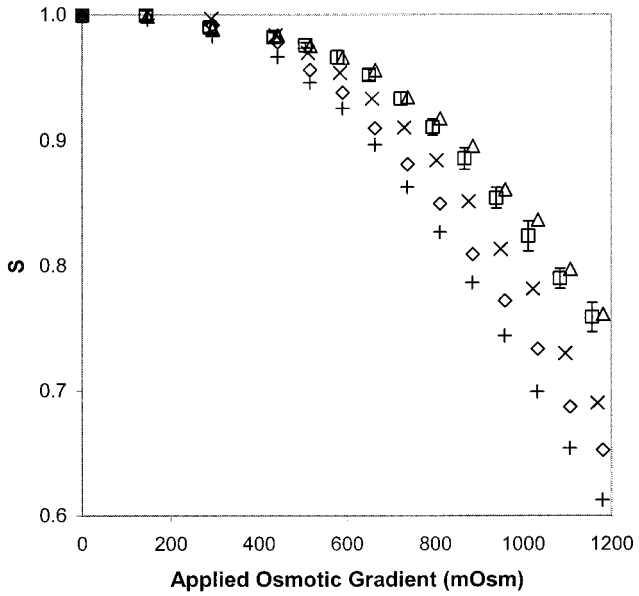

Figure 2. Leakage profiles for vesicles made of DOPC (triangles), SOPC (squares), DL_2PC (crosses), POPC (diamonds), and DL_3PC (pluses). Error bars shown for SOPC are measured standard deviations and are representative for all lipids.

Figure 1 provides supporting evidence that leakage occurs not via passive diffusion but through gross membrane defects. Passive diffusion through a lipid membrane is extremely sensitive to both the size and the charge of the solute.[19] Trials were run in which monovalent ions, divalent ions, and carbohydrates served as internal solutes in addition to the fluorescent marker. If the passive diffusion mechanism was the prevalent means of leakage, it is expected that solutes of different size or charge would each leak at different rates. In this situation, the amount of CF expelled from a vesicle would be a function of the other solutes present in the lumen. However, from the data in Figure 1, it is seen that the moderately large and highly charged fluorescent tag (CF^{2+}, MW = 376 g/mol) leaked at the same relative rate for all solutions examined (the minor solute effects seen are likely artifacts related to solution nonidealities that were ignored in the calculation of solution osmolality). We conclude that the vesicle contents are expelled indiscriminately and that gross membrane defects thus represent the most likely leakage mechanism.

The small error bars shown in Figure 1 show that when using identical solute conditions, the leakage assays are extremely repeatable. To minimize error from the artifact noted above, all further leakage data discussed will be based on identical internal solutions, as described in the Experimental Section.

Altering the membrane composition has a small but significant effect on leakage, as is demonstrated in Figure 2. For data clarity, only the error bars for SOPC are shown. These errors are based on experimental standard deviations and are nearly identical with those for all lipids.

The order of the lipids is DL_3PC, POPC, DL_2PC, SOPC, and DOPC from the weakest (first to leak) to the strongest (last to leak). Lipids are listed in this order in Table 1 along with pertinent structural and thermodynamic data. The order of leakage is independent of the amount of disorder present in the bilayer, as indicated by the number of double bonds present, as well as the cohesive attraction between lipids, as indicated by the main-chain transition temperature. Also listed in the table are the mechanical properties of the lipid membranes determined using the micropipet aspiration method. There is an apparent correlation between leakage and membrane elasticity, K, which implies that leakage may be understood through an examination of the mechanical stability of stressed vesicles.

Mechanical equilibrium requires that the osmotic pressure difference across an interface equal the Laplace pressure. For an elastic material such as a vesicle membrane, this is supported by the membrane tension:

$$\Delta P = (C_{in} - C_{out})RT = 2K\alpha/r \qquad (1)$$

Here ΔP is the osmotic pressure drop across the membrane, C_{in} and C_{out} are the solute concentrations on the vesicle inside and outside, respectively, R is the ideal gas constant, T is the absolute temperature, K is the membrane areal stretch elasticity, α is the instantaneous membrane areal strain (defined as $\Delta A/A_0$), and r is the instantaneous vesicle radius. The mechanism proposed for osmotically induced leakage states that vesicles swell, leak, and then reseal at equilibrium.[8,10] At low osmotic gradients, vesicles swell with water but maintain internal solutes. Performing a volume balance on the swelled vesicle allows the relation to the vesicle's initial state:

$$\frac{V_0}{V}C_{in,0} - C_{out} = \frac{2K\alpha}{rRT} \qquad (2)$$

where V_0 is the initial vesicle volume (i.e., at zero applied gradient), V is the instantaneous volume, and $C_{in,0}$ is the initial vesicle interior concentration. Allowing for vesicle leakage and casting the equation in dimensionless form gives

$$\frac{V_0}{V}S + \frac{C_{in,0} - C_{out}}{C_{in,0}} = \frac{2K\alpha}{rC_{in,0}RT} + 1 \qquad (3)$$

where S is the fraction of solutes that remains in the vesicle after equilibration, as defined in the Experimental Section. With this equation, one must only predict the vesicle size (i.e., V and α) after vesicle leakage and resealing to predict leakage as a function of membrane material properties.

One such prediction was put forth by Hallett et al.,[8] who suggested that vesicles swell until they reach their critical areal strain. Once this strain is reached, the vesicles remain at a constant size while releasing internal contents until mechanical equilibrium can be achieved. Incorporating this into eq 2 results in the equation

$$\frac{S}{1 + \frac{3\alpha_c}{2}} + \frac{C_{in,0} - C_{out}}{C_{in,0}} = \frac{2K\alpha_c}{rC_{in,0}RT} + 1 \qquad (4)$$

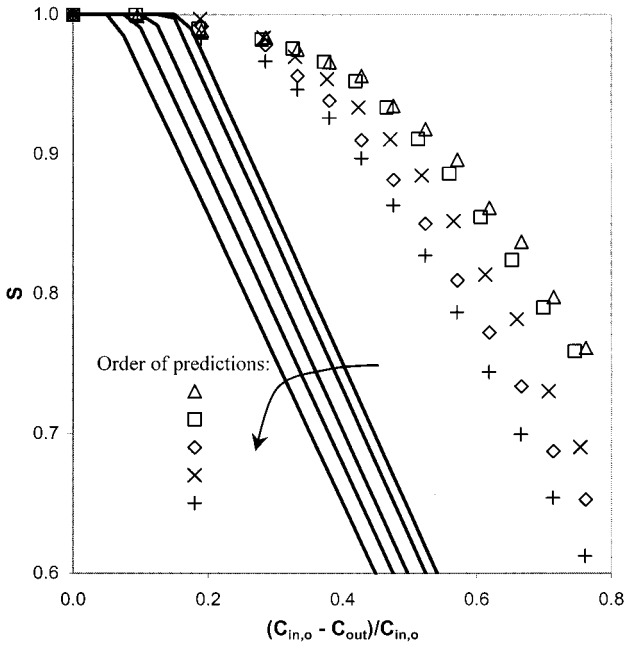

Figure 3. Comparison between experimental data (shown by symbols) and model predictions (shown by lines) for vesicle leakage profiles. Symbols correspond to DOPC (triangles), SOPC (squares), DL_2PC (crosses), POPC (diamonds), and DL_3PC (pluses).

where α_c is the critical areal strain. Note that this assumes small α_c, which is verified by micropipet measurements (see Table 1). Because the vesicle radius once leakage begins is constant (rendering the right-hand side of the equation constant), there should be a linear relationship between the leakage amount and the applied osmotic gradient. One may indeed use a linear function to fit the large-gradient experimental data in Figures 2 and 3. We note here, however, that the data appear to show some curvature and might, in fact, be fit by a more complicated nonlinear function (see below).

According to eq 4, osmotic leakage profiles may be predicted if membrane mechanical properties are known. It is generally accepted that the micropipet aspiration method pioneered by Evans and Needham[20] offers the most accurate measurement of vesicle mechanical properties. Values for the elastic modulus for all lipids used here[13,21] and critical areal strain for all lipids other than POPC[12] have previously been determined using this technique. However, the elasticity of POPC, reported as 227 ± 47 mN/m,[21] is suspect; POPC is similar in composition to egg PC, for which elasticities of 140−167 mN/m have been reported.[22,23] We therefore performed micropipet experiments on POPC vesicles and find that the elastic modulus is 178 ± 7 mN/m and the critical areal strain is 0.037 ± 0.006. Based the improved agreement with egg PC and reduced error (measured here by the standard deviation), our POPC elasticity is deemed the correct value and will be used hereafter.

Model predictions using the micropipet mechanical property values from Table 1 are shown in Figure 3 along with experimental results for each lipid. There is a general qualitative agreement between the predicted order of leakage (DL_3PC > DL_2PC > POPC > SOPC > DOPC) and the experimentally observed order (DL_3PC > POPC > DL_2PC > SOPC > DOPC), with the small discrepancy in the order of DL_2PC and POPC. This suggests that the model possesses some validity. However, there is clearly very poor quantitative agreement

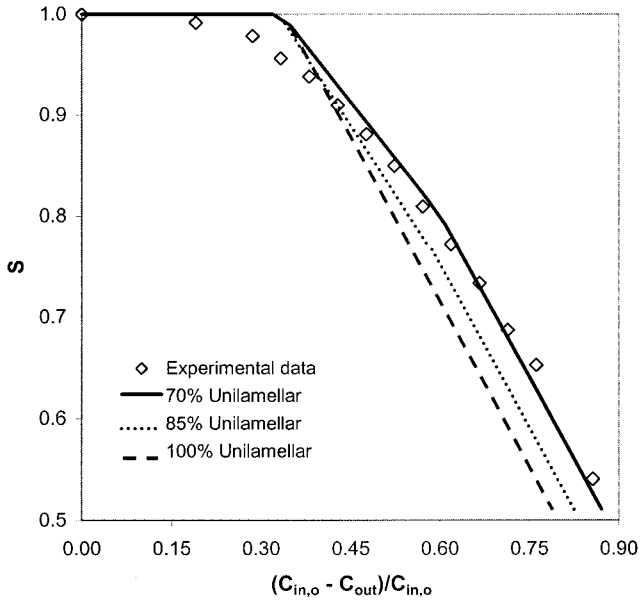

Figure 4. Experimental (shown by symbols) and predicted (shown by lines) leakage for POPC vesicles using the two-population model and mechanical properties estimated using light scattering. Model predictions are shown for 100% unilamellar, 85% unilamellar/15% bilamellar, and 70% unilamellar/30% bilamellar vesicles.

between the predictions and the data, demonstrating the poor ability of the current model to predict osmotically induced vesicle leakage.

One possible improvement to this model has been offered in which the effects of vesicle polydispersity are addressed.[8] This correction, however, is a minor one that cannot satisfactorily improve the fit to our data (data not shown). A more viable reason for the poor model fit rests on the applicability of micropipet mechanical results. The vesicles used in the micropipet experiments are ~200 times larger than the ones used in osmotic leakage experiments. While it is the usual assumption that vesicle properties are independent of vesicle size, some studies have found that elasticity decreases with increasing vesicle radius in the nanometer-sized regime.[4,9,24] We have performed preliminary light scattering experiments that indicate that the elastic modulus for our 100 nm POPC vesicles is approximately 560 mN/m, compared to the value of 178 mN/m we report using micropipet aspiration. The relationship between elasticity and vesicle size, or perhaps more appropriately, vesicle curvature, is not well understood and is currently under investigation in our laboratory.

We examined the possibility that the model does not agree with the experimental data because the micropipet-derived mechanical properties used as inputs are invalid. The use of K and α_c values commensurate with our preliminary light scattering results indeed shift the predicted leakage curves toward the experimental data (see the dotted line in Figure 4). This suggests that micropipet values of elasticity are not valid for nanometer-sized vesicles, and much larger values such as those from the light scattering experiments are more appropriate. However, the predicted curve and experimental data still show poor agreement. Alternatively, we may use eq 4 to determine vesicle mechanical properties through fitting the linear portion of experimental leakage profiles. Least-squares fits for all lipids studied result in physically unreasonable results such as negative values for critical areal strains. Other possible problems with the model must therefore also be considered.

The leakage model assumes that the vesicles are unilamellar and initially spherical. Studies using cryo-transmission electron microscopy (cryo-TEM) have shown that vesicles created using extrusion are nearly spherical in shape.[15,25] However, studies based on an entrapped volume assay[10] and light scattering[26] reported that vesicles created using extrusion are not perfect spheres; instead, they are "flattened disks" that have less than optimal surface-to-volume ratios. This implies that they may accept a significant amount of water at constant area before membrane dilation is necessary, simply by adopting a more spherical shape. While introduction of this possibility into the model does result in model predictions superficially shifting toward experimental data, it also increases the difference between the slopes of the two curves. This results in critical areal strains found by curve fitting to become even more negative, and hence more unrealistic.

Similarly, there have been conflicting reports as to the unilamellar nature of vesicles created using high-pressure extrusion. Both cryo-TEM data[15] and ^{31}P NMR studies[8,15] have shown vesicles created to be essentially unilamellar. However, using freeze-fracture electron microscopy, Monnard et al.[25] found that POPC vesicles made by extrusion were largely a mixed population of unilamellar and bilamellar vesicles. The percentage of bilamellar vesicles was set by the salt concentration in the vesicular solution and was as high as 36%.

We have thus modified the existing leakage model to account for leakage from vesicles with mixed populations of unilamellar and multilamellar membranes. Populations are each assumed to leak independently and respond to osmotic stresses as described by eq 4. As shown by micropipet aspiration, multilamellar vesicles have elasticities that approximately scale by their number of lamellae (i.e., for egg PC vesicles with one bilayer, $K = 140$ mN/m, while vesicles with two bilayers have $K = 227$ mN/m[22]). On the basis of earlier discussion, we suspect the mechanical properties found using the micropipet to be invalid for 100 nm vesicles and therefore must rely on light scattering estimates. Because we currently have light scattering data only for POPC, at present we will restrict ourselves to modeling this lipid. As a first approximation, we assume that our vesicles consist of two groups: unilamellar vesicles with an elasticity of 560 mN/m and bilamellar vesicles with an elasticity of 1120 mN/m. Both populations are assumed to have critical areal strains of 0.047 as derived from light scattering. Because the results of Monnard et al.[25] do not extend to the high salt concentrations used here, we will treat the ratio of the two populations as a fitting parameter.

Figure 4 shows the comparison of the experimental results for POPC with predictions of the two-population model. A population of 70% unilamellar and 30% bilamellar vesicles gives good agreement with the experimental data. In addition, we see that the possible origin of the curvature of the experimental leakage data noted earlier is simply from the linear combination of the two independent populations, with an accompanying discontinuity. We do note, however, that, as seen in Figure 4, the model predictions are sensitive to the population percentages, making independent determination of these parameters critical for rigorous model testing. Once these parameters are available, the two-

population model should permit the quantitative prediction of osmotically induced leakage of vesicles of any composition. This information should ultimately assist in the development of lipid membranes with well-tuned release properties as well as shed light on the molecular basis for the effect of changing the lipid composition on membrane behavior.

Conclusions

While osmotically forced leakage has been the subject of previous studies, the importance of the lipid composition as a controlling factor has been ignored. In this paper, we present the first systematic study of osmotic leakage while varying the lipid composition and show that indeed a significant effect is seen. Additionally, we have shown that an existing model for the leakage phenomenon is insufficient and introduce an improved model that accounts for the possibility of a mixed vesicle population. Finally, preliminary results suggest that while the micropipet method is a valuable source of membrane mechanical information, the properties found might not be applicable to nanometer-sized vesicles such as those commonly used in swelling studies.

Dedication

This tribute to John Quinn gives me (T.K.V.) a chance to express my deepest thanks to my mentor and friend. John took me under his wing (big wing that it is!) when I started my academic career at the University of Pennsylvania. His support, inspiration, and insights—on scientific as well as life matters—have been invaluable to me. John and I co-advised two University of Pennsylvania Ph.D. students (Conor Hanley and Kevin Girard), who worked on membrane applications of Langmuir−Blodgett films, a field that John had pioneered some 20 years prior. In classic Quinn form, our work involved simple yet elegant experiments; it also served as a catalyst for much of my current research on the properties of lipid membranes. John often claims that the secret to success is simply to surround yourself with good people and not get in their way. The real truth is that people have become good by being around John, who, in his own inconspicuous manner, shows them the way.

Acknowledgment

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Literature Cited

(1) Finkelstein, A. *Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes*; Wiley: New York, 1987; Vol. 4.

(2) Morris, C. Mechanosensitive Ion Channels. *J. Membr. Biol.* **1990**, *113*, 93.

(3) Ahkong, Q.; Lucy, J. Osmotic Forces in Artificially Induced Cell-Fusion. *Biochim. Biophys. Acta* **1986**, *858*, 206.

(4) Li, W.; Aurora, T.; Haines, T.; Cummins, H. Elasticity of Synthetic Phospholipid Vesicles and Submitochondrial Particles During Osmotic Swelling. *Biochemistry* **1986**, *25*, 8220.

(5) Needham, D.; Zhelev, D. The Mechanochemistry of Lipid Vesicles Examined by Micropipette Manipulation Techniques. In *Vesicles*; Rosoff, M., Ed.; Marcel Dekker: New York, 1996.

(6) de Haas, K.; Blom, C.; van den Ende, D.; Duits, M.; Mellema, J. Deformation of Giant Lipid Bilayer Vesicles in Shear Flow. *Phys. Rev. E* **1997**, *56*, 7132.

(7) Dobereiner, H.; Kas, J.; Noppl, D.; Sprenger, I.; Sackmann, E. Budding and Fission of Vesicles. *Biophys. J.* **1993**, *65*, 1396.

(8) Hallett, F.; Marsh, J.; Nickel, B.; Wood, J. Mechanical Properties of Vesicles. II. A Model for Osmotic Swelling and Lysis. *Biophys. J.* **1993**, *64*, 435.

(9) Hantz, E.; Cao, A.; Escaig, J.; Taillandier, E. The Osmotic Response of Large Unilamellar Vesicles Studied By Quasi-Elastic Light-Scattering. *Biochim. Biophys. Acta* **1986**, *862*, 379.

(10) Mui, B.; Cullis, P.; Evans, E.; Madden, T. Osmotic Properties of Large Unilamellar Vesicles Prepared by Extrusion. *Biophys. J.* **1993**, *64*, 443.

(11) Rutkowski, C.; Williams, L.; Haines, T.; Cummins, H. The Elasticity of Synthetic Phospholipid Vesicles Obtained by Photon-Correlation Spectroscopy. *Biochemistry* **1991**, *30*, 5688.

(12) Olbrich, K.; Rawicz, W.; Needham, D.; Evans, E. Water Permeability and Mechanical Strength of Polyunsaturated Lipid Bilayers. *Biophys. J.* **2000**, *79*, 321.

(13) Rawicz, W.; Olbrich, K.; McIntosh, T.; Needham, D.; Evans, E. Effect of Chain Length and Unsaturation on Elasticity of Lipid Bilayers. *Biophys. J.* **2000**, *79*, 328.

(14) Murga, M.; Bernik, D.; de Valdez, G.; Disalvo, A. Permeability and Stability Properties of Membranes Formed by Lipids Extracted from Lactobacillus Acidophilus Grown at Different Temperatures. *Arch. Biochem. Biophys.* **1999**, *364*, 115.

(15) Hope, M.; Bally, M.; Webb, G.; Cullis, P. Production of Large Unilamellar Vesicles by a Rapid Extrusion Procedure—Characterization of Size Distribution, Trapped Volume and Ability to Maintain a Membrane-Potential. *Biochim. Biophys. Acta* **1985**, *812*, 55.

(16) Angelova, M.; Soleau, S.; Meleard, P.; Faucon, J.; Bothorel, P. Preparation of Giant Vesicles by External AC Electric Fields. Kinetics and Applications. *Prog. Colloid Polym. Sci.* **1992**, *89*, 127.

(17) Longo, M.; Waring, A.; Hammer, D. Interaction of the Influenza Hemagglutinin Fusion Peptide with Lipid Bilayers: Area Expansion and Permeation. *Biophys. J.* **1997**, *73*, 1430.

(18) Benachir, T.; Lafleur, M. Osmotic and pH Transmembrane Gradients Control the Lytic Power of Melittin. *Biophys. J.* **1996**, *70*, 831.

(19) Paula, S.; Volkov, A.; Van Hoek, A.; Haines, T.; Deamer, D. W. Permeation of Protons, Potassium Ions, and Small Polar Molecules Through Phospholipid Bilayers as a Function of Membrane Thickness. *Biophys. J.* **1996**, *70*, 339.

(20) Evans, E.; Needham, D. Physical Properties of Surfactant Membranes: Thermal Transitions, Elasticity, Rigidity, Cohesion, and Colliodal Interactions. *J. Phys. Chem.* **1987**, *91*, 4219.

(21) Troiano, G.; Stebe, K.; Raphael, R.; Tung, L. The Effects of Gramicidin on Electroporation of Lipid Bilayers. *Biophys. J.* **1999**, *76*, 3150.

(22) Kwok, R.; Evans, E. Thermoelasticity of Large Lecithin Bilayer Vesicles. *Biophys. J.* **1981**, *35*, 637.

(23) McIntosh, T.; Advani, S.; Burton, R.; Zhelev, D.; Needham, D.; Simon, S. Experimental Tests for Protrusion and Undulation Pressures in Phospholipid Bilayers. *Biochemistry* **1995**, *34*, 8520.

(24) Sun, S.; Milon, A.; Tanaka, T.; Ourisson, G.; Nakatani, Y. Osmotic Swelling of Unilamellar Vesicles by the Stopped-Flow Light-Scattering Method—Elastic Properties of Vesicles. *Biochim. Biophys. Acta* **1986**, *860*, 525.

(25) Monnard, P.; Berclaz, N.; Conde-Frieboes, K.; Oberholzer, T. Decreased Solute Entrapment in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine Liposomes Prepared by Freeze/Thaw in the Presence of Physiological Amounts of Monovalent Salts. *Langmuir* **1999**, *15*, 7504.

(26) Jin, A.; Huster, D.; Gawrisch, K.; Nossal, R. Light Scattering Characterization of Extruded Lipid Vesicles. *Eur. Biophys. J. Biophys. Lett.* **1999**, *28*, 187.

(27) Silvius, J. *Lipid−Protein Interactions*; John Wiley & Sons: New York, 1982.

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