# Influence of Lipid Chemistry on the Osmotic Response of Cell Membranes: Effect of Non-Bilayer Forming Lipids

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The cell membrane can withstand significant osmotic stress before pores open to relieve the tension. The amount of stress that the membrane can withstand is determined by the lipid chemistry; we have examined the effect of four non-bilayer forming lipids on the stability of phosphatidylcholine (PC, a bilayer forming lipid) membranes. Large unilamellar vesicles were subjected to hypo-osmotic gradients and the leakage of their contents was quantified. Three micelle forming lipids, lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and lysophosphatidylglycerol (LPG), were studied along with one inverted-micelle forming lipid, phosphatidylethanolamine (PE). The inclusion of the lyso lipids resulted in greater leakage from the vesicles, while the inclusion of the PE resulted in less leakage from the vesicles. From the leakage data, the pressure difference at the point of pore closure was determined along with the critical surface tension and the line tension. Results will be presented detailing how the shape and percentage of non-bilayer forming lipids affects leakage.

## Introduction

Lipids provide the primary support structure for the cell membrane, assembling into two opposed leaflets known as the lipid bilayer. The lipids in the cell membrane possess a wide range of hydrocarbon chain lengths and polar headgroups. To shield the hydrophobic tails from water, lipids assemble into aggregated structures; the bilayer structure adopted by lipids in cell membranes is one of several possible aggregate structures.[1−6] The particular aggregate structure that a given lipid (or any amphipathic molecule) will adopt depends on the volume and length of the hydrophobic tails with respect to the area of the polar headgroup.[1,2] Examples of several aggregated structures are shown in Figure 1.

Many of the lipids found in cell membranes are non-bilayer forming lipids. The role of these lipids is not entirely understood, but it is thought that their incorporation is necessary for a number of cellular functions, including vesicle fusion, vesicle budding, and channel gating.[7−10] Vesicle fusion and budding both involve mechanical changes out of the plane of the bilayer, while channel gating involves mechanical changes in the plane of the bilayer. At the same time, the inclusion of non-bilayer forming lipids must not detract from the primary function of the lipid bilayer: to serve as a permeability barrier. An understanding of how this balance is struck between facilitating secondary functions, yet not destroying the membranes' primary function, is largely lacking. To gain a more global insight into how lipid shape affects membrane function, hypo-osmotic gradients were used to osmotically stress large unilamellar vesicles. The use of osmotic gradients provides a mechanism with which to perturb membranes in a systematic fashion; by analyzing the response of membranes containing differing amounts of non-bilayer forming lipids, their effect on structure, stability and function can be addressed. In this paper, the membranes studied always contained phosphatidylcholine (PC),

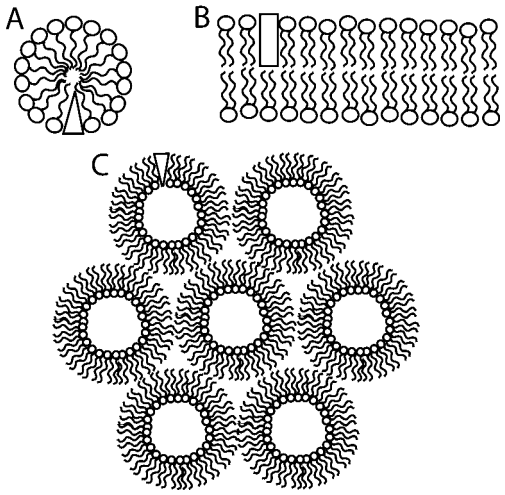


**Figure 1.** Schematic illustrations of the aggregate structures that amphiphilic molecules can adopt: (A) micelle, (B) bilayer, and (C) inverted micelle.

a bilayer forming lipid. Three micelle forming lipids, lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylglycerol (LPG), and one inverted-micelle forming lipid, phoshatidylethanolamine (PE), were included into the PC membranes in varying amounts.

Lipid bilayers typically form closed shells, referred to as either vesicles or liposomes, which range in diameter from 20 nm to several microns. When vesicles are placed into a hypo-osmotic environment, they expand due to the incoming water. The need to shield the hydrocarbon chains from water places a limit on how far the vesicles can stretch; past a certain point pores will open up in the membrane.[11−22] Pore formation has a twofold effect: it decreases the average area per lipid, shielding the hydrocarbon tails from water, and it allows the impermeable solutes inside the vesicle to escape, reducing the osmotic pressure across the membrane. The pores close when the osmotic

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pressure is reduced to the point that it is balanced by the Laplace pressure and a stationary state with no net flux of water across the membrane is established.[22] Results will be presented that show that the inclusion of micelle forming lipids lowers the concentration difference at which pores close and that the inclusion of inverted-micelle forming lipids has the opposite effect. By determining the concentration difference at the point of pore closure, it is possible to calculate the tension of the stretched membrane and the line tension of the pore.[22] Using a very simple assay, we will show that the values we calculate for membrane and line tension are in good agreement with those previously reported in the literature for similar systems.[14,15,17,23,24]

## Experimental Methods

**Material.** Chloroform stock solutions of L-α-phosphatidylcholine from egg yolk (eggPC), L-α-phosphatidylethanolamine made by transphosphatidylation of egg lectin in the presence of ethanolamine (eggPE), 1-palymitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC), 1-palymitoyl-2-hydroxy-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (LPG), and lysophosphatidylethanolamine from egg (LPE) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and were used without further purification. The (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) (HEPES), Sephadex G-50, and Triton X-100 detergent were purchased from Sigma Chemical Co. (St. Louis, MO). The 5-(and-6)-carboxyfluorescein was purchased from Molecular Probes (Eugene, OR). The carboxyfluorescein (CF) buffer was composed of 100 mM carboxyfluorescein, 700 mM NaCl, and 50 mM HEPES; it was adjusted to pH 7.4 with 1550 mM NaOH. An iso-osmotic buffer was composed of 750 mM NaCl, 50 mM HEPES; it was adjusted to pH 7.4 with 1550 mM NaOH.

**Vesicle Preparation.** Large unilamellar vesicles (LUVs) were prepared by extrusion. Briefly, mixtures of different lipids at appropriate molar ratios in chloroform were dried under nitrogen and held under vacuum for 1 h; the dried lipids were resuspended in the 100 mM CF, 700 mM NaCl, 50 mM HEPES buffer. The lipid suspension was then extruded through polycarbonate membranes with 50 nm pores a minimum of 15 times. Following extrusion, the LUV solution was centrifuged for 5 min at 14 000 rpm (Epenndorf Minispin Plus). The solution was then run down a Sephadex G-50 size exclusion column, equilibrated with iso-osmotic buffer, to remove any CF not encapsulated in the vesicles.

**Vesicle Stressing Assay.** This assay is similar to one in the literature.[13,15,18] The iso-osmotic buffer was diluted with a 50 mM HEPES (adjusted to pH 7.4 with 50 mM NaOH) solution to create the buffers used for stressing the vesicles. To osmotically stress the vesicles, 30 or 50 $\mu$L of the vesicle solution was placed into a cuvette and 1970 or 1950 $\mu$L, respectively, of a lower osmolarity buffer was added. The solution in the cuvettes sat for 5 min before measurements were made. When the CF escapes from the vesicles, it dequenches and the rise in fluorescence can be used to quantify the amount of leakage. Previous papers have shown that the solutes are expelled indiscriminately.[13,15,18] The fluorescence was measured on a Jobin Yvon Fluorolog-3 (excitation $\lambda = 491$ nm, emission $\lambda = 519$ nm) equipped with a single excitation monochromator and dual emission monochromators. The amount of vesicle leakage was calculated with the following equation:

$$\text{Leakage (\%)} = 100 \times \left(\frac{F_{\text{signal}} - F_{\text{baseline}}}{F_{100\%} - F_{\text{baseline}}}\right)$$

where $F_{\text{baseline}}$ is the fluorescence of the vesicles placed into an iso-osmotic environment, $F_{\text{signal}}$ is the fluorescence of the stressed vesicles, and $F_{100\%}$ is the fluorescence after vesicle destruction by the addition of Triton X-100. The experiments were done at room temperature and repeated several times. For all compositions studied, the $F_{100\%}$ and $F_{\text{baseline}}$ values were very similar. This indicated that neither the encapsulation efficiency nor the leakage before stressing changed with composition.

**Dynamic Light Scattering.** A Coulter N4 dynamic light scattering system was used to determine the radii of the extruded vesicles. Data were fit using a cumulant analysis and the results were consistent with a monodisperse population. The vesicles were measured, in equiosmolar solutions, to have radii of ∼45 nm; the vesicles containing PE were generally measured to be ∼0−5% larger than the vesicles containing lyso lipids. However, the error in the measurements was large enough as to make it unclear whether the observed variation was significant. The measured radii seem on the large size given that the pores are 50 nm in diameter. There are two possible explanations: First, dynamic light scattering is not very sensitive to small objects and is highly sensitive to large objects; therefore, despite our best efforts to make sure no large vesicles are present a tiny fraction could skew the results. Second, it has been shown that extrusion produces vesicles that are larger than the pore size and that the smaller the pore the larger the difference.[25,26] As vesicles extruded by hand through 100 nm pores have measured radii that are ∼1.5 times the pore radii,[25,26] we expect that vesicles extruded through 50 nm pores to have radii of at least 37 nm.

## Results

**Inclusion of Micelle Forming Lipids.** In Figure 2, we show leakage versus external osmolarity for varying concentrations of LPC, LPG, and LPE in eggPC (phosphatidylcholine from egg). Vesicles were made by extruding through 50 nm pores, as detailed in the Experimental Methods; the internal contents comprised 100 mM carboxyfluorescein, 700 mM NaCl, and 50 mM HEPES at pH 7.4. In all three plots, the leakage from 100 mol % eggPC vesicles (black squares) is shown. If the concentration difference across the membrane is not large, then the Laplace pressure balances the osmotic pressure and there is no net flux of water; this can be seen in the data where zero leakage is observed at low osmotic differences. When the Laplace pressure is exceeded the vesicles swell, pores open, and the entrapped salt and carboxyfluorescein escape. As described in the Experimental Methods, leakage can be quantified by measuring a rise in the fluorescence. It can be seen that the incorporation of lyso lipids into the eggPC vesicles always results in greater leakage from the vesicles. Error bars are not shown in these plots, as they are the same size as the markers.

Figure 2a shows the incorporation of LPC. It was impossible to form vesicles containing 40 mol % or greater of LPC. The addition of 10 mol % LPC results in a significant jump in the leakage as compared to 100 mol % eggPC; additionally, the vesicles begin to leak at smaller pressure differences across the membrane. The addition of 20 and 30 mol % continues the trend toward more leakage and pores opening at smaller pressure differences. The effect of the incorporation of LPG is shown in Figure 2b. Vesicles containing 50 mol % or greater of LPG could not be formed. As in the case of LPC, the addition of LPG results in greater leakage from the vesicles. Compared with LPC, however, the incorporation of LPG is slightly less of a perturbation. For example, it is necessary to go to 20 mol % LPG to see the amount of leakage seen with 10 mol % LPC.

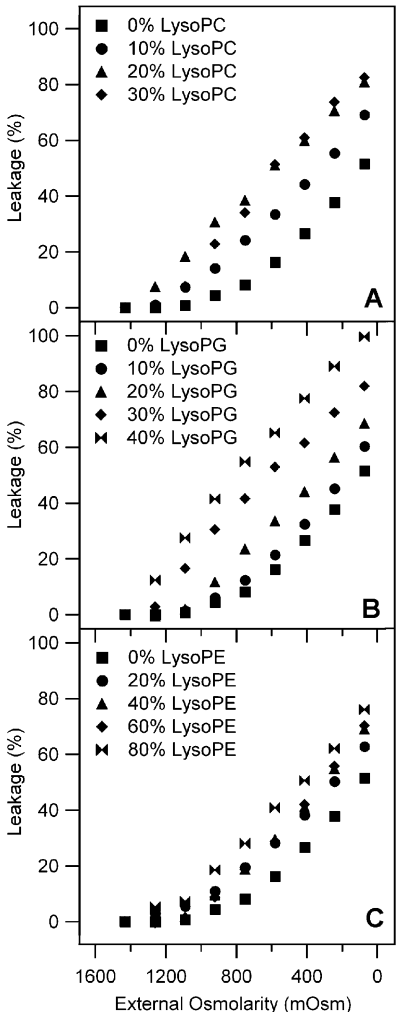


**Figure 2.** Effect of lipid composition on the leakage from vesicles subjected to hypo-osmotic gradients. Results from eggPC vesicles containing increasing amounts of (A) LPC, (B) LPG, and (C) LPE are shown.

Interestingly, when 40 mol % LPG is incorporated, 100% leakage of the contents is observed. The incorporation of LPE is shown in Figure 2c; it can clearly be seen that LPE can be incorporated to a much greater extent than either LPC or LPG. Vesicles containing 100 mol % LPE would not pass through the size exclusion column; it has been previously observed that vesicles containing 100% PE headgroups will aggregate at physiological pH, rendering them unusable for our experiments.[27] As with LPC and LPG, the addition of LPE results in vesicles that expel a greater percentage of their contents. However, this percentage is not nearly as great as it is for LPC and LPG. The LPC and LPG were both of a single chain length, 16:0. The LPE, on the other hand, was mixed 16:0 and 18:0.

**Inclusion of Inverted-Micelle Forming Lipids.** In Figure 3, we show leakage plots for differing PC/PE mixtures; 50 nm vesicles with a starting internal composition of 100 mM carboxyfluorescein, 700 mM NaCl, 50 mM HEPES at pH 7.4, same as for the lyso lipid/PC mixtures. The PE used for these experiments was made from the transphosphatidylation of egg lectin in the presence of ethanolamine; therefore, both the PC and PE contain the same tail distribution. Error bars are shown only on the 80 mol % eggPE/20 mol % eggPC mixture; they are of a similar magnitude on the rest of the mixtures. The inclusion of PE significantly reduces the amount of leakage from the vesicles. PE can aggregate in the inverted micelle structure,

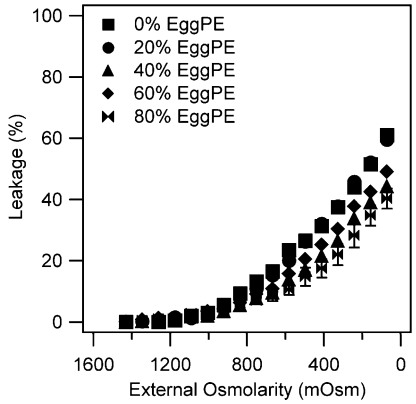


**Figure 3.** Effect of lipid composition on the leakage from vesicles subjected to hypo-osmotic gradients. Results from eggPC vesicles with increasing amounts of eggPE are shown.

making it a non-bilayer forming lipid; as such, it was initially surprising that its inclusion would result in vesicles that expelled smaller, and not larger, fractions of their contents. As with the 100 mol % LPE, we had difficulty getting 100 mol % PE vesicles through the column and consequently were unable to do experiments with them.

## Discussion

When vesicles are subjected to hypo-osmotic gradients, they swell to a new volume as water rushes in; the expansion of the volume is resisted by the intermolecular interactions between the lipids. As the vesicles swell the membrane thins; water penetrates deeper and deeper until ultimately a water bridge is formed across the membrane and a pore is formed.[21] The contents of the vesicles are freely expelled from the inside of the vesicles through the pore and the stress is gradually reduced. Our results show that, depending on the material properties of the membrane, differing amounts of the contents will leak out before pore closure. In a recent paper, Levin and Idiart[22] showed that the concentration difference across the membrane, $C_{\text{diff}}$, at the point of pore closure can be related to the membrane surface tension by the following relation:

$$C_{\text{diff}} \approx \frac{2\sigma_c}{10^3 k_B T N_A R_o} \tag{1}$$

where $\sigma_c$ is the critical tension of the stretched membrane, $k_B$ is the Boltzmann constant, $N_A$ is Avegadro's number, and $R_o$ is the unstretched vesicle radius. Assuming that the barrier to pore opening is on the order of $k_BT$, the line tension can be calculated from the surface tension:

$$k_B T \approx \frac{\pi\gamma^2}{\sigma_c} \tag{2}$$

where $\gamma$ is the line tension. Therefore, to determine the critical surface tension and the line tension, we need to first determine the concentration difference across the membrane upon pore closure (It is an open debate as to whether one pore is open the entire time or if pores flicker open and close. In any event, pore closure in this context refers to the point where leakage stops.). The data were fit as follows.

The initial concentration inside the vesicle is given by $C_{\text{in,o}}$, the initial volume by $V_o$ and the outside concentration by $C_{\text{out}}$. As the vesicle swells, the concentration inside the vesicle

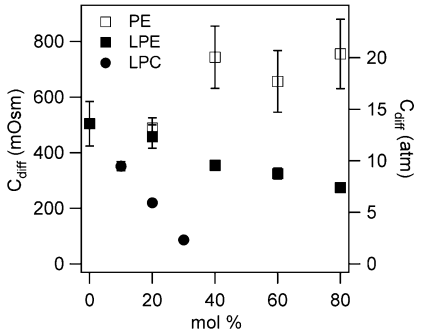


**Figure 4.** Effect of lipid composition on pore closure. The pressure differences across the membrane at the point of pore closure, $C_{diff}$, is plotted for eggPC with increasing amounts of non-bilayer-forming lipids.

changes to some instantaneous concentration given by $C_{in}$. At some point a pore opens and contents begin to leak. The concentration inside the vesicle at that point is given by

$$C_{in,o}\frac{V_o}{V} \tag{3}$$

where $V$ is the internal volume at the point of rupture. The contents leak until

$$C_{in} - C_{out} = C_{diff} \tag{4}$$

where $C_{diff}$ is the concentration difference across the membrane at the point when leakage stops; at that point the concentration inside the vesicle is $C_{in}$. The fraction of contents that leak, $L$, can be determined by subtracting the current concentration, $C_{in}$, from the concentration at the point of leakage and dividing by the concentration at the point of leakage:

$$L = \frac{C_{in,o}\frac{V_o}{V} - C_{in}}{C_{in,o}\frac{V_o}{V}} \tag{5}$$

which can be further rearranged to the following form:

$$L = \left(1 - \frac{C_{diff}V}{C_{in,o}V_o}\right) + \left(-\frac{V}{C_{in,o}V_o}\right)C_{out} \tag{6}$$

The equation is of the same form as that of Hallett et al..[13] Instead of solving for the surface compressibility modulus as they do, we solve for $C_{diff}$, the pressure difference across the membrane at the point of pore closure. This equation indicates that there is a linear relationship between the percent leakage and the external osmolarity. Due to the fact that the vesicles are not all of exactly the same size, the data in Figures 2 and 3 do not immediately become linear upon leakage. The larger vesicles begin to leak first, giving rise to an initial curvature in the data. Once all of the vesicles have opened, the data fits, with high confidence, to a straight line (For a monodisperse population of vesicles the distribution of sizes is roughly symmetric about the average value; therefore, the larger vesicles roughly average out the smaller vesicles.). The data in Figures 2a, 2c, and 3 were fit using this model and the calculated values for $C_{diff}$ are shown in Figure 4. We were unable to fit the LPG data, Figure 2b; this will be discussed below. Using 45 nm for $R_o$, the critical surface tension of the membrane and the line tension of the pore can be calculated; the results are given in Table 1. Our dynamic light scattering experiments indicate that there may be some variation in vesicle size with composition, the PE containing vesicles being slightly larger than the lyso containing vesicles. These variations would cause the calculated values for PE containing vesicles to be slightly larger; conversely, they would cause the calculated values for the lyso containing vesicles to be slightly smaller. Thus, if anything, the observed tends will be further accentuated.

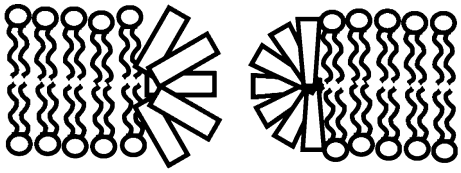

**Figure 5.** Schematic illustrations of lipids bending around in a pore to shield the hydrophobic tails from water. Two-tailed lipids are represented on the left side and one-tailed lipids on the right.

**TABLE 1: Material Property Values Determined from Leakage Data: $C_{diff}$, Pressure Difference Across Membrane at the Point of Pore Closure; $\sigma_c$, Critical Surface Tension; $\gamma$, Line Tension of Lipid Pore**

| membrane composition | $C_{diff}$ (mOsm) | $C_{diff}$ (atm) | $\sigma_c$ (mN/m) | $\gamma$ (pN) |
|---|---|---|---|---|
| eggPC | $500 \pm 80$ | $12 \pm 2$ | $28 \pm 4$ | $6.0 \pm 1.2$ |
| 10 mol % LPC | $350 \pm 20$ | $8.5 \pm 0.4$ | $19 \pm 1$ | $5.0 \pm 0.5$ |
| 20 mol % LPC | $220 \pm 10$ | $5.3 \pm 0.1$ | $12.2 \pm 0.3$ | $4.0 \pm 0.3$ |
| 30 mol % LPC | $86 \pm 4$ | $2.1 \pm 0.1$ | $4.8 \pm 0.2$ | $2.5 \pm 0.3$ |
| 20 mol % LPE | $460 \pm 40$ | $11 \pm 1$ | $25 \pm 2$ | $5.7 \pm 0.9$ |
| 40 mol % LPE | $350 \pm 10$ | $8.6 \pm 0.2$ | $20 \pm 1$ | $5.0 \pm 0.4$ |
| 60 mol % LPE | $330 \pm 20$ | $7.9 \pm 0.5$ | $18 \pm 1$ | $4.8 \pm 0.6$ |
| 80 mol % LPE | $270 \pm 10$ | $6.7 \pm 0.2$ | $15.2 \pm 0.4$ | $4.4 \pm 0.4$ |
| 20 mol % PE | $490 \pm 40$ | $12 \pm 1$ | $27 \pm 2$ | $5.9 \pm 0.8$ |
| 40 mol % PE | $740 \pm 110$ | $18 \pm 3$ | $41 \pm 6$ | $7.3 \pm 1.4$ |
| 60 mol % PE | $660 \pm 110$ | $16 \pm 3$ | $36 \pm 6$ | $6.9 \pm 1.4$ |
| 80 mol % PE | $760 \pm 120$ | $18 \pm 3$ | $42 \pm 7$ | $7.4 \pm 1.5$ |

The measured critical surface tension, $\sigma_c$, of eggPC is $28 \pm 4$ mN/m and the measured line tension, $\gamma$, is $6.0 \pm 1.2$ pN. Measurements of a variety of different lipids with a variety of different techniques consistently find that the line tension of a lipid pore is $\sim 1-20$ pN;[14,17,23,24] our values are right in that range. Chernomordik et al.[23] measured both the critical surface tension and line tension of eggPC and eggPC/LPC mixtures; those experiments, however, were done in the presence of decane, making it difficult to compare our values. To ascertain whether our measured values are reasonable, we have looked at two more compositions for which surface and line tension values exist in the literature. Using a similar osmotic stressing assay, the critical tension of a 55 mol % eggPC/45 mol % cholesterol mixture was determined to be 40 mN/m,[15] while using our method we determined the critical surface tension of a 60 mol % eggPC/40 mol % cholesterol mixture to be 43 mN/m. This is in good agreement. With dynamic tension spectroscopy, the critical surface tension under fast loading conditions was determined to be 30 mN/m for dilinoleoyl (18:2) PC and the line tension was determined to be 6.2 pN;[24] we measured 25 mN/m and 5.8 pN respectively for dilinoleoyl (18:2) PC, again in good agreement.

For the lyso lipids, $C_{diff}$, and consequently $\sigma_c$ and $\gamma$, decrease as more of the one-tailed lipids are incorporated into the vesicles. To see why this is so consider the illustration in Figure 5. When pores open, the lipids curl around to shield the hydrophobic tails from water.[21] On the lefthand side of the illustration barrel-shaped lipids, i.e., two-tailed lipids, can be seen in the pore. On the righthand side of the illustration triangle-shaped lipids, i.e., lyso lipids, can be seen in the pore. It is immediately apparent that the triangle-shaped lipids are better accommodated

in the pore. Their inclusion therefore lowers the line tension; this has the consequence of stabilizing the pore and allowing a greater extent of the contents to leak from the vesicle. The inclusion of the each of the three lyso lipids results in different amounts of leakage, as would be expected, given that their headgroups areas are different: 62 Å$^2$ for PC,[28] 66 Å$^2$ for PG,[29] and 52 Å$^2$ for PE.[30,31] The larger the head the more triangular the lipid; consequently, one would predict that LPC and LPG would lower the line tension by roughly the same amount, while LPE would also lower the line tension but not to the same extent as LPC and LPG. This is what is experimentally observed.

The $C_{diff}$ values for LPC can be fit to a straight line that intercepts zero at 37 mol % LPC. This indicates that vesicles comprising greater than 37 mol % LPC (in eggPC) will not form, explaining why our attempts to create vesicles with 40 mol % LPC failed. Vesicles composed of 40 mol % LPG were observed to exhibit 100% leakage, Figure 2b; as one would then expect, our attempts to make vesicles with 50 mol % LPG failed. Unlike LPC and LPE, we were unable to fit the LPG data. The PG headgroup is negatively charged; as a consequence, the area is very sensitive to the salt concentration. In our assay, the salt concentration is different at each of the data points; therefore, the headgroup area and the shape of the lipid are also, making it impossible to fit the data to a straight line.

The inclusion of PE into the PC vesicles results in an increase in $C_{diff}$, and consequently an increase in $\sigma_c$ and $\gamma$. Two-tailed lipids lean more toward a barrel shape than a triangle shape, due to increased volume in the tail region. The volume and length of the tail region in eggPC and eggPE are identical (as we used eggPE made from eggPC, Experimental Methods), but the PE headgroup is significantly smaller than PC; PE sits right on the border between being a perfect barrel and an inverted triangle. As a consequence, the PE is not as well accommodated in the pore as PC is and its inclusion raises the line tension. Thus, the inclusion of inverted-micelle forming lipids results in vesicles that expel smaller amounts of their contents under osmotic stress. Interestingly, the line tension does not increase until 40 mol % PE is included. It has been speculated that the pore edge may be enriched or depleted in the inclusion lipids (as compared to their fraction in the vesicles as a whole).[12,14] The lack of change in line tension when 20 mol % PE is included indicates that the PE is in fact depleted from the pore. At the higher percentages of PE, it probably continues to be depleted relative to the bulk fraction, but we are unable to observe to what extent. Conversely, the lyso lipids are probably enriched in the pore, but again we are unable to observe if this is true. Further theoretical work will be carried out to determine quantitatively the extent of incorporation and depletion.

Recently Bouchard and co-workers[14,17] have made direct measurements in a series of elegant experiments of the effect of the inclusion of cholesterol and Tween-20 on the line tension of membranes. They observed that the inclusion of cholesterol, which has a similar shape to PE, causes pores to close more rapidly, while Tween-20, which has a similar shape to lyso lipids, decreases the line tension, allowing the pores to remain open longer. Their measurements were made using giant unilamellar vesicles bathed in a glycerol solution. To stress the vesicles they were illuminated with white light. This paper shows that similar information about line tension can also be gained using a very simple assay. Our results are in general agreement with the known changes in headgroup area between PG, PC, and PE. There are, however, many lipid chemistries for which it is not as easy to predict the effect they will have on membrane structure, organization, and function. Several examples include polyunsaturated tails, which are important for vision among other processes;[32,33] highly branched tails, which have been found in archaea that inhabit extreme conditions;[34,35] and the sphingosine linkage, which is thought to help drive the formation of lipid rafts.[36] The methods described in this paper provide a simple mechanism by which to tackle these important and outstanding questions.

## Conclusions

The effect of non-bilayer forming lipids on the osmotic response of cell membranes was examined in this paper. It was found that the inclusion of micelle forming lipids resulted in greater leakage from large unilamellar vesicles under hypo-osmotic conditions, while the inclusion of inverted-micelle forming lipids had the opposite effect. A method was developed to quantify the effect of inclusion and it was shown that this could be used to determine the critical surface tension of the membrane and the line tension of the lipid pore.

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