Differential Effects of Membrane Order on Membrane Permeability

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Phospholipid membranes segregate into lateral domains of liquid ordered (lₒ) and liquid disordered (l_d) phases when cholesterol and mixed species of lipids with saturated and unsaturated acyl chains are present. To examine membrane permeability and rate of vesicle rupture when in pure lₒ, l_d, pure, and mixed lₒ/l_d phases, lipids were prepared based on the ternary phase diagram of POPC, sphingomyelin, and cholesterol. These lipids were suspended with 2% methanol or carbonated aqueous (CM) and formed by extrusion at 4°C. Using a stopped-flow fluorimeter, changes in CF fluorescence were measured when lipids were exposed to sudden osmotic gradients, pH gradients, or 0.1% Triton. Acyl chain and phospholipid headgroup packing were assessed in all compositions with time-resolved measurements of DPH fluorescence lifetime and anisotropy. Water permeability was highest in the pure l_d phase, and a factor of more than 100 lower in the pure lₒ phase. Proton permeability was lowest in the pure l_d phase, and approximately five-fold higher in the lₒ phase. The rate of membrane rupture was higher in the pure l_d phase than in the pure lₒ phase, with inconsistent results in the coexistence region. Water permeability was found to correlate with acyl chain packing, decreased with increased membrane order. Proton permeability increased exponentially with increasing membrane order.

**RESULTS**

**Water Permeability**

The permeability coefficient of water through the membrane is plotted against both order as predicted by the ternary phase diagram and percent cholesterol in the membrane. There is a notable difference between the first two samples in the completely l_d phase, indicating that in this region there is a stronger correlation between percent cholesterol in the membrane than bulk membrane order.

**Proton Permeability**

Similar to the results seen with water permeability, there is a marked change in proton permeability while the membrane is in the completely l_d phase. Travelling through the coexistence region, there appear to be plateaus in the different intercoexisting regions. Once the membrane is in complete l_d phase, there is a marked increase in permeability with addition of cholesterol that indicates a stronger correlation to percent cholesterol.

**Vesicle Rupture by 0.1% TritonX-100**

The results of vesicles ruptured by Triton were the least consistent from trial to trial, but are notable in that there is a clear relationship between percent cholesterol and the rate of vesicle rupture. The results are most consistent within the coexistence region of the ternary phase diagram compared to percent cholesterol.

**DISCUSSION**

There is a clear relationship between permeability and ensemble membrane order indicated by anisotropy decay measurements of DPH. Water permeability coefficients found in this study were comparable to those found in previous studies (Gensure et al., 2004), decreasing when POPC in the membrane is replaced by PtdCho and Chol. Proton permeability was found to be much lower than that of previous studies, although the trend of increasing permeability with increased percentage of POPC was also seen by Gensure et al. This difference could be explained by a different methodology. In this study, a pH gradient mix was monitored for 250 seconds to ensure the system had come to equilibrium.

**REFERENCES**

4. Oregon Nanoscience and Microtechnologies Institute

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**ABSTRACT**

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**INTRODUCTION**

Particular lipids were chosen for this study that are commonly found in biological membranes. This study focuses on changes in membrane permeability with lipid composition. Previous work has shown changes in permeability with addition of cholesterol. These lipids have also been extensively studied as constituents of a ternary phase diagram (Fig. 1), believed to predict phase behavior based on percent composition. At 2°C, pure POPC membranes are in the liquid disordered (l_d) phase. At increased temperatures, addition of cholesterol and sphingomyelin, the membranes exhibit liquid ordered (lₒ) or solid (l_s) characteristics as shown in Figure 2.

**METHODS**

**Experimental Preparation**

Samples were prepared with POPC, PtdCho and CHOL. From Sigma-Aldrich. Lipid vesicles were prepared by mixing lipid suspensions with house buffer containing 2% methanol or carbonated aqueous (CM) and extruded with a luer lock needle using an Avanti mini-extruder at 65°C. Lyophilized samples were reconstituted with house buffer containing both carbon dioxide and methane (95:5), extruded with an luer lock needle using an Avanti mini-extruder at 65°C for two cycles. Samples were allowed to cool slowly to room temperature overnight. With house buffer containing 2mM carboxyfluorescein, freeze/thawed 4X, and extruded with an Avanti mini-extruder at 65°C for two cycles. Samples were allowed to cool slowly to room temperature overnight. Preparation of liposomes was confirmed by transmission electron microscopy.

**Flow Fluorimetry**

Exclusion Column Chromatography

Average liposome size was determined by using size-exclusion chromatography of 100% l_d PC, pure POPC membranes, 100% l_d PC, and mixed 50% l_d/l_s PC membranes with house buffer containing both carbon dioxide and methane (95:5), extruded with an luer lock needle using an Avanti mini-extruder at 65°C for two cycles. Samples were allowed to cool slowly to room temperature overnight. Preparation of liposomes was confirmed by transmission electron microscopy.

There is a clear relationship between permeability and ensemble membrane order indicated by anisotropy decay measurements of DPH. Water permeability coefficients found in this study were comparable to those found in previous studies (Gensure et al., 2004), decreasing when POPC in the membrane is replaced by PtdCho and Chol. Proton permeability was found to be much lower than that of previous studies, although the trend of increasing permeability with increased percentage of POPC was also seen by Gensure et al. This difference could be explained by a different methodology. In this study, a pH gradient mix was monitored for 250 seconds to ensure the system had come to equilibrium.