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Differential Effects of Membrane Order on Membrane Permeability

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ABSTRACT

Phospholipid membranes segregate into lateral domains of liquid ordered (Lo) and liquid disordered (Ld) 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, POPC, PC, sphingomyelin, and cholesterol were co-dissolved in a final 1:1:1 liposome ratio at 1% in water. Perturbation of the ternary phase diagram of POPC, sphingomyelin, and cholesterol. These LUVs were loaded with 2mM carboxylfluorescein (CF). Proton permeability was lowest in the pure Lo phase, and approximately five-fold higher in the Lo + Ld phase. The rate of membrane rupture was higher in the pure Ld phase than in the pure Lo phase, with inconsistent results in the coexistence region. Water permeability was found to correlate with acyl chain packing, decreasing 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 Lo 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 result seen with water permeability, there is a marked change in proton permeability while the membrane is in the completely Lo phase. Travelling through the coexistence region, there do appear to be plateaus in the differently interconnected regions. Once the membrane is in the complete Ld phase, there is a marked increase in permeability with addition of cholesterol that indicates a stronger correlation to percent cholesterol. Vescicle 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 are only the results that show consistent behavior in the 100% Lo and 100% Ld regions. In these regions, change in percent cholesterol does not significantly change the speed of vesicle rupture.

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 (Gemue et al., 1989). Proton permeability was found to be much lower than that of previous studies, although the trend of increasing permeability with percent cholesterol was also seen by Gemue et al. This difference could be explained by a difference in membrane composition. In this study, no pH gradient mix was monitored for 250 seconds to ensure the system had come to equilibrium.

CONCLUSIONS

1. There is a strong correlation between membrane permeability and the DPH anisotropy-derived order parameter S shown in Fig. 9; water permeability decreases with order and proton permeability increases with order.
2. Within the 100% Lo region of the ternary phase diagram, changes in percent cholesterol result in changes in water permeability (Fig. 6).
3. Within the 100% Lo and 100% Ld regions of the ternary phase diagram, changes in percent cholesterol result in changes in proton permeability (Fig. 7).
4. Within the 100% Lo and 100% Ld regions of the ternary phase diagram, changes in percent cholesterol do not affect the rate of vesicle rupture by Triton (Fig. 8).

REFERENCES


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