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DECREASING TEMPERATURE BELOW Tc OR INCREASING CHOLESTEROL ENHANCES VESICLE-FUSION BILAYER MEMBRANE FUSION

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ABSTRACT

Lipid composition plays an important role in fusion of vesicles to membranes, an essential process for exocytosis. Lipid head group, tail structure, and sterol content all impact the complex phase behavior of membranes. To determine the effect of lipids on fusion, we performed fusion assays on the nystatin/ergosterol (nys/erg) fusion assay and stimulated fusion with a sol (corticosterol) protein. Vesicles and erg fuses with a planar membrane producing characteristic spike increases in membrane conductance. Using PEPC (7:5) membranes, we varied cholesterol from 0-40 mol% and observed significant increases in fusion rates. In one series of experiments, membranes were formed with 0 mol% cholesterol, repainted with 20 mol%, then repainted with 0 mol%. The 20 mol% cholesterol showed a marked increase in fusion rates over both pre- and post-controls. Likewise, increased fusion rates were observed in DPPC/cholesterol (0:1) membranes under lowering temperature below the phase transition (Tc). These data are consistent with the liquid disordered phases observed in vesicle fusion, and shows how membrane fusion can be affected by lipid behavior.

INTRODUCTION

Lipid rafts have received much attention recently. Formation of rafts, or the general formation of lipid domains in such rafts, are likely important in the docking and fusion of vesicles to cell membranes. Such rafts form as specialized domains in cell membranes that differ from the surrounding lipids in cholesterol content as well as physical properties. These domains are understood to participate in various physiological functions. These domains, composed of specific lipid phases, are defined by the physical characteristics of membrane fluidity, lipid order, and lipid packing or spacing. In this study we examine fusion rates as a function of lipid phase. Lipid phase was changed by varying cholesterol content and temperature as shown in Figure 1. Our data support the hypothesis that changes in phase dramatically affect vesicle fusion.

METHODS

BILAYER MEMBRANE CHOLESTEROL ENHANCES VESICLE FUSION

We tested if adding cholesterol to the planar bilayer would alter fusion rates. First, a membrane containing no cholesterol was formed by “painting” lipids over a hole, then repainted as many variables as possible while maintaining constant; we used the same setup and repainted the membrane with lipids containing varying amounts of cholesterol. Finally, the membrane was repainted with the same lipids used before. In each condition, membrane capacitance and fusion rates were recorded. An example experiment with 0-20% cholesterol is shown in Figure 2. Although it is unlikely that each repainting totally replaces the original lipid composition, the observation that the final repainting lowered the fusion rate back to similar levels observed in the original conditions confirms that the majority of the lipids are replaced.

In experiments where temperature was changed, DPPC and (D)PC (10/90 mol%) bilayer membranes were formed. These membranes have a Transitions temperature, Tc, for each lipid composition. This allowed us to see the effects of lipid phase transitions on fusion rates.

Artificial vesicles were made containing nystatin and ergosterol. The nystatin-ergosterol complex forms in the vesicles that allow us to detect membrane current activation. The nystatin/ergosterol current was monitored while changing the membrane temperature below Tc. Artificial vesicles were made containing nystatin and ergosterol. The nystatin-ergosterol complex forms in the vesicles that allow us to detect membrane current activation. The nystatin/ergosterol current was monitored while changing the membrane temperature below Tc.

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PHYSICAL PROPERTIES OF MEMBRANE REACTIONS

CONCLUSIONS

Fusion of vesicles to planar bilayers is a model of exocytosis and would be expected to be altered by lipid properties of both membranes. Cholesterol and temperature both affect lipid properties and determine lipid phase. Holding sterol in the vesicle membrane constant (~25% ergosterol), the cholesterol and temperature of the bilayer was varied. We observed that:

• Cholesterol (10-40 mol%) in the bilayer greatly enhanced fusion rates of vesicles (Figure 3).
• Lowering the temperature below Tc (41°C for DPPC) also greatly enhanced fusion rates (Figure 4).
• These data are consistent with the liquid disordered phase (Ld) inhibiting vesicle-membrane fusion (Figure 1).
• The correlation between lipid phase and vesicle fusion may provide a role for lipid rafts in vesicle fusion.

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REFERENCES