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DECREASING TEMPERATURE BELOW Tt OR INCREASING CHOLESTEROL ENHANCE VESICLE-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 utilized the nystatin/ergesterol (nys/erg) fusion assay and stimulated fusion with a salt (osmotic) gradient. With this assay, vesicles containing nys and erg fuse with a planar membrane producing characteristic spike increases in membrane conductance.Using PE/PC (7:3) 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 membrane showed a marked increase in fusion rates over both pre- and post- controls. Likewise, increased fusion rates were observed in DPPC/cholesterol (9:1) membranes upon lowering temperature below the phase transition (T_t). These data are consistent with a liquid disordered lipid phase suppressing 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 present in such rafts, are likely important in the docking and fusion of vesicles to a cell membrane. 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 affects vesicle fusion.

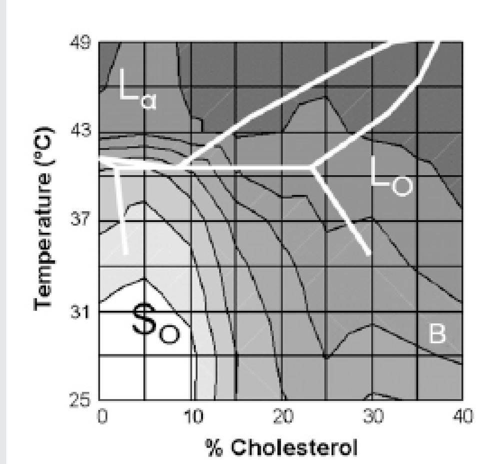

Figure 1. Effects of cholesterol and temperature on nystatin fluorescence intensity (Wilson-Ashworth, 2006). The characteristic change from a solid-ordered (S_o) to a liquid-disordered (L_α) or liquid-ordered (L_o) phase at 41 C of DPPC lipids is modified by cholesterol content.

METHODS

An planar lipid bilayer was made separating two chambers in saline buffer. Vesicles were allowed to fuse to the membrane with varying amounts of cholesterol. The composition of the target membrane consisted of POPE and POPC (7:3 ratio) dissolved in decane with 0-40mol% of Cholesterol. An osmotic gradient (750:300mOmM) was formed and vesicles allowed to fuse spontaneously with the planar membrane. Fusion rates were compared for different amounts of cholesterol.

In experiments where temperature was changed, DPPC and Chol (10mol%) bilayer membranes were formed. These membranes have a Transitions temperature, T_t, of ~41C and are in a gel phase at room temperature. 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 form channels in the vesicles that allow us to detect exocytosis by measuring the bilayer current when the vesicles fuse. This electrical current is measured as “spikes” on a graph depicting current (pA) versus time, and directly count each fusion event.

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 in the bilayer chamber. To be sure as many variables as possible remained 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 0-20-0% 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.

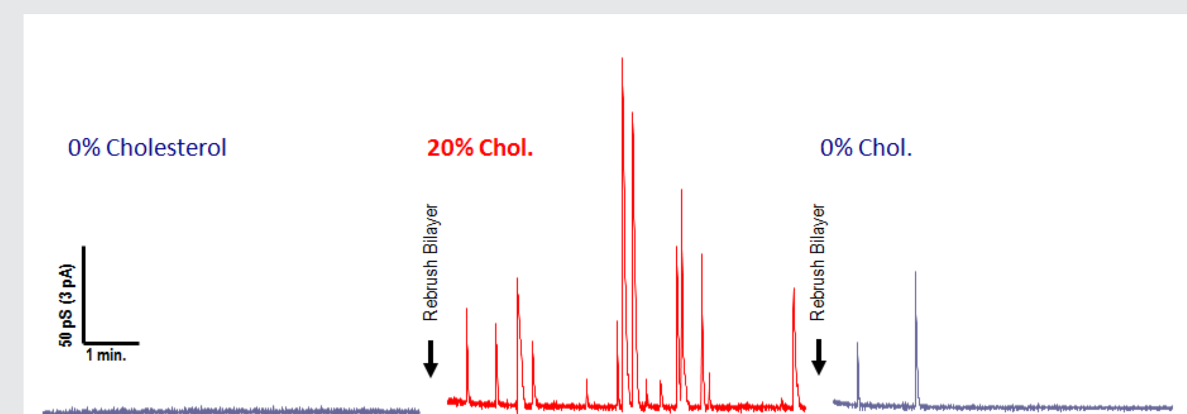

Figure 2. Example traces from our lipid bilayer fusion assay. Nystatin/Ergosterol fusions are shown in a target membrane containing 0mol% cholesterol, repainted with 20mol% cholesterol, and then repainted with 0mol% cholesterol.

A series of experiments were performed with the middle cholesterol content changing from 10% to 40%. In these experiments, the addition of cholesterol always increased fusion rates dramatically compared to no cholesterol (see **Figure 3A**). However, these experiments did not let us determine the difference in fusion rates between two membranes containing differing amounts of cholesterol. Therefore, we also performed experiments starting with some cholesterol and then rebrushing with higher cholesterol. **Figure 3B** shows the first of these experiments with 10-30-10mol% cholesterol. Although the higher cholesterol (30mol%) still increased fusion rates over the 10mol%, the increase was not as dramatic as compared to no cholesterol.

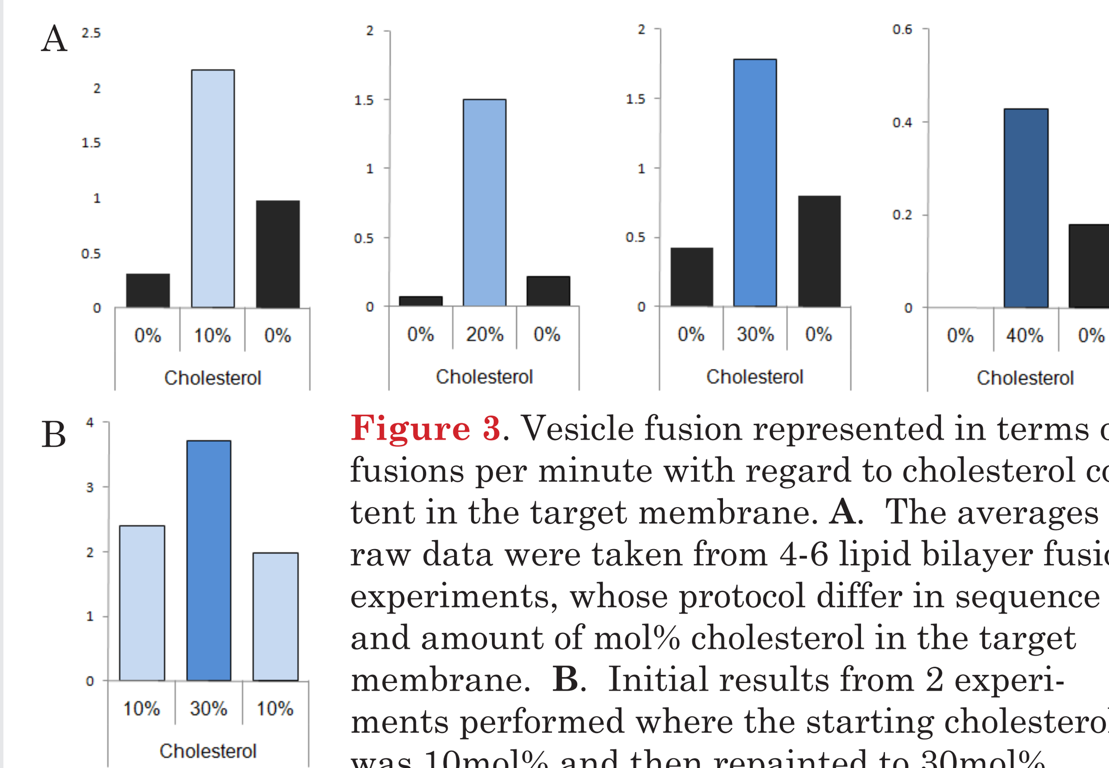

Figure 3. Vesicle fusion represented in terms of fusions per minute with regard to cholesterol content in the target membrane. **A.** The averages of raw data were taken from 4-6 lipid bilayer fusion experiments, whose protocol differ in sequence and amount of mol% cholesterol in the target membrane. **B.** Initial results from 2 experiments performed where the starting cholesterol was 10mol% and then repainted to 30mol%.

PLANAR BILAYER RESULTS

To further test how lipid phases affect fusion rates we fused vesicles to DPPC:Cholesterol (9:1) bilayers both below and above the phase transition (T_t~41C). According to the phase diagram shown in **Figure 1**, lowering the temperature below T_t should shift the lipids from the liquid disordered (L_α) to the solid ordered (S_o) phase. As shown in **Figure 4**, fusion spikes were not observed above T_t (pink vertical bars), but clearly observed below T_t (white vertical bars). It is unknown why fusion rates increased with repeated cycling below T_t.

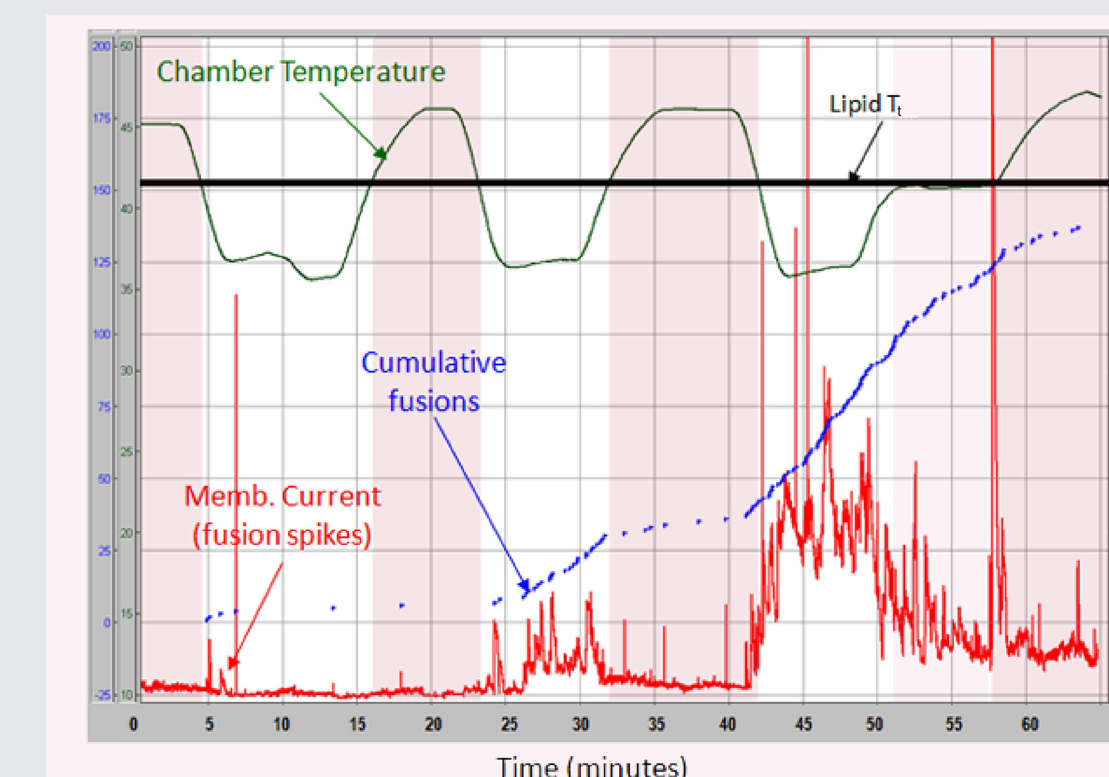

Figure 4. Observation of vesicle fusion (red spikes) is blocked by raising the temperature above T_m of the bilayer lipids. The blue points demarcate fusion events and highlights regions of increased fusion (rising slope). Note that higher temperatures (pink zones) correlate with reduced rates of observed fusion (flat red and blue lines). Vesicles are composed of PE:PC:PS:Erg and nystatin. Bilayer membrane is composed of DPPC + 10mol% cholesterol which has a gel-to-fluid transition at ~41 C (black line).

CONCLUSIONS

Fusion of vesicles to planar bilayer membranes 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 (10-40mol%) in the bilayer greatly enhanced fusion rates of vesicles (**Figure 3**).
- Lowering the temperature below T_t (41 C for DPPC) also greatly enhanced fusion rates (**Figure 4**).
- These data are consistent with the liquid disordered phase (L_α) 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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