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Self-Adhesion among Phospholipid Vesicles

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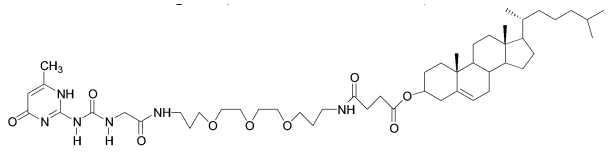
We have previously quipped that "life is a sticky business"...that substrate and enzyme, antigen and antibody, tRNA and mRNA, leukocyte and endothelial cell, bacteriophage and bacterium, and sperm and egg are all pairs that stick to each other.[1] The occasion for this remark was a study of giant vesicles that, via appropriately selected lipids or polymeric coatings, can be induced to adhere electrostatically.[1] Stickiness among bilayers also occurs when one vesicle population contains a donor while a second population contains an acceptor as with the following pairs: (a) adenine/uridine-substituted lipids;[2] (b) positive dendrimer/negative lipid;[3] and (c) barbituric acid-substituted lipids/2,4,6-triaminopyridine-[4] or adenine-substituted lipids.[5] Vesicles have likewise been made to cluster when ferrous ion[6] or avidin[7−9] is added to bilayers containing a terpyridine ligand or biotinylated lipid, respectively. Even DNA can bind to zwitterionic vesicles and promote their aggregation.[10] Specific membrane−membrane interaction that controls self-assembly and models cell−cell adhesion is clearly an active field of bioorganic chemistry and biophysics.[11]

Many studies of molecular recognition among membranes are based on two dissimilar vesicles. We, on the other hand, will describe vesicle adhesion as generated by a single membrane-bound adhesive agent (**1** or **2**, drawn below). All our vesicles, in other



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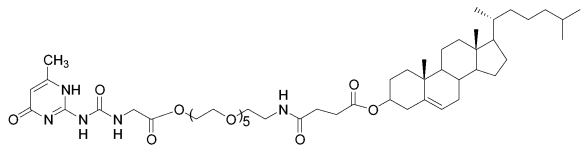
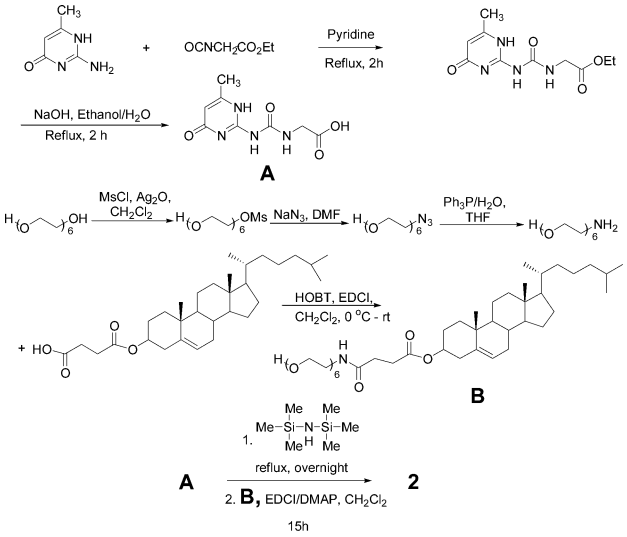


2

words, were identical in composition. Adhesion depended on the remarkable ability of the 2-ureido-4[1*H*]-pyrimidinone unit ("Upy") in **1** and **2** to dimerize via four strong hydrogen bonds.[12] Thus, its association constant in chloroform saturated with water is 1×10^7 M^{-1}.[12] Of course, the association constant in pure water, where all our vesicle experiments were carried out, should be less favorable than this. On the other hand, vesicles whose surface is covered with Upy units have the advantage with regard to their stickiness of being polyvalent. Multiple simultaneous interactions are well-known to enhance the associative process in both chemistry and biology.[13] And because our vesicles were comprised of only a single adhesive agent, inter- vs intravesicular competition for hydrogen bonding sites could be explored.

Adhesive agents **1** and **2** have three distinct sections: At one end lies a cholesterol derivative whose hydrophobicity serves to embed the molecules into the vesicle bilayer. At the other end is located the Upy unit that, as mentioned, hydrogen bonds with itself. Separating the two moieties is a hydrophilic spacer that can guide a vesicle-bound Upy into the water. The synthesis of **2** is given in Scheme 1. Both **1** and **2** were characterized by $^1H/^{13}C$ NMR, HRMS, and EA following chromatography on silica.

Scheme 1. Synthesis of Compound **2**



Vesicles were prepared by dissolving 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (16 mg) in 2 mL of chloroform. Evaporating the solvent by rotary evaporator under reduced pressure created a lipid film that was hydrated via a 10 min stirring with 10 mL of purified water. The resulting suspension was then extruded 19 times, back and forth, through a 100 nm pore Whatman Nuclepore membrane[14] to form vesicles with an average hydrodynamic diameter of 70 nm as revealed by dynamic light scattering (data collected for 10 min).

When POPC was admixed with compound **1** (95: 5 w/w) prior to film formation, we found it impossible to extrude the suspension through the 100 nm membrane. Applying considerable manual force to the syringes served only to cause the apparatus to leak. This was our first indication that compound **1** could indeed cross-link the bilayers and thereby impede flow through the membrane pores. We next added solid **1** to a suspension of preformed POPC vesicles, but **1** was not solubilized. Moreover, **1** was too insoluble in water-miscible solvents to permit the addition of **1** in a small amount of cosolvent to the POPC vesicles. This turn of events led us to synthesize **2**, which, with its longer and more hydrophilic spacer, dissolves in water at sufficient concentrations to admix with preformed POPC vesicles. Upon mixing the aqueous components, the opalescence of the vesicle suspensions became immediately apparent. Figure 1 shows the increase in turbidity at 400 nm as various amounts of **2** were added to 1 mM vesicular POPC. Since the total amount of light scattering is proportional to the mass of

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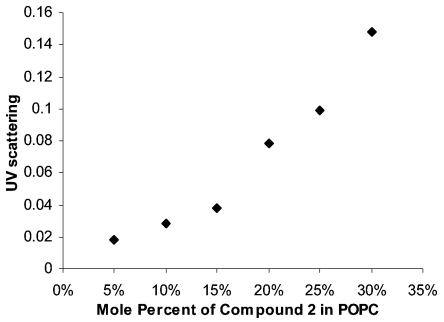

Figure 1. Change in UV scattering at 400 nm as various percentages of **2** were added to 1 mM POPC vesicles.

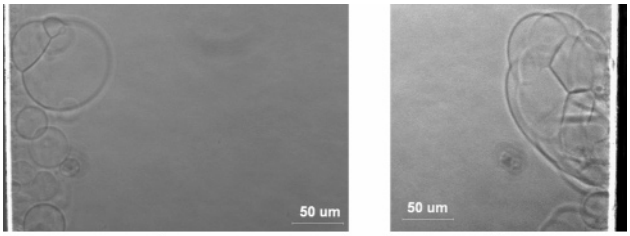

Figure 2. Light microscope pictures (phase contrast) of giant vesicles composed of (A) POPC + 5% **2** and (B) POPC + 10% **2**. The vesicles were formed on a Pt wire where a lipid film was subjected to 100 mV−10 V and 3−10 Hz.

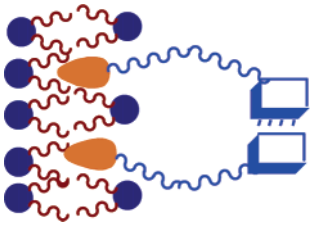

Figure 3. Schematic of intravesicular hydrogen bonding. Orange cone = steroid (inserted into a bilayer); blue rectangle = Upy. The average Upy/bilayer distance has not been established.

the particles squared,[15] this provided strong evidence that **2** was indeed functioning as an adhesive agent. It remained only to secure light and electron microscopy pictures to confirm this conclusion.

Giant vesicles, 30−250 μm in diameter and composed of POPC plus 5−30% of **2**, were prepared by an electroformation method described previously.[16] As seen in Figure 2, adhesions occur at 5% **2** and, to an even greater extent, at 10% **2**. But they are less common at 20% **2** and totally absent at 30% **2**. The simplest explanation is that when the vesicle loading reaches a level above 20%, pairs of adhesive agent are sufficiently close together that intravesicular hydrogen bonding (Figure 3) becomes feasible. Assuming a random distribution of the adhesive agent with a cross-sectional area equal to that of POPC, simple geometric considerations show mean distances between adhesion molecules of 4.3, 3.1, 2.1, and 1.7 nm at **2**/POPC percentages of 5, 10, 20, and 30, respectively. A 1.7 nm intermolecular distance at the vesicle surface apparently allows an intravesicular association that competes successfully with intervesicular adhesion.

Cryo-high-resolution scanning electron microscope pictures of POPC vesicles without **2** and with 10% **2** are shown in Figure 4A and B, respectively. Cryo methods were necessary so as not to involve water evaporation at high vacuum that would artifactually promote aggregation. The adhesive nature of the vesicles containing **2** is again evident. Adhered vesicles were also found using 30% **2**. It may be that the much greater curvature of the small extruded vesicles (and hence a greater Upy separation), relative to that of the giant vesicles, accounts for the difference in the ability to engage in intravesicular association.

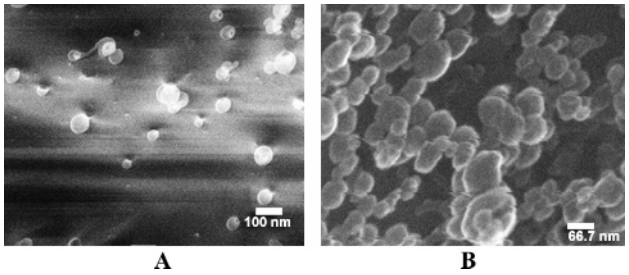

Figure 4. Cryo-HRSEM images of extruded POPC vesicles with (A) no added **2** and (B) 10% added **2**.

In summary, we have synthesized a compound that binds to a phospholipid bilayer via a hydrophobic steroid thereby projecting a strong multi-hydrogen bonding unit into the surrounding water. As shown by light scattering, light microscopy, and cryo-HRSEM, this latter unit self-adheres and induces membrane−membrane attachments, as found in many biological systems.

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Supporting Information Available: Synthetic details for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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