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Internally Catalyzed Separation of Adhered Lipid Membranes

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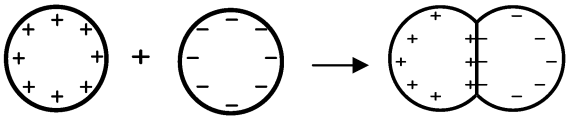
When a giant vesicle[1] composed of POPC[2] (rendered anionic with 5 mol % POPG[2]) touches a giant POPC vesicle (rendered cationic with 5 mol % of DDAB[2]), one sees under the light microscope an electrostatically driven adhesion (eq 1).[3] The bilayer adhesion endures for an afternoon. Accordingly, it is possible to investigate environmental factors that might eliminate the adhesive forces and, thereby, cause the vesicles to separate from each other. If the vesicles no longer stick to surfaces of correspondingly opposite charges, then it follows that the vesicles' charges have been deleted at some point prior to separation. Informative experiments on lipid membrane adhesion and its reversal were conceived on this basis.

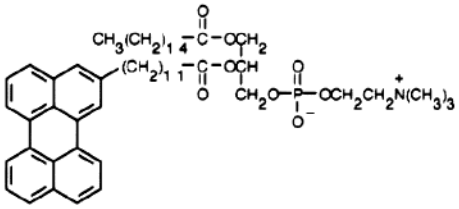


(1)

Physical principles underlying vesicle adhesion can be found in a variety of sources.[4−7] Interest in the subject no doubt stems in large part from cell−cell interactions that dominate many aspects of life.[8,9] To our knowledge, Silvius et al. were among the first to mix two populations of submicroscopic vesicles having opposite charge.[10] Aggregation, leakage, and lipid-mixing ensued. Jullien, Lehn, et al. inferred, mainly by fluorescence and NMR, that submicroscopic vesicles bearing opposite charges exchange lipids by direct contact.[11] MacDonald and Pantazatos observed, by fluorescence video microscopy, that giant vesicles of opposite charge fuse rapidly without leakage.[12] As will be seen, fusion of our giant vesicles also occurred but, more frequently, we observed reversal of the adhesion process caused by internal membrane dislocations. Whether separation of transiently adhered cells, common in biology, can operate by a similar mechanism has not yet been established.

Giant vesicles, 20−100 μm in diameter, were prepared in a water-filled electroformation cell[1] as follows. One Pt wire was coated with anionic lipid, while the second Pt wire was coated with cationic lipid. Application of an alternating current (3 Hz, 0.8 V) produced giant vesicles along both wires. A holding pipet was used to guide an anionic vesicle across the cell to a cationic vesicle still perched on the wire. Thereupon, the adhered vesicle pair was detached from the wire and moved to the center of the cell where adhesion duration times could be measured.

Our experiments rested upon one key observation: when 0.5 mol % perylene-substituted phospholipid **1** was incorporated into an anionic giant vesicle (94.5 mol % POPC and 5 mol % POPG), vesicle adhesion to a cationic vesicle (95 mol % POPC and 5 mol % DDAB) became transitory (Figure 1). The time that the vesicles of opposite charge remained in contact depended upon the concentration of **1**. Thus, as seen in Figure 2, the vesicles drifted apart in times ranging from 90 min at 0.1 mol % **1** to 5 min at 1.0 mol % **1**.



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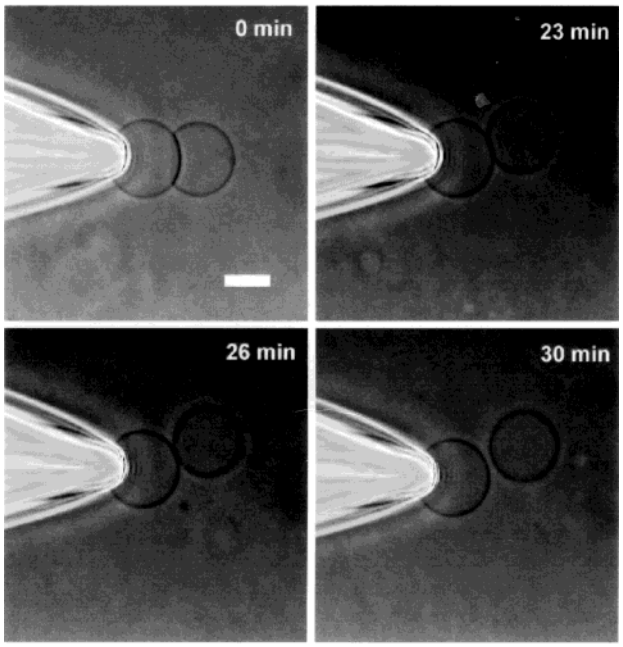

Figure 1. Adhesion between an anionic vesicle (held by a pipet) and a cationic vesicle as viewed by phase-contrast light microscopy (see ref 1). Since the anionic vesicle contains 0.5 mol % **1** (relative to total phospholipid), the adhesion becomes transient, and within 30 min the vesicles drift apart in the thermal currents. Bar = 20 μm.

It is important to emphasize that loss of vesicle adhesion in the presence of **1** is a dynamic process—membrane molecules reorganize in the second/minute time regime once two vesicles of opposite charge adhere. This reorganization subsequently induces vesicle departure. If the effect had been related to a static structural alteration in the anionic vesicle prior to its adhesion, this would have manifested itself as an immediate absence of adhesion. Note that four other substituted phospholipids (labeled with BODIPY, diphenylhexatrienyl, and rhodamine groups)[13] failed to catalyze reversal of the adhesion. Despite its low concentration, perylene's steric bulk seemingly disrupts, in a time-dependent fashion, the normal electrostatic attraction holding the vesicles together.

A clue to the mechanism of the adhesion reversal came from charge determinations. Thus, after a **1**-catalyzed separation of the two vesicles, the initially cationic vesicle was guided by micropi-

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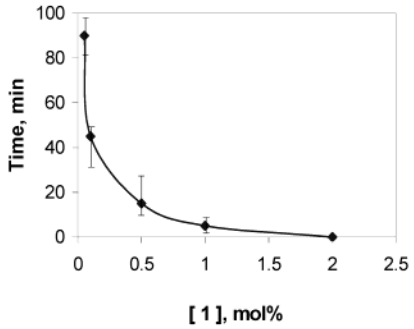


Figure 2. Time required for an anionic vesicle (93−95 mol % POPC, 5 mol % POPG, and 0−2 mol % **1**) to drift away from a cationic vesicle (95 mol % POPC and 5 mol % DDAB) as a function of the mol % of **1**. Data points are the average of several experiments, and the line is a visual guide.

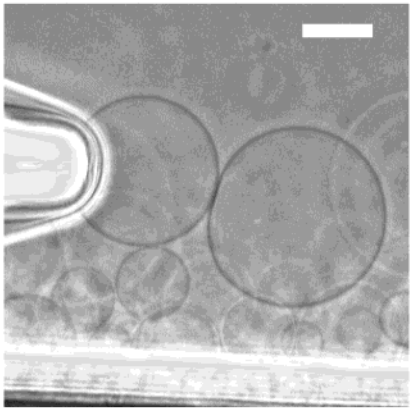
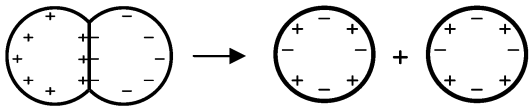


Figure 3. Phase-contrast photo showing that an initially cationic vesicle (held by the micropipet), which had drifted away from a **1**-loaded anionic vesicle, no longer adheres when pressed against an anionic vesicle, implying loss of cationic charge. Bar = 25 μm.

peting methods to an anionic vesicle. The two did not stick (Figure 3). Similarly, the initially cationic vesicle did not stick to an anionic vesicle. This absence of sticking was observed at many locations around the periphery of the two vesicles (not only at the former junction region). The conclusion is inescapable; the vesicles with 5 mol % of anionic POPG or cationic DDAB have lost electric charge responsible for the electrostatic attraction. Charge-loss catalyzed by **1** is substantial (>80%) because two vesicles with only 1 mol % POPG and DDAB, respectively, but without any **1**, retain their adhesion for over 2 h.

If **1** is placed in the cationic DDAB-loaded POPC vesicles, the anti-adhesive effect is even more efficient than when added to the anionic POPC/POPG vesicles. At 0.1 mol % **1** adhesion occurs, but it is followed by separation and drifting apart only 2−3 s later (constituting a 10^4-fold reduction in adhesion-time). If both the anionic and cationic vesicle membranes contain 0.5 mol % **1**, then the anionic vesicle tends to burst and coat the cationic vesicle, a process which was not further investigated.

One might imagine that membrane disruption caused by low levels of **1** can facilitate the relocation of cations and anions so as to form high ionic-strength patches ("rafts")[14] at the juncture region. Although possible, this would serve only to enhance electrostatic attraction, not destroy it. The simplest mechanism for the reversal in adhesion invokes an interchange of anions and cations (eq 2). The vesicles, having lost much of their charge, now drift apart. Perylene promotes this interchange by physically disrupting the tight membrane packing that ordinarily impedes intervesicular transport. It seems surprising that such a small concentration of perylene (as low as 0.1 mol % of the total lipid) can so radically affect the membrane dynamics. (The results should serve as a warning to those using membrane probes). One wonders if, similarly, living cells might produce low levels of membrane-bound lipid or protein that induce so-called "kiss-and-run" vesicle events by promoting the release of adhesive elements.[15]



(2)

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(2) POPC = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (from Avanti); DDAB = didodecyldimethylammonium bromide (from Fluka).
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(13) Molecular Probes: D-3803 = 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a, 4a-diaza-*s*-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (BODIPY FL C5-HPC); D-3805 = 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a, 4a-diaza-*s*-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphate, diammonium salt (BODIPY FL C5-HPA); D-476 = 2-(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-*sn*-glycero-3- phosphocholine (DPH HPC); T-1391 = *N*-(6- tetramethylrhodaminethiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TRITC DHPE).
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