Induced separation of a binate vesicle into two independent entities

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Two lipid vesicles, one residing in the aqueous interior of the other, separate into independent vesicles upon increasing the temperature or osmotic pressure; phase-contrast microscopy provides the details of the process.

Giant vesicles are spherical lamellar structures having diameters greater than one micrometer. Single giant vesicles can be isolated, manipulated and observed in real time by light microscopy. By contrast, small vesicles (so-called 'SUVs'), used conventionally in most vesicle research, are sub-microscopic and thus evaluatable in solution only by indirect spectroscopic means. Capitalizing upon this size advantage of giant vesicles, researchers have recently reported diverse membrane events ranging from cytomimetic phenomena[1] to surface reactions.[2]

Giant vesicles undergo a variety of morphological changes brought about by mechanical,[1] thermal,[3–5] chemical,[6–8] electrical[9] and pressure[10] alterations in the environment. Thermally-induced shape changes have, perhaps, garnered the most attention from the scientific community. For example, it was shown[5] that temperature affects the vesicle area-to-volume ratio, leading to budding and other shape transitions. Mathematical models explaining the various membrane phenomena are also available.[11,12]

Both natural phospholipids and synthetic lipids can self-asemble into giant vesicles. Didodecyldimethylammonium bromide (DDAB) is a good example of the latter. Use of 'unnatural' lipids is important because it provides a large array of compounds with which to relate membrane properties to molecular structure. We ourselves have employed DDAB, among various synthetic lipids, to observe vesicle aggregation, fusion, endocytosis and birthing.[1]

On occasion, a population of DDAB giant vesicles will contain a 'binate' vesicle that consists of one sphere within another of slighly larger size. Usually the components of the binate vesicle have a common area of contact as portrayed in Fig. 1.

When the system is subjected to heat or osmotic pressure, the binate vesicle separates into two independent vesicles. Since the initial and final surface areas and volumes of the vesicles can be quantified, one has a prime opportunity to monitor the translocation of lipid and vesicular water during the morphological change. Tracking the fate of components in model membrane systems is relevant to cell biology where membrane reorganization occurs in a controlled but poorly understood manner.

A few experimental details will clarify the procedure. DDAB and cholesterol were mixed by dissolving the solids in $CHCl_3$–MeOH and removing the solvent under reduced pressure. The resulting film was sonicated in deionized water and lyophilized to yield a white powder of intimately mixed lipids. Less than 0.1 mg of lipid mixture was smeared onto a glass microscope slide within the confines of a cemented 14 mm i.d. O-ring. Approximately 0.5 ml of deionized water was added to the sample, a glass coverslip applied, and the excess water drained. About 3 h of incubation at 20 °C produced a colony of giant vesicles.

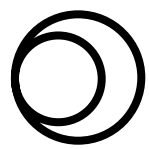

Fig. 1 Schematic representation of a binate vesicle

For heating experiments, the microscope slide was mounted on a hollow brass plate through which flowed water from a constant temperature bath. Temperatures were read from a thermocouple residing directly in the vesicle medium. Dilution experiments, in which the osmotic pressure was altered, were performed by taking up to 25–75 μl of vesicle suspension into a micropipet and gently releasing it into 0.5 ml of deionized water.

Vesicle transformations were observed with phase-contrast optics using a Nikon Diaphot-TMD inverted microscope. Images were recorded with the aid of a Dage-MTI CCD-72 solid-state camera connected in series to a Panasonic AG-1960 SVHS, a Hamamatsu Argus-10 image procesor, and a Sony black and white monitor. Image processing and radii determination from calibrated distances were accomplished using Image-Pro Plus software on a Micron Millenia workstation, while images were printed using a Tektronix Phaser 440 dye sublimation printer.

Fig. 2 shows the effect of heating a 95% DDAB–5% cholesterol vesicle from 20 to 29 °C. In Fig. 2(A), one sees a binate vesicle (adhered to a smaller single vesicle) with considerable apparent contact between the inner and outer bilayers (arrows). The outer vesicle seemingly 'peels' away from the inner vesicle [Fig. 2(B)–(E)] while assuming various non-spherical shapes in the process. In less than 1 min, two unattached spherical vesicles are formed [Fig. 2(F)].

Table 1 records the volumes and surface areas for vesicles in Figs. 2(A) and 2(F). Since the total volume of the two vesicles in Fig. 2(F) is 15.3 pl [compared to only 13.9 pl for the outer vesicle in Fig. 2(A)], 1.4 pl of water were incorporated into the system during the separation process. External water must have

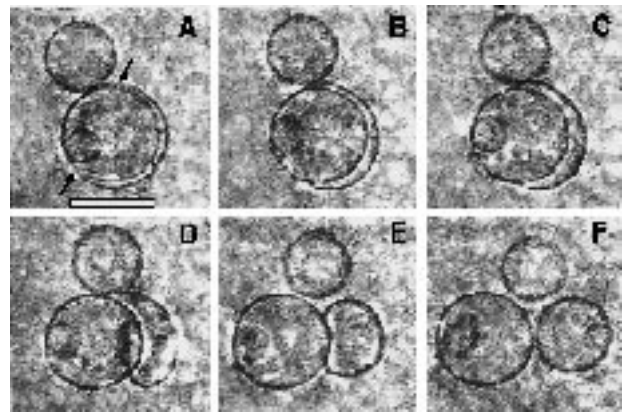

Fig. 2 Progressive separation of the outer shell of a binate vesicle induced by a temperature increase. Arrows in micrograph (A) define the region of membrane contact between the inner and outer vesicle before separation became evident. T = 29 °C raised from 20 °C; time from (A) to (F) = 1 min; bar = 25 μm.

Table 1 Vesicle radii, internal volumes and surface areas in Figs. 2 and 3[a]

	Before separation		After separation	
	Vesicle-1	Vesicle-2	Vesicle-1	Vesicle-2
Fig. 2				
Radius/μm	13.7	14.9	13.7	10.3
Volume/pl	10.7	13.9	10.7	4.6
Surface area/μm²	2360	2790	2360	1330
Fig. 3				
Radius/μm	24.4	27.8	23.5	24.1
Volume/pl	60.8	90.0	54.3	58.6
Surface Area/μm²	7450	9680	6940	7300

[a] Vesicle-1 represents the inner 'static' vesicle and vesicle-2 represents the outer 'active' vesicle.

entered the 'active' vesicle-2 exclusively because the volume of the 'static' vesicle-1 remained constant at 10.7 pl. Since the permeation rate of water through bilayers is generally much too slow to explain the observed influx,[13] it is likely that water enters vesicle-2 *via* an intervesicular gap as was observed previously in a laser-induced vesicle expulsion.[14]

Vesicle separation in Fig. 2 also led to a sizable decrease in the total surface area (from 5150 to 3690 μm² based on the assumption that the 'active' vesicle was originally an intact sphere). Even if the 'active' vesicle is taken to be an incomplete sphere [terminating at the arrows in Fig. 2(A)], there is a net decrease in lipid surface area of about 570 μm² as the new vesicle in Fig. 2(F) (of area 1330 μm²) is formed. One possible explanation is that lipid is deposited upon the 'static' inner vesicle by the 'active' outer vesicle. If this is true, then the deposited lipid must exist as a 'patch' on the surface of the 'static' vesicle because insufficient lipid was made available to coat the receptor vesicle entirely. Formation of bilayer patches residing on intact vesicles has been observed previously.[15]

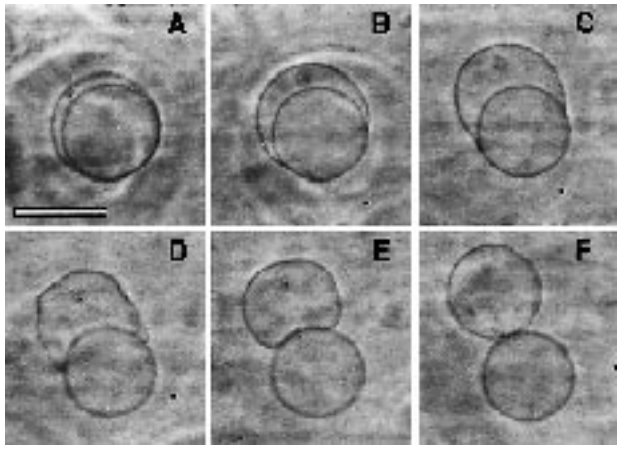

Fig. 3 Separation of a binate vesicle caused by dilution. Note the extreme undulation depicted in micrograph (D). T = 20 °C; time from (A) to (F) = 3 min; bar = 50 μm.

Lipid material could also have been lost to the bulk medium as sub-microscopic vesicles.[14]

An osmotic pressure change at constant temperature, created by dilution with deionized water, also induces vesicle separation (Fig. 3). Once again Table 1 reveals a volume increase requiring the input of external water. Thus, the total volume in Fig. 3(F) (112.9 pl) exceeds that of the outer periphery in Fig. 3(A) (90.0 pl). The need to supply external water is evident in Fig. 3(D) showing a flaccid and strongly undulating intermediate. Entry of water at narrow juncture gaps must be osmotically driven by monomeric DDAB and impurities that invariably exist in the inner vesicular regions.

Within the limits of uncertainty in the measurements, it appears that the surface area data are adequately explained by the two vesicles in Fig. 3 sharing a common membrane (*i.e.* the 'active' vesicle consisting of an incomplete sphere attached to an intact inner vesicle). Two-thirds of 9680 μm² plus a small osmotic swelling gives a value close of 7300 μm², the surface area found for the newly formed vesicle. The 7% volume decrease seen with the 'static' vesicle may reflect a compaction of bilayer lipid upon departure of the attached membrane.

Control experiments showed that vesicles composed of DDAB without cholesterol also manifested the separation phenomenon upon exposure to elevated temperatures and osmotic pressures. Thus, cholesterol-rich domains, if they exist at all, do not seem to play a role here in the observed membrane reorganization.

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Notes and References

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