Unbinding Transition of a Biological Model Membrane

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We have discovered a temperature-driven unbinding transition of fluid membranes in samples of digalactosyldiacylglycerol (DGDG) swollen in 0.1*M* NaCl aqueous solution. Single bilayers were observed by phase contrast light microscopy. The transition is reversible and without apparent hysteresis. Its temperature is only a few °C lower for the single membranes of two vesicles than for multilayer systems, but it varies strongly among samples. The bending rigidity of DGDG bilayers was also measured and found to be $(0.12-0.21)\times10^{-12}$ erg.

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Many of the lipids occurring in biological membranes have long been known to form giant vesicles, often unilamellar, when they are exposed to a large excess of water, provided their hydrated bilayers are in the fluid state.[1] This is true not only of electrically charged materials but also of neutral ones such as phosphatidylcholine (PC) and digalactosyldiacylglycerol (DGDG). The bilayers of all those lipids are frequently called biological model membranes.

Extensive microscopic studies of egg lecithin, a natural PC, have shown that vesicles also appear in NaCl solution, though very slowly at 0.5*M*, and that with or without salt the visibly undulating bilayers do not adhere to each other when coming into contact.[2] Later on, it was found that osmotic inflation of PC vesicles results in temporary membrane adhesion.[3-5] Occasional mutual adhesion, always associated with lateral tension, was also noted during the swelling process.[3-6] The tension manifests itself by weakening the undulations and was computed from the characteristic length ξ of a rounding of the membrane next to the contact area by use of the relationship $\sigma=k_c/\xi^2$, where k_c is the bending rigidity of the membrane. Apart from their scatter, the contact angles of adhesion appeared to be constant, the tensions ranging from 3×10^{-4} to 3×10^{-6} dyn cm^{-1}. They were around 40° for symmetric adhesion, i.e., two single membranes with equal contact angles, and up to 70° for the single membrane adhering to a bundle of others.

The separation of unstressed membranes in the face of van der Waals attraction has been assigned to their thermal undulations,[7] which also permits one to explain why lateral tension induces mutual adhesion.[4-6] In the case of unstressed model membranes, van der Waals and undulation forces are similar in strength so that a phase transition between a free and a bound state is conceivable. Recently, Lipowsky and Leibler[8] named it unbinding transition and predicted it to be continuous, in contrast to what may be naively expected. Their calculation employs a renormalization-group technique and is based on a realistic interaction potential including hydration forces.

In the following we report on the discovery of the first example of a membrane unbinding transition. The system is DGDG bilayers in NaCl solution and the agent is temperature. DGDG differs from PC by its polar head consisting of two sugar rings in a row. The material (extracted from whole-wheat flour) was purchased from Sigma (Munich) and used without further purification. No impurities, i.e., less than 1%, were present as judged from thin-layer chromatography. We spread some μg of material on an object slide, added about 40 μl of aeqeous solution, put a cover glass on top, and sealed the approximately 40-μm-high cell to prevent evaporation. All observations were made with a phase contrast microscope equipped with camera and video system. The object plane was usually in the middle of the sample cell, the depth of focus being ±1 μm. Membranes are visible where they are parallel to the optical axis in the object plane.

DGDG swelled in pure water at room temperature, forming within hours giant unilamellar vesicles and other large membrane structures. Well-developed thermal undulations showed the bilayers to be in the fluid state (which extends down to roughly −50 °C).[9] Adhesion was seen only together with lateral tension. The example in Fig. 1 represents a branched structure of single membranes merging one by one into bundles at mutually adhering membranes. Undulations are barely visible and contact roundings near the limit of optical resolution, which points to considerable tension. We found the contact angles to be 45°–60° for symmetric adhesion and about 85° for the single membrane adhering to a stack. Apart from the larger contact angles, DGDG in pure water behaved as egg lecithin.

Salt concentrations of 0.1*M* in most cases prevented DGDG from forming vesicles at room temperature. Within the first few hours the crystalline powder imbibed liquid to a certain extent, swelling slightly and smoothing its contours. However, giant vesicles and other lamellar structures developed after the temperature was sufficiently raised. Returning to lower temperatures resulted in spontaneous mutual adhesion at many places. The

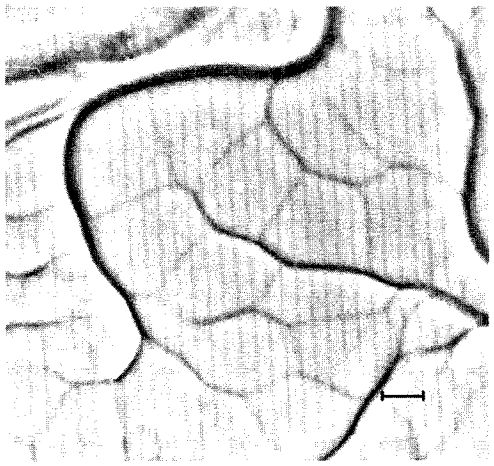

FIG. 1. Adhesion induced by lateral tension of single giant DGDG membranes in pure water. Note the merging of single membranes (thinnest lines) into pairs, triplets, and thicker bundles. The barely resolved contact roundings in the immediate vicinity of contact areas indicate lateral tensions on the order of 10^{-5} dyn/cm. The contact angles, made by the contact areas and the asymptotic directions of the free membrane, are seen to be 45° or more. The bar represents 10 μm.

process was reversible and apparently without hysteresis, occurring for a given structure in a temperature interval of less than 1 °C. The binding temperature varied strongly from sample to sample (and, to a lesser extent within samples). Even in identical experiments (DGDG from the same bottle, always 0.1M NaCl, equal glass slides) it scattered from 75 to 10 °C and was sometimes not found at all above the experimental limit of 6 °C. We will return to this point below.

Figures 2(a) and 2(b) show the binding transition in an array of eight parallel DGDG membranes. The first photograph [Fig. 2(a)] was taken at a temperature just above the transition. The membranes are separate and display thermal undulations. As the temperature is being lowered (about 0.1 °C/min), one sees adhesion of all eight membranes at first locally. While the adhesion spreads with a sharp front, the water between the membranes is driven into a small number of compact water pockets where the spreading seems to stop [Fig. 2(b)]. The single membranes of the water pockets are still undulating. The unbinding at rising temperature progressed less dramatically, the water returning in a diffuse way. Going up and down through the transition several times, we found its temperature to be the same within ± 0.5 °C, apart from a slow decrease with time of 1 °C in 2 d.

Figure 3(a) shows a somewhat distorted multilayer system of barely discernible DGDG membranes in NaCl solution just above the binding transition. A slight decrease of temperature resulted in the drastically different

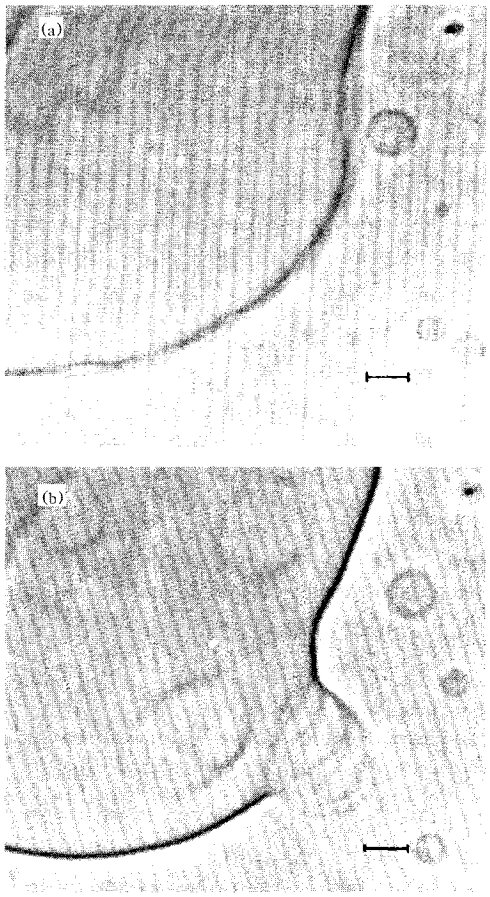

FIG. 2. Binding transition of eight membranes in 0.1M NaCl, (a) $T=22.8$ °C, above the binding temperature, and (b) $T=22.1$ °C, below the binding temperature. The adhesion squeezes the water between the membranes (a) into nearly spherical water pockets (b), but has little effect on single membrane undulations. The bars represent 10 μm.

structure of Fig. 3(b) displaying spontaneous adhesion. Although the membranes are seen to run from one bundle to another as in Fig. 1, there are striking differences from induced adhesion. They consist in strong undulations indicating very low lateral tensions and in the characteristic divergence from a point of the last eight (or six) membranes at the tips of the bundles. There are also important differences from the array shown in Fig. 2(b). While adhesion starts again with eight membranes, the total number of membranes is much larger. Moreover, the absence of adhesion involving fewer than eight membranes is unambiguous at most of the starlike brush ends. These and similar observations in other samples suggest that there is little difference in binding temperature between an infinite stack and a bundle of eight membranes. The linear growth of the bundles during cooling started at many places. A decrease of temperature by about 10 °C below the binding transition

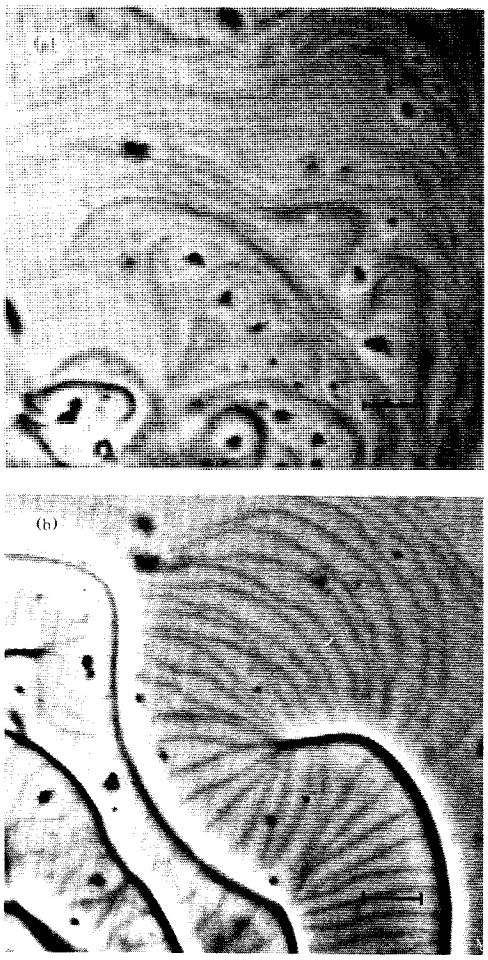

FIG. 3. Binding transition of a multilayer system in $0.1M$ NaCl, (a) $T=23.1\,^{\circ}C$, above the binding temperature, and (b) $T=22.4\,^{\circ}C$, below the binding temperature. The membranes are barely discernible above the transition (a), while single membranes (thin lines) and bundles indicating adhesion (thick lines) are seen below it (b). Fluctuations were strong on both sides of the transition. The bars represent 10 μm.

produced strong tensions and pair adhesion. The resulting patterns resembled that of Fig. 1.

We also observed reversible adhesive contacts of unilamellar and other vesicles. The binding transition of two single membranes seemed to be only a few °C below that of eight and more membranes. Immobile vesicles are not shown here because of a risk of artifacts caused by possible adhesion to the glass. The adhesive contacts of freely floating vesicles were rare and difficult to record.

It is surprising that the membranes bind in NaCl solution which is known to screen part of their van der Waals attraction.[10] On the other hand, NaCl has been found to reduce the repulsive hydration forces in multilayer systems.[11] Webb, Tilcock, and Green[12] reported that sonicated DGDG vesicles aggregate if pure water is exchanged for salt solution.

For a simple check of whether DGDG membranes may be expected to bind, we compare the energy of van der Waals interaction of two membranes in the half-space approximation

$$g_{\mathrm{vdW}} = -A_H/12\pi z^2$$

to that of undulation forces in a stack of membranes[7,13]

$$g_{\mathrm{und}} = 3\pi^2(k_BT)^2/128k_c\bar{z}^2.$$

Here z is a uniform and $\bar{z}$ is a mean spacing. The Hamaker constant A_H as measured by Marra[10] is 7.5×10^{-14} erg in pure water and 3.1×10^{-14} erg in $0.2M$ NaCl solution. We obtained the bending rigidity k_c from a Fourier analysis of the thermal undulations of very extended (≥ 100 μm) essentially straight membrane contours.[14] The measured values of k_c varied between 0.12×10^{-12} and 0.21×10^{-12} erg. Inserting the larger A_H and the largest k_c in the above equations and putting $z=\bar{z}$, one finds van der Waals attraction to be approximately 10% larger than repulsion by undulations, while repulsion comes out 4 times stronger than attraction for the lower bounds of the two parameters. Occurrence of a binding transition seems possible on the basis of this estimate which is in line with the result of the renormalization-group calculations.[8] Accordingly, the apparent absence of hysteresis may be taken to suggest that the binding transition of DGDG obeys the theory of Lipowsky and Leibler.[8] The variation of the binding temperature could then be attributed to that of the bending rigidity.

It has been argued elsewhere[5] that the large contact angles of induced mutual adhesion cannot be understood in terms of the statistical mechanics of undulations. To resolve the conflict, the membranes were postulated to be much rougher on a submicroscopic scale, i.e., to contain a larger area reservoir, than is to be expected on the basis of their undulations. A model of the suspected roughness of electrically neutral biological model membranes has been proposed.[15] The conjectures and their origin, with a first mention of the binding transition of DGDG, were summarized previously.[16] The enormous variation of the binding temperature among the samples seems to be further evidence for a high sensitivity and, thus, complexity of these membranes.

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[1]For a recent study, see R. M. Servuss, Z. Naturforsch. **43c** 44 (1988).

[2]See the discussion in W. Helfrich, Z. Naturforsch. **33a**, 305

(1978), for a summary of early observations including further references.

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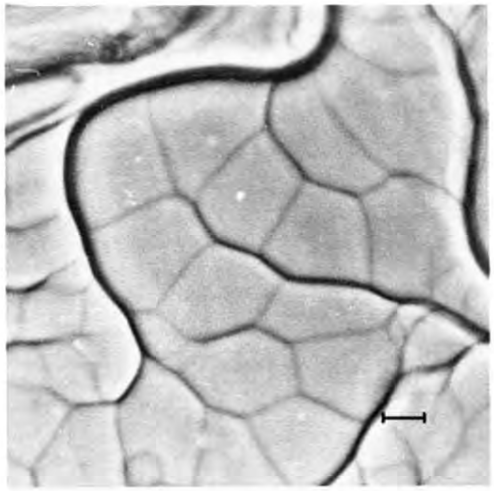

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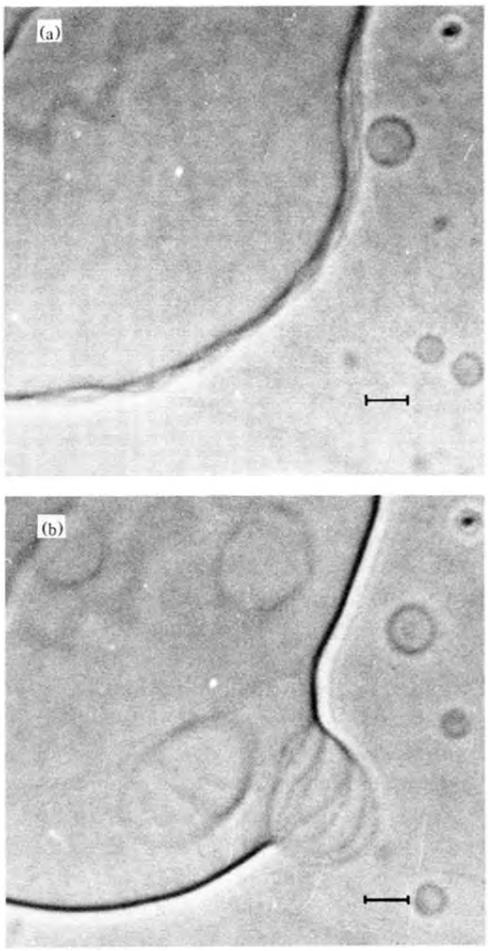

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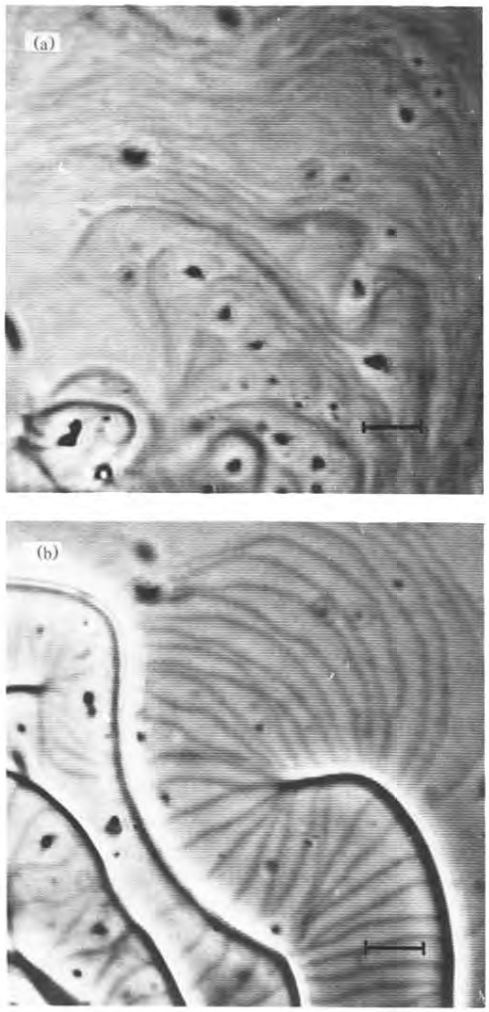

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