Temperature dependence of formation of a supported phospholipid bilayer from vesicles on SiO_2

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Adsorption of egg-phosphatidylcholine vesicles and bilayer formation on a SiO_2 surface was investigated in the temperature range 278–303 K using the quartz crystal microbalance-dissipation technique. The critical coverage for the vesicle→bilayer transition is found to decrease with increasing temperature. The temperature dependence of the time-scale characterizing this transition can be represented in the Arrhenius form. Higher temperatures produce a bilayer with fewer trapped, nonruptured vesicles.

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The cell membrane (biomembrane) is one of the most important constituents in living organisms. Via specialized biomolecules, incorporated in the phospholipid bilayer that forms the membrane, it mediates all communication between the intracellular and extracellular spaces, and thus also cell-cell communication [Fig. 1(a)]. The high interest in understanding the properties of the cell membrane has spurred intense research to build simplified, yet representative models of it [1–3]. These attempts are driven both by curiosity—to study the function and mechanistic aspects of the biomembrane [4,5] and by (potential) applications in technology and life sciences. Examples are biomembranes as functional elements in biosensors and biochips [6,7] for diagnostics and drug screening, biomimetic photosynthesis [8] and various medical applications such as nonfouling surfaces [9,10].

Two prominent examples of biomembrane model systems are unilamellar phospholipid vesicles (liposomes) [11,12] [Fig. 1(b)] and supported phospholipid membranes (SPBs) [13–15] [Fig. 1(c)]. In the former a bilayer of amphiphilic phospholipid molecules form a spherical shell, separating an ''intracellular'' liquid volume from the ''extracellular'' space, while SPBs are planar, extended bilayers of the same composition as vesicles, but adsorbed on a suitable solid surface. SPBs are commonly prepared by a method pioneered by McConnell *et al.* [16], where vesicles are allowed to interact with a suitable surface, that induces rupture and fusion of the vesicles to a coherent bilayer [Fig. 1(b)→Fig. 1(c)]. Theoretical and experimental efforts are now accelerating to understand and control the formation kinetics and properties of the SPBs [13,17–19]. This is a prerequisite for reliably building SPBs as model systems in basic research and for exploiting them in applications.

Our laboratory and others have shown that vesicle-to-bilayer transformation occurs for egg-yolk phosphatidylcholine (egg-PC) small unilamellar vesicles only on certain surfaces [19–21] where SiO_2 and mica are prototype systems. In the present work we have systematically studied the temperature dependence of this process, and thereby obtained unique insight into the vesicle-to-bilayer transformation kinetics. Complementary measurements were made on TiO_2 where the same vesicles instead of a SPB, form a monolayer of intact vesicles [22].

The experiments were performed using the quartz crystal microbalance-dissipation (QCMD) technique [23–25]. It measures real time kinetics of vesicle adsorption/transformation [18] through a simultaneous measurement of two output signals from a piezoelectric quartz crystal oscillator sensor, on one side facing the liquid containing the vesicle solution. The measured signals from the sensor crystal, oscillating in thickness shear mode at a fundamental resonance frequency of 5 MHz are the change in frequency, Δf, and the change in damping, ΔD (or Q factor), as functions of time. These values are obtained at a frequency of 1 Hz, from the recorded decay curve of the freely (open circuit) oscillating crystal, when the driving power to the crystal is interrupted by a fast relay.

In the present case, Δf gives information about adsorbed mass, either directly by using the Sauerbrey relation [26] or by applying a theoretical model to correct for limitations of the Sauerbrey relation [27,28], and ΔD gives information about the viscoelastic properties of the adlayer. Rigid and thin elastic films cause no or very small ΔD, while soft and viscous films cause large ΔD. For details see Keller and Kasemo [20] and Höök *et al.* [28].

The experiments were performed by injecting a solution of small, monodisperse egg-PC (melting temperature $-15\,^{\circ}C$) vesicles, prepared and characterized as described earlier [20,29,30], into the measurement cell containing the QCMD sensor. The sensor was precoated with a SiO_2 film deposited by *e*-beam evaporation (or TiO_2 in some experiments), also described earlier [18,20]. Δf and ΔD were recorded as functions of time until the surface was saturated by a SPB (or by intact vesicles on TiO_2). The experiments were performed at several temperatures in the range 278–303 K (the range is set by the current experimental equipment). Two types of vesicle preparations were used: sonicated unilamellar vesicles (SUVs) of ~25 nm mean diameter, and ex-

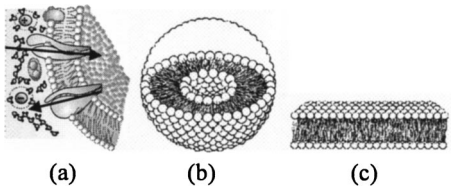

FIG. 1. (a) Cell membrane, (b) phospholipid vesicle (liposome), (c) supported phospholipid membrane.

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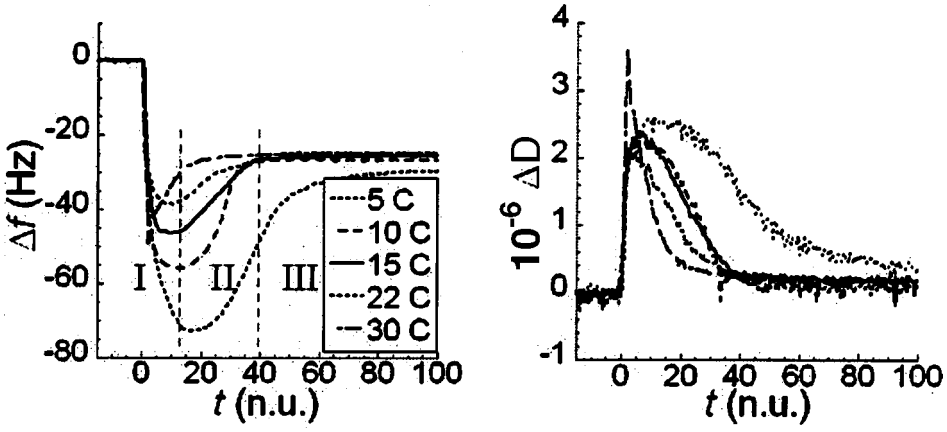

FIG. 2. $\Delta f(t)$ (left) and $\Delta D(t)$ (right) shown as functions of time for vesicle adsorption on SiO_2 at five different temperatures. The time axis has been normalized to remove the effect of temperature on the bulk diffusion [31].

truded vesicles (EUVs) of ~40 nm mean diameter. For each preparation the experiment was repeated on two crystals and at least four times at each temperature. The surfaces were cleaned with SDS and UV/ozone treatment between the runs.

Figure 2 shows one representative set of measurements. The temperature dependent kinetics of vesicle adsorption and transformation (to SPB), are displayed as Δf and ΔD versus time [31] curves at five different temperatures, for 40 nm EUVs. Qualitatively similar results were obtained for 25 nm SUVs (not shown), except that the curve shapes are somewhat different.

The shapes of the $\Delta f(t)$ and $\Delta D(t)$ curves reveal three stages [18,20]: intact vesicle adsorption (I) followed by rupture and formation of a patchy SPB film (II), which eventually is completed (III). The change in sign of $\partial f/\partial t$ at the beginning of stage II originates from the fact that the mass uptake at this point is dominated by vesicle rupture and release of water (sensed as a mass) trapped inside and between vesicles, instead of being dominated by adsorption of nonruptured vesicles [20]. Thus, the mass loss after the minimum is caused by loss of water, which dominates over the simultaneous supply of additional lipid mass from the bulk solution. The simultaneous sign change of dD/dt at approximately the same time as in df/dt reflects the transition from soft, water filled, dissipative vesicles (causing high ΔD) to the much less dissipative, flat bilayer [18,20]. Step II does not start until a critical surface coverage of adsorbed vesicles, Θ_c, is obtained [18].

Identical experiments on TiO_2 do not show minima/maxima in the Δf and ΔD signals. Instead, they monotonically rise to asymptotic values about 2 and 2.5 times higher than on SiO_2, respectively, for the same vesicle size. Experiments conducted with different vesicle sizes at 295 K (results not further discussed here) show that the asymptotic Δf and ΔD signals do not change with increasing vesicle size on SiO_2 [22]. This is consistent with bilayer formation as the end result at saturation. In contrast, both signals rise monotonically on TiO_2 as expected for intact vesicle adsorption.

The most interesting feature in Fig. 2 is the strong temperature dependence that, to the best of our knowledge, has not been quantitatively measured or analyzed before. (Membrane spreading on a glass surfaces has been reported to possess a temperature dependence [32] and a very weak temperature effect on the electrical properties of hybrid bilayers has also been reported [33].) With increasing temperature the minimum in Δf and maximum in ΔD move to shorter exposure times with smaller amplitude, i.e., at higher temperatures a smaller vesicle concentration is required on the surface to induce rupture and SPB formation. This was also shown by interrupting vesicle adsorption at a low temperature before the Θ_c for that temperature was reached, and then increasing the temperature, without vesicles in the solution. During the temperature rise, rupture/fusion was induced (because the critical coverage is lower at higher temperatures, Fig. 2), as deduced from the observed f and D shifts. The data thus clearly demonstrate a thermally activated transition from adsorbed vesicles to a supported bilayer, which could be interpreted as a coverage dependent activation energy for the process.

The final frequency (mass uptake) and dissipation shifts at saturation increase slightly with decreasing temperature. At 303 K Δf_∞ is -25 Hz, exactly corresponding to a coherent, fully covering bilayer. At 278 K the value is -29 Hz. The explanation for the higher mass load at the lower temperatures is most likely trapped, nonruptured vesicles that cannot fuse into the bilayer (probably due to steric hindrance). The ''extra'' 4 Hz at 278 K correspond to about 5% coverage of nonruptured vesicles on the SPB saturated surface.

We have above demonstrated a thermally activated phase transition from surface bound vesicles to a SPB on SiO_2, which occurs at a T-dependent critical coverage. This implies that vesicle-vesicle interaction is important for the transition. Since the transition does not occur on TiO_2 under otherwise identical conditions we conclude that *the transition is caused by a combination of vesicle-surface and vesicle-vesicle interaction*, the former being stronger on SiO_2.

Our mechanistic picture based on earlier work [35,36], which is consistent with the present and previous data [18,20,36], is the following: Single vesicles deform when they adsorb on the studied surfaces, but do not rupture at low coverage, i.e., the deformation and associated strain induced by the surface interaction (probably dominated by van der Waals interaction [34]) is alone insufficient to induce rupture in the studied T range. For TiO_2 this holds for all coverages. On SiO_2 there is additional vesicle deformation when the local or global coverage (see below) exceeds the one where vesicles can maintain the isolated vesicle shape. The additional deformation causes more strain and a lowering of the

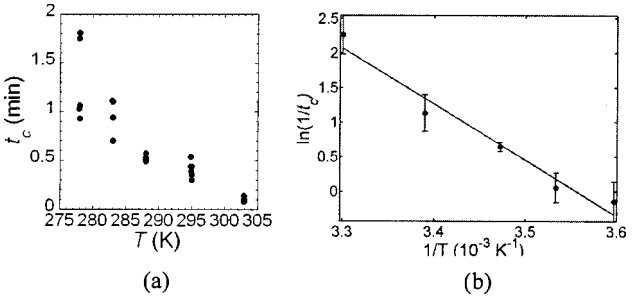

FIG. 3. (a) Time t_c to reach the minimum in Δf (cf. Fig. 2) vs temperature for 40 nm EUVs. (b) Arrhenius-type plot of $\ln(1/t_c)$ vs T^1, where the slope yields an estimate of the activation energy E_a for the onset of bilayer formation.

activation barrier for rupture and fusion. The rupture point in the vesicle is probably where the radius of curvature is smallest, as discussed by Seifert [34]. After initial rupture and fusion the continued vesicle→SPB transition is enhanced in an autocatalytic manner by vesicle-SPB interaction as discussed earlier [18]. Since the rupture process is thermally activated, the required critical surface coverage for the phase transition is higher, and takes longer time to reach, the lower the temperature (Fig. 2).

The time t_c to reach the critical coverage is displayed as a function of temperature in Fig. 3(a). Extrapolation by the eye suggests that spontaneous rupture in the zero coverage limit might occur at 310–320 K. However, a least square fit with a second-order polynomial places this point at considerably higher temperatures. A corresponding extrapolation in the low-temperature limit, to estimate a temperature where intact vesicles adsorb at all coverages (as on TiO_2), is not meaningful because of the larger data scatter at low T. Both these limits will be explored experimentally in the future.

A detailed Arrhenius-type analysis of the averaged data points in Fig. 3(a) to obtain the (apparent) activation energy, E_a, for the coverage dependent vesicle rupture is beyond the scope of this paper, but we make an attempt below. For this purpose a kinetic model is required, where the critical surface coverage of vesicles, $\Theta_c(T)$, at the frequency minimum, $\Delta f_{\min}$, in Fig. 2, can be extracted from the experimental data, since $\Theta_c(T)$ is the relevant ''control parameter'' [18]. Extraction of Θ_c from $\Delta f_{\min}$ is complicated by trapped water around the vesicles (as discussed in Ref. [22] and by Höök *et al.*, [28]).

We estimate the activation energy range by plotting the logarithm of the inverse of the time, t_c, to reach $\Delta f_{\min}$ versus T^1 [Fig. 3(b)]. This is motivated by a simple, first-order rate equation, assuming (i) mass transport limited adsorption of vesicles (demonstrated earlier by SPR [18]) and (ii) a rate-limiting step of rupture of adsorbed vesicles to form bilayer patches. The rate equation can be written in terms of the vesicle surface coverage, Θ, as $d\Theta/dt \approx C\sqrt{D(T)/\pi t} - k_{\mathrm{bil}}\Theta$, where C is a known constant, k_{bil} the rate constant for bilayer formation (vesicle rupture), and $D(T)$ is the temperature dependent bulk diffusion coefficient. Intact vesicles give the dominant mass contribution in the range of interest and the rate equation above for the vesicle surface coverage is therefore a good approximation for the QCM mass signal. Thus, $\Delta f_{\min}$ approximately corresponds to $d\Theta/dt \approx 0$. Mass transport limited adsorption yields $\Theta_c \propto \sqrt{t_c}$ for the critical vesicle coverage. This gives a value for E_a in the range 0.65–0.78 eV/molecule (63–78 kJ/mol), with a slight difference between EUVs and SUVs (the apparent activation energies for the latter are a little higher).

Further articulation of this mechanistic picture will be given in forthcoming mean-field and Monte Carlo simulations [37]. The correct physical interpretation of the critical coverage is yet uncertain, since it is not known if the vesicle-vesicle interaction and critical coverage are (i) caused by a global effect of squeezing a new vesicle into the existing adlayer of vesicles, which adjust collectively, or (ii) due to a local clustering of vesicles caused by their mutual van der Waals attraction. Although distinctly different physically, both mechanisms are at the present level of detail compatible with the available data. Microscopic information, e.g., atomic-force microscopy data, will help to refine the model.

The picture above is consistent with the observation that vesicles do not rupture on TiO_2, but stay intact at all coverages, if we accept that the vesicle-surface interaction is weaker on TiO_2. The latter is supported experimentally by comparing data for the two investigated surfaces, which show that vesicles during the initial adsorption phase, when they are intact on both surfaces, are much less deformed on TiO_2 than on SiO_2 [22]. (The key observation is that dD/df in the initial adsorption phase is smaller for SiO_2 compared to TiO_2, signalling more flattened and rigid vesicles on SiO_2.)

In summary, we have demonstrated a thermally activated vesicle-to-bilayer transition on SiO_2, with cooperating vesicle-surface and vesicle-vesicle interaction as central ingredients, manifested in a T-dependent critical coverage for the transition. From a practical viewpoint we have also shown that the highest temperatures produce the ''best'' SPBs in terms of residual nonruptured vesicles, because there is, in contrast to the low-temperature data, no sign of trapped vesicles at 303 K.

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