DOI: 10.1002/cphc.200800555

Phase Transition Induced Adhesion of Giant Unilamellar Vesicles

Thomas Franke,[b] Christian Leirer,[a, c] Achim Wixforth,[a] and Matthias F. Schneider*[a]

Dedicated to Erich Sackmann on the occasion of his 75th birthday.

Cell and vesicle adhesion is believed to be dictated by the balance between a local interaction potential, which represents the sum of all attractive and repulsive forces and the elastic energy. Changing the mechanical properties of the membrane therefore offers a sensitive tool to control vesicle adhesion. Here, we take advantage of the dramatic changes in area per molecule, fluidity and compressibility during lipid phase transition to alter vesicle adhesion. We demonstrate that driving a giant unilamellar vesicle (GUV) through its phase transition by increasing the temperature leads to a wetting transition of the vesicle onto a pure glass substrate. Analysing vesicle shape and the adhesion area shows that the vesicle is strongly adhered and that the wetting process follows exactly the melting transition of the lipid membrane. We provide evidence that the linear relationship between change in area and enthalpy during lipid phase transition can be applied to individual vesicles as its application correctly extracts the heat capacity profile of DPPC vesicles from our adhesion experiments. It clearly demonstrates that this wetting process is driven by the coupling of mechanical and thermodynamic properties in lipid membranes.

1. Introduction

Cell adhesion represents a crucial step for a variety of biological processes, such as vesicle fusion, intracellular trafficking or wound healing[1] to name a few. Furthermore, in order to develop new materials for implants or stents, a more profound experimental understanding of the adhesion governing forces would be particularly beneficial.

From a biological point of view cell adhesion was often pictured as the specific interaction between ligand and receptor (lock–key mechanism), which pull the two membranes together to form an adhesion zone,[2] sometimes tightly enough to induce fusion.[3] However, Sackmann and his co-workers[4–6] have shown that cell adhesion can only be fully understood by including the mechanical properties of the biomembrane into the model. Using GUVs containing a lipid matrix with ligand proteins and an artificial glycocalix as model systems, they were able to reveal the role of mechanical properties for the adhesion of biomembranes.[7,8]

Lipowsky and Seifert[9–11] thoroughly studied the adhesion of fluid vesicles from a theoretical point of view. For modest adhesion potentials they established a phase diagram including continuous as well as discontinuous transitions between free and bound vesicles of different shape. The transition depends on the adhesion potential W_a and the reduced volume v_r and takes place at [Eq. (1)]:

$$W_a = 2\kappa/R^2, \tag{1}$$

where κ represents the bending rigidity and R the vesicle radius. For strong adhesion ($W > 10^{-4}\,\mathrm{J\,m^{-2}}$) at constant volume constraint, they found that the bound vesicle adopts the simple shape of a spherical cap.[12] Recently, Lipowsky extended his studies to investigate the temperature dependence of fluid vesicle adhesion.[13] They found that, at least for low temperatures, the ratio between adhesion area A_{adh} and total area A_0 of the vesicle is a linear function of T/κ, where T is the temperature and κ the bending rigidity, as shown in Equation (2):

$$\alpha = \frac{\Delta A_{adh}}{A_0} \propto \frac{T}{\kappa} \tag{2}$$

In this work, we studied adhesion of DPPC vesicles, which undergo a gel to fluid phase transition. We observe strong adhesion of the vesicle to the glass substrate forming a spherical cap. We follow the dynamics of the adhesion area as well as the macroscopic shape of the vesicle and find that the increase in area obeys the same behaviour as the enthalpy during lipid

[a] C. Leirer,[+] Prof. Dr. A. Wixforth, Dr. M. F. Schneider[+]
University of Augsburg, Experimental Physics I
Biological Physics Group
Universitaetstr. 1, D-86159 Augsburg (Germany)
Fax: (+49) 821-5983227
E-mail: matthias.schneider@physik.uni-augsburg.de

[b] Dr. T. Franke[+]
University of Augsburg, Experimental Physics I
Microfluidics Group
Universitaetstr. 1, D-86159 Augsburg (Germany)

[c] C. Leirer[+]
Niels Bohr Institute
University of Copenhagen, DK-2100, Copenhagen (Denmark)

[+] These authors contributed equally to the work.

chain melting. We propose that the major source driving the wetting transition is the supply of excess area during melting accompanied by the decrease in bending rigidity of the membrane.

Experimental Section

DPPC dissolved in chloroform (20 mg mL^{-1}) was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA) and was used without further purification. For fluorescent studies we added Texas Red DHPE (Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine,triethylammonium salt, Invitrogen, USA) at a concentration of 0.1 mol%. Vesicle electroformation and micropipette aspiration were performed as described elsewhere[14,15] and a practical introduction to vesicles is given by Dimova et al.[16]

All aqueous solutions were prepared with ultrapure water (pure Aqua, Germany) with a specific resistance of 18.2 MΩ. For vesicle preparation we spread a small amount of lipid (10 µl of a 1 mg mL^{-1} solution) in chloroform on an ITO coated glass substrate and left it in a vacuum for at least 6 h to remove any traces of organic solvent. A teflon spacer was used to separate two ITO slides forming a swelling chamber. Subsequently, pure water or an aqueous sucrose solution (200 mM) was added and an AC field of 1 V mm^{-1} and 10 Hz was applied between the conducting ITO slides. The assembled chamber was fixed and placed in a thermal water bath which was heated to 50 °C. The electroswelling process was completed after 3 h and giant unilamellar vesicles up to diameters of 100 µm were harvested for direct use in the adhesion experiment.

The micropipette manipulation setup was mounted on an inverted phase contrast and fluorescence microscope (Axiovert 200 M with Texas Red filterset, Zeiss, Oberkochen,) and equipped with a conventional CCD camera. A micromanipulator (MP285, Sutter Instrument) was mounted on the microscope which allowed control of a glass capillary position in all three dimensions independently. The micropipettes were manufactured with a programmable, horizontal pipette puller (P97, Sutter Instrument) and subsequently processed with a custom-made microforge. All adhesion experiments were performed in an open chamber consisting of two parallel glass coverslides separated by a 2 mm teflon spacer in which a vertical substrate wall could be introduced. The temperature within the experimental chamber was controlled using two flat Peltier elements (Conrad electronic SE, Hirschau) mounted onto each glass slide and recorded with a small thermocouple (accuracy 0.3 K). To examine adhesion a selected vesicle was partially sucked into the micropipette and maneuvered into the proximity of the substrate wall. Pushing the vesicle slightly against the wall finally lead to adhesion. Afterwards the micropipette was removed from the system.[17]

2. Results and Discussion

2.1. Phase Transition Induced Wetting

In Figure 1 a series of phase contrast images of a GUV DPPC vesicle near the glass wall is shown. Gently pressing the wrinkled gel-like vesicle against the wall was sufficient to immobilize it (Figure 1 a), presumably due to the reduced repulsive undulation forces in this phase. Quenching into the fluid phase by rapidly increasing the temperature from 35 °C to 45 °C the vesicle undergoes a wetting transition (Figure 1 b). We would like to note, that driving the vesicle through the phase transition in the absence of a wall leads to various morphological transitions[18–20] due to the drastic change in reduced volume and elastic properties. Following the melting process, the adhesion area (adhesion disk) grows until the entire vesicle reached the fluid phase (Figure 1 b, lower right). A similar experiment was performed by Gruhn et al.[21] However, in their experiments the excess area was controlled osmotically.

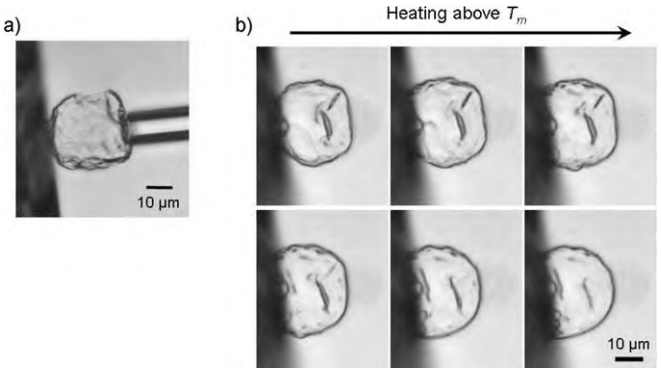

Figure 1. a) A DPPC vesicle in the gel phase is slightly pressed against a glass substrate. The vesicle partially adsorbs to the surface. b) Phase contrast images of a GUV DPPC vesicle during phase transition. A sudden increase in temperature (~1 °C s^{-1}) induces a wetting transition. The total area increases by ~ 20%. When the vesicle reaches the fluid phase it forms a spherical cap with a contact angle close to 90° resembling strong adhesion (images taken at intervals of ~0.5 s, from 39.5 °C to 42 °C). The last picture in Figure 1 b is taken above the phase transition temperature in the fluid phase. In this elastic, fluid regime a vesicle is not assumed to adopt the shape of a spherical cap due to bending elastic energy. This shape is a direct consequence of vesicle adhesion.

During the wetting process the gain in adhesion energy must balance the cost in bending energy.[4] The adhesion energy increases with the contact area, while the bending energy increases with bending rigidity κ. Importantly, both terms are affected by the lipid membrane phase transition.

As has already been mentioned the vesicle seems to adhere strongly since the overall shape of the vesicle changes during the process. For this case it has been shown, that vesicle adhesion is dominated by the competition between adhesion and surface energy.[11] As the vesicle starts to increase its area during phase transition, more and more surface is adsorbed to the glass wall (Figure 1 b), without stretching the membrane significantly. The final shape of the adhered vesicle resembles a spherical cap as predicted by Lipowsky[10] for strong adhesion under constant volume constrained. The contact angle θ is a measure for the adhesion strength g_{adh} according to the Young-Dupre equation $g_{adh} = \sigma(1 - \cos\theta)$, where σ represents the surface tension, at least in the case for strong adhesion, which is indicated by the shape of a spherical cap.[10] Measuring the total area of the vesicle in the fluid phase A_{fl}, using the phase contrast images of Figure 1 b, reveals a ~ 20% increase in area during the experiment ($A_{fl} \approx 1{,}20\ A_g$, with the area of the gel like vesicle A_g). This increase is expected from the lipid chain melting and indicates the absence of any significant membrane extension.[22]

2.2. Time Evolution of the Adhesion Area

In order to understand whether the observed process can be understood in terms of thermodynamic equilibrium we monitored the adhesion process using a heating rate of only $0.1\,^{\circ}C\,s^{-1}$. To study the growth dynamics of the adhesion disc in more detail we used fluorescently labelled vesicles and focused specifically on the vesicle substrate interface. Placing DPPC vesicles with 200 mM internal sucrose solution in the chamber filled with 200 mM glucose solution promotes initial sedimentation of the vesicles onto the lower coverslide, without deforming the vesicles significantly. In Figure 2a the growth of the adhesion disc during phase transition of such a fluorescently labelled vesicle is shown [images i)–vi)]. Here, the temperature increase takes place slowly ($\sim 0.1\,^{\circ}C\,s^{-1}$) and did not affect the final appearance of the adhered vesicle indicating an equilibrium state. In Figure 2b, the adhesion area measured is plotted against temperature. The adhesion area A_{adh} increases along with the melting process linearly from $0.6\times 10^{-10}\,m^2$ to $5.9\times 10^{-10}\,m^2$ by a factor of 10 over a temperature range of $3\,^{\circ}C$ (Figure 2b). From the increase in A_{adh} and the change in total vesicle area $\Delta A(T)$, we were able to derive $\alpha(T)$ from the experiment [Eq. (2)], which in turn, by using the relations presented in Gruhn's work,[13] allows us to calculate $\kappa(T)$. However, since our system is predominantly heterogeneous and does change phases during heating, we doubt the meaning of such a comparison. On the other hand, it is worth noting, that when taking numbers from literature for the bending rigidity in the gel and the fluid phase[23–25] the linear relationship [Eq. (2)] predicts a 10-fold increase in $\alpha(T)$, which is in good agreement with our experimental result.

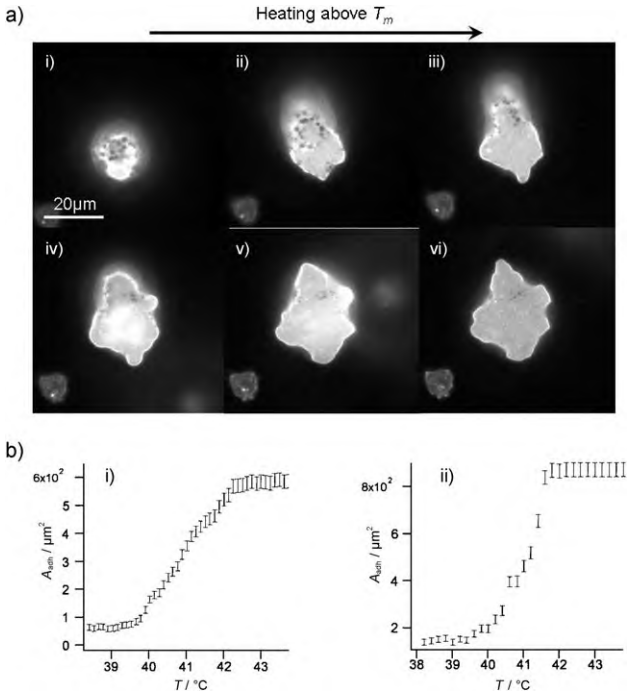

Figure 2. a) Fluorescence images i)–vi) of the evolution of the adhesion area ΔA_{adh} taken at a very slow heating rate ($0.1\,^{\circ}C\,s^{-1}$). The total area $A(T)$ increases from $1.39\cdot 10^3\,\mu m^2$ to $1.71\cdot 10^3\,\mu m^2$ (~ 22%). b) The area of adhesion A_{adh} of two vesicles (i) and (ii) plotted as a function of temperature. A_{adh} increases by a factor of 10 in a temperature interval of $3\,^{\circ}C$. Error bars are increasing with the size of the adhesion area.

The contribution of gravitational effects due to density differences of the sugar solutions can be estimated by the energy density $W_g = gA\Delta\rho/6\pi$, A being the total surface of the vesicle, $\Delta\rho$ the difference of sugar solution inside and outside the vesicle and g the constant of gravity. For a typical vesicle of radius $R = 10\,\mu m$ the adhesion energy density has to be corrected by the term $W_g \approx 10^{-8}\,J\,m^{-2}$ and is therefore only relevant in the regime of ultralow adhesion.[21,26]

2.3. Mechanical and Thermodynamic Coupling

Using a linear relationship between changes in total area $\Delta A(T)$ and enthalpy $\Delta H(T)$ during lipid phase transition [Eq. (3)], first demonstrated by Heimburg et al.:[22]

$$\Delta A(T) = \gamma \Delta H(T) \tag{3}$$

Figure 2b allows us to calculate the heat capacity c_p of the membrane from the change in area during lipid melting using Equation (4):

$$c_p = \frac{dH}{dT} = \gamma^{-1}\frac{dA}{dT} \tag{4}$$

and accounting for the total number of lipids in the vesicle. In Figure 3 the calculated molar heat capacity profile, taking $\gamma \approx 0.89\,m^2\,J^{-1}$ [22] and an average size of a single lipid in the fluid phase of $0.65\,nm^2$, is shown and compared with calorimetry measurements of small unilamellar vesicles (SUV). Despite some differences in size and geometry the data show excellent

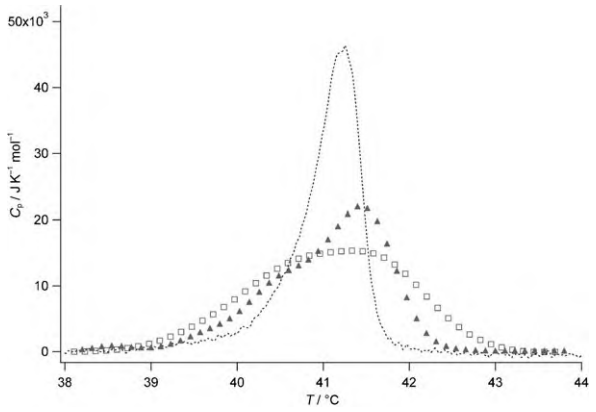

Figure 3. Heat capacity calculated from Figure 2b (triangles and squares) compared with the heat capacity profile of SUV of DPPC (dotted line) measured by differential heat calorimetry (DSC) at a scan rate of $10\,^{\circ}C\,h^{-1}$. For the calculation an average size of a single lipid in the fluid phase of $0.65\,nm^2$ and $\gamma = 0.89\,J\,m^{-2}$ were taken. Clearly, all three graphs follow the same qualitative behaviour indicating that the adhesion area is a consequence of the coupling between enthalpy and area during chain melting. The change in heat extracted from Figure 2b is $37\pm 4\,kJ\,mol^{-1}$ (squares, 2a) and $33\pm 4\,kJ\,mol^{-1}$ (triangles, 2b) and for the DSC plot $36.4\,kJ\,mol^{-1}$.

agreement strongly supporting that the linear relationship [Eq. (3)] can be applied to individual GUVs. Furthermore, integrating our experimentally derived curve reveals a change in melting enthalpy ΔH of 36.6 ± 4 kJ mol^{-1} very close to the precise heat extracted from calorimetry experiments of 36.4 kJ mol^{-1}.

4. Conclusions

In summary, we show how thermodynamic control over the mechanical properties of the lipid membrane enables the triggering of strong adhesion of GUV DPPC vesicles onto a glass substrate. We propose that the main driving force is the change in area and bending rigidity during lipid phase transition. While the decrease in bending stiffness of the bilayer allows only the initiation of the adhesion process, the rate of area increase determines the dynamics of the growth of the adhesion disc. We show that the adhered excess area follows the same linear relationship in individual vesicles as has been reported earlier by Heimburg et al.[22] for ensembles. Furthermore, we would like to propose that using vesicles in their phase transition provides a simplified model of a heterogeneous membrane with soft microdomains embedded into a cytoskeleton-supported rigid matrix (or vice versa).

Finally, considering that phase transitions can also take place in small vesicles (~100 nm) or even locally in membrane segments, induced not by temperature but rather by pH-changes or protein binding, biomembrane adhesion might be supported by changes in phase state and accompanied elastic properties.

Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft DFG (SCHN 1077, SPP 1313 and SPP1164), the Elite Netzwerk Bayern (CompInt) and the German Excellence Initiative via the Nanosystems Initiative Munich (NIM). M.F.S., C.L. and T.F. were supported by the Bayrische Forschungsstiftung.

Keywords: giant unilamellar vesicles · lipids · membranes · phase transitions · vesicle adhesion

[1] P. Bongrand, *Physical Basis of Cell-Cell Adhesion*, CRC, Boca Raton, **1988**.
[2] P. Chan, M. Lawrence, M. Dustin, L. Ferguson, D. Golan, T. Springer, *J. Cell Biol.* **1991**, *115*, 245–255.
[3] S. W. Schneider, *J. Membr. Biol.* **2001**, *181*, 67.
[4] E. Reister-Gottfried, K. Sengupta, B. Lorz, E. Sackmann, U. Seifert, A. Smith, *Phys. Rev. Lett.* **2008**, *101*, 208103.
[5] A. Smith, U. Seifert, *Soft Matter* **2007**, *3*, 275.
[6] A. Smith, K. Sengupta, S. Goennewein, U. Seifert, E. Sackmann, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6906.
[7] Z. Guttenberg, B. Lorz, E. Sackmann, *Europhys. Lett.* **2001**, *54*, 826–832.
[8] E. Sackmann, *Science* **1996**, *271*, 43.
[9] T. Franke, R. Lipowsky, W. Helfrich, *Europhys. Lett.* **2006**, *76*, 339.
[10] R. Lipowsky, U. Seifert, *Langmuir* **1991**, *7*, 1867.
[11] U. Seifert, *Phys. Rev. A* **1991**, *43*, 6803.
[12] *Morphology of vesicles,* U. Seifert, R. Lipowsky in *From Vesicles to Cells, Vol. 1A* (Eds.: E. Sackmann, R. Lipowsky), Elsevier, Amsterdam, **1996**.
[13] T. Gruhn, R. Lipowsky, *Phys. Rev. E* **2005**, *71*, 011903.
[14] M. Angelova, D. S. Dimitrov, *J. Electroanal. Chem. Interfacial Electrochem.* **1988**, *19*, 323.
[15] M. I. Angelova, D. S. Dimitrov, *Prog. Colloid Polym. Sci.* **1988**, *76*, 59.
[16] R. Dimova, S. Aranda, N. Bezlyepkina, V. Nikolov, K. Riske, R. Lipowsky, *J. Phys. Condens. Matter* **2006**, *18*, S1151.
[17] D. Needham, D. Zhelev in *Giant Vesicles, Perspectives in Supramolecular Chemistry, Vol. 6* (Ed.: P. L. L. a. P. Walde), Wiley, New York, **2000**.
[18] C. T. Leirer, B. Wunderlich, V. M. Myles, M. F. Schneider, *Biophys. Chem.* **2009**, DOI: 10.1016/j.bpc.2009.04.002.
[19] C. T. Leirer, B. Wunderlich, A. Wixforth, M. F. Schneider, *Phys. Biol.* **2009**, *6*, 016011.
[20] M. F. Schneider, D. Marsh, W. Jahn, B. Kloesgen, T. Heimburg, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14312.
[21] T. Gruhn, T. Franke, R. Dimova, R. Lipowsky, *Langmuir* **2007**, *23*, 5423.
[22] T. Heimburg, *Biochim. Biophys. Acta Biomembr.* **1998**, *1415*, 147.
[23] T. Heimburg, *Thermal Biophysics of Membranes*, Wiley-VCH, Weinheim, **2007**.
[24] K. Mecke, T. Charitat, F. Graner, *Langmuir* **2003**, *19*, 2080.
[25] R. Dimova, B. Pouligny, C. Dietrich, *Biophys. J.* **2000**, *79*, 340.
[26] Z. Guttenberg, B. Lorz, E. Sackmann, A. Boulbitch, *Europhys. Lett.* **2001**, 826.