R. Lipowsky · D. Richter · K. Kremer (Eds.)

The Structure and Conformation of Amphiphilic Membranes

Proceedings
of the International Workshop
on Amphiphilic Membranes, Jülich, Germany,
September 16–18, 1991

With 150 Figures

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo
Hong Kong Barcelona
Budapest

Contents

Vesicle–Substrate Interaction Studied by Reflection Interference Contrast Microscopy

J. Rädler and E. Sackmann

Physik Department, Biophysics Group E22, Technische Universität München, James Franck-Str., W-8046 Garching, Fed. Rep. of Germany

Abstract. Giant DMPC-Vesicles interacting with a supported DMPC-bilayer are investigated by Reflection Interference Contrast Microscopy (RICM). Spherical vesicles whose shape fluctuations are suppressed by osmotic pressure can be observed to fluctuate like Brownian particles above the substrate. The interaction potential can be determined from the measured distribution function of distances. In the case of adhesion, the contact contour of deflated vesicles can be calculated. Contact roundings for of weak adhesion as well as contact angles for strong adhesion have been measured.

1 Introduction

The adhesion of vesicles to a wall has recently become of interest in the context of the variety of shape transformations that free vesicles exhibit [1]. Clearly, in the case of adhering vesicles the equilibrium shape and the dynamics of the fluid membrane is strongly dependent on the strength of the vesicle wall interaction [2]. We investigated repulsive vesicle wall interaction as well as vesicle adhesion.

2 Experimental setup and methods

The experimental setup is depicted in Fig.1. The vesicles are studied by reflection interference contrast microscopy. By this technique interference is observed between the object beam reflected at the membrane and the reference beam reflected at the glass-buffer interface. In the case of an osmotically swollen, spherical vesicle the interference pattern are known as Newton rings and the absolute sphere-substrate distance can be measured from the position of the fringes. Consequently, fast image processing allows monitoring distance fluctuations in real time [3].

Furthermore, the interference fringes at the edge of the contact zone of adhering vesicles of arbitrary shape can be used to calculate the vesicle contour close to the surface. Thus the nature of the equilibrium shape of adhering vesicles in the contact zone can be studied; i.e. the contact angle for strongly adhering vesicles or smooth contact curvatures in the case of weak adhesion.

Special thought must be given to the preparation of the surface of the substrate. Since optical techniques require glass as the underlying substrate, surface

Springer Proceedings in Physics, Vol. 66 The Structure and Conformation of Amphiphilic Membranes
Editors: R. Lipowsky · D. Richter · K. Kremer

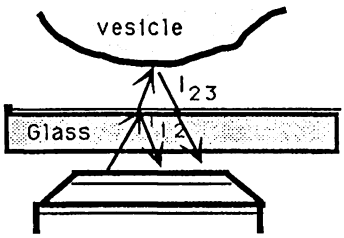

Figure 1: Principle of the reflection interference contrast microscopy. The interference between the object beam I_{23} and the reference beam I_{12} is observed.

roughness and unspecific electrostatic effects are considerable shortcomings in vesicle adhesion experiments. Most glass effects, however, are suppressed by silanizing the glass surface, i.e. chemically attaching C_{18} hydrocarbon chains to the surface and depositing a lecithin monolayer on top. Using this kind of substrate supported monolayer has the advantage of studying the symmetric lecithin-lecithin interaction that has already been measured by other techniques.

3 Repulsive interaction

Prieve et al. [4] showed recently that the interaction of a sphere which by Brownian motion fluctuates above a repulsive surface can be measured by sampling the distance distribution function of the random motion. The distribution function of distances contains the interaction potential simply by the Boltzmann relation. Giant DMPC vesicles are observed not to adhere to DMPC supported monolayers at low ionic strength ($< 20mM$) and osmotically swollen vesicles show distance fluctuations. The negative logarithm of the measured distance distribution is shown in Fig. 2. The solid line indicates a best fit to the theoretical interaction potential including electrostatic, Van der Waals and gravitational forces. The figure also shows that under the same conditions but increased ionic strength the theoretical interaction becomes attractive as experimentally observed (dashed line).

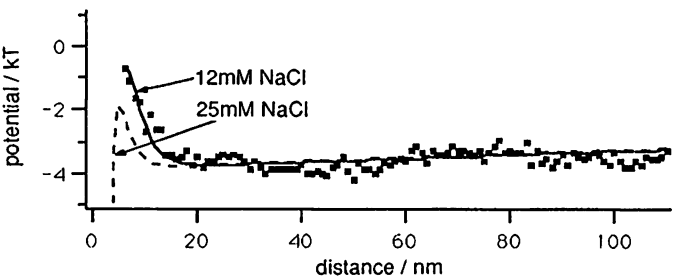

Figure 2: The interaction potential obtained from the distance fluctuations of a spherical DMPC vesicle above a DMPC supported monolayer in 100mM inositol plus 12mM NaCl. The solid line depicts the theoretical interaction potential for $\Psi = 1.8mV$. Increasing the ionic strength to 25mM leads to adhesion due to Van der Waals forces as indicated by the dashed line.

4 Weak adhesion

Adhesion is observed, if the ionic strength in a system of DMPC vesicles on a DMPC supported monolayer surface is increased to 20mM. However, the adhesion is weak which is obvious from visible surface undulations on the vesicle. Fig. 3 depicts the contour of the vesicle in the contact zone. The shape exhibits a contact rounding that is fitted by a circle of contact radius R_K (dashed line). The contact curvature has been predicted to depend on the ratio of bending to adhesion energy [6, 2] :

$$R_K = \sqrt{2K_C/\gamma} \tag{1}$$

Taking K_C to be $10^{-19}J$ the adhesion energy turns out to be on the order of $1.2 \cdot 10^{-8}J/m^2$. This value is small compared to $10^{-5}J/m^2$ measured by micropipette [6] and surface force technique [7]. However, in those cases tension is applied to the membranes, while in contrast at very low tension γ is reduced due to steric repulsion of the undulating membrane [8].

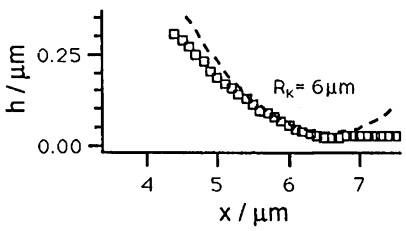

Figure 3: Calculated contour from the interference fringes of a DMPC-vesicle on a DMPC supported monolayer. The contour rounding gives an estimate for the adhesion energy. The dashed line depicts a circle of contact radius R_K (distorted scale !).

5 Strong adhesion

Strong interaction is achieved by incorporating negative charges into the supported monolayer and positve charges into the vesicle. Fig. 4 shows an example of a vesicle (SOPC:Chol:DODAB) (49:49:2 mol%) adhered to a supported monolayer (DMPC:PS) (98:2 mol%). It depicts the reconstructed profile of the vesicle. The vesicle is only slightly deflated and the contour is close to the spherical shape (dashed line). However, the contact zone exhibits sharp contact angles. The equilibrium shape of adhering vesicles has been calculated by Seifert and Lipowsky [2]. In agreement with their predictions we also found the following shapes schematically depicted in figure 5.

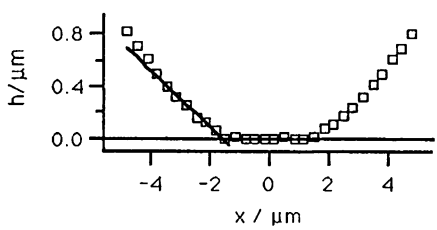

Figure 4: Reconstructed contour of a strongly adhering vesicle. The contact zone shows a contact angle.

Figure 5: Schematic representation of experimentally found shapes.

Acknowledgments

We like to thank A. Zilker for support with the image processing system. We also gratefully acknowledge the financial support by the DFG SA 246/20 and by the Fond der chemischen Industrie.

References

[1] Kaes,J. and Sackmann, E., Bio. Phys J. **60** (1991) 1

[2] Seifert,U. and Lipowsky,R., Phys.Rev A **42** (1990) 4768

[3] Raedler,J.and Sackmann,E. submitted

[4] Prieve,D.C. and Frey,N.A., Langmuir **6** (1990) 396

[5] Evans,E.A. and Parsegian,V.A., in Surface Phenomena in Hemorheology : Copley,A.L. and Seaman,G.V.F.,Eds., N.Y. Acad. Sci. (1983) 13

[6] Evans,E.A. and Metcalf,M., Biophys. J. **46** (1984) 423

[7] Marra,J. and Israelachvili,J., Biochemistry **24** (1985) 4608

[8] Servuss,R.M. and Helfrich,W. J.Phys. France **50** (1989) 809