Experimental Evidence of the Electrostatic Contribution to the Bending Rigidity of Charged Membranes

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We address the issue of the origin of the bending rigidity of a charged membrane formed from amphiphilic molecules. Electrostatic effects are investigated by direct measurement of the force necessary to deform a catanionic membrane as function of the ionic strength of the medium by means of an atomic force microscope (AFM). Using continuum mechanical modeling of membrane deformation, we derive the bending rigidity of the catanionic membranes and monitor for the first time its decrease in response to increasing salt concentration.

Bilayer membranes are ubiquitous in living systems, and their control is therefore central for life sciences, biological, and biotechnological applications. Physically, these membranes are two-dimensional entities with interactions partly mediated via the third dimension (e.g., counterion cloud). The bending rigidity (K_C), which relates the bending of a membrane to deformation forces, is a key parameter for membrane shape and plays an important role in processes like fusion and adhesion.[1] Still, experimental data on bending rigidities from direct force-deformation measurements are scarce and so far only available for uncharged membranes.[2] However, there is ample indirect evidence that points toward pronounced differences between charged and uncharged amphiphilic membranes with respect to bending rigidity. Swelling of a smectic phase upon addition of water is well-known to be very different for charged or uncharged lipids.[3] The same is true for equations of state relating osmotic pressure to spacing: maximum water content in bilayer stacks at zero osmotic pressures is typically 50%[4] with neutral lipids and exceeds 95% with charged lipids.[5] The dominant repulsive force for neutral lipids is the ubiquitous hydration force, while the dominant force is the electrostatic force for charged systems in the absence of salt. The most convenient molecular systems to quantify effects of molecular forces on self-assembly are salt-free catanionics,[6–8] since structural charge can be tuned at will by only varying the molecular ratio of the two components, without introducing large protruding head-groups as by using lipid-surfactant mixtures. This has led to the first "balance" of hydration force compared to electrostatics[8] as well as to the demonstration of the preeminence of hydration force versus molecular protrusion.[9]

The situation is less clear for the effect of surface charge on the stability of vesicles. Spontaneous thermodynamically stable vesicles are proven to exist since 1985 for charged lipids with low critical micellar concentration[3] but measurements of bending constants versus surface charge using scattering are scarce.[10–12] Large rigidity ($>100k_BT$) is expected since the in-plane coher-

ence lengths are large.[13] Effects of small size of crystallites limit the application of fitting the scattering, since diffraction peaks are broadened by other effects than Caillé broadening. A direct approach based on nanomechanics, i.e., effective mechanical bending on a sub-micron-scale of the membrane by the AFM probe, has been proven to be reliable and less limited than fitting Gaussian or power-law shape of Bragg peaks in the powder regime.[14] Here, we use this technique to demonstrate that the theoretical expectations[15] are indeed met for charged bilayers with controlled surface charge, screened by salt. These theoretical works have predicted an electrostatic contribution to the bending rigidity of charged membranes which is independent of geometry[16] and boundary conditions.[1,17] The total bending rigidity (K_C) can thus be expressed as follows:

$$K_C = K_C{}^{nel} + K_C{}^{el}$$

where $K_C{}^{el}$ and $K_C{}^{nel}$ are, respectively, the electrostatic part and the nonelectrostatic part of the bending rigidity. We will test in this work if this additive approach is valid for real "stiff" systems ($K_C \gg k_BT$) and provide quantitative data.

As previously shown, immobilization of catanionic polyhedrons needs substrates with suitable surface charge densities in order to prevent a collapse of the microstructure.[14] In this study, the immobilization (Figure 1) was performed on silicon surfaces prepared by silanization with octadecyltrichlorosilane (OTS) followed by a controlled UV/ozone oxidation. The control of the irradiation time allows the elaboration of surfaces with different charge densities.[18]

Hollow faceted polyhedrons are formed by mixing myristic acid ($C_{13}H_{27}COO^-$, H^+) and cetyltrimethylammonium hydroxide ($C_{16}H_{33}N(CH_3)_3{}^+$, OH^-) with an excess of the anionic component and without added salt.[13,19] These polyhedrons are positively charged (ζ-potential $= +50$mV), and the ionic strength of the solution, measured by conductivity, is close to 10^{-5} M. AFM force measurements were carried out on catanionic polyhedrons with increasing monovalent salt concentration. In accordance with expectations from shell mechanics, we found a linear force deformation relation for the polyhedrons, when the load was applied in the center of the facets and when the

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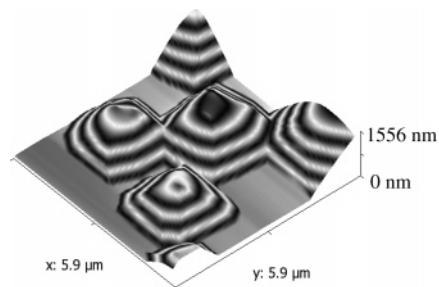[‡] Université du Maine.
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Figure 1. AFM image showing the immobilization of hollow facetted catanionic polyhedrons on functionalized OTS self-assembly monolayer.
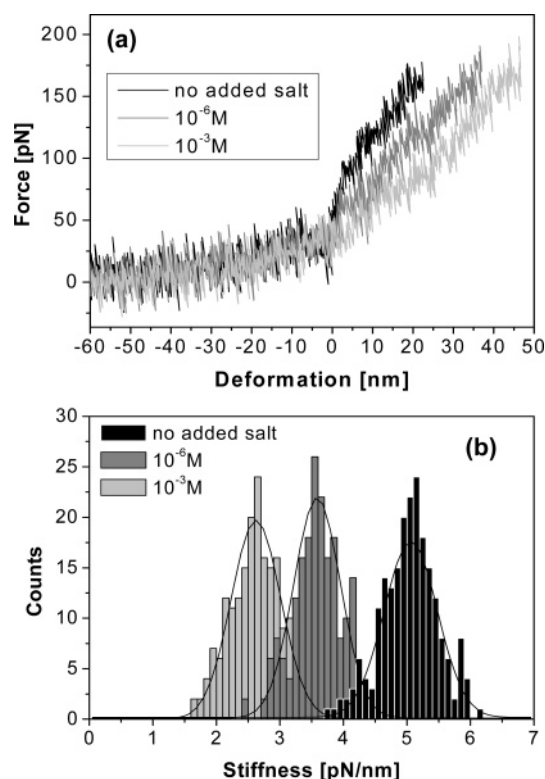

Figure 2. (a) Evolution of the stiffness of the catanionic membrane when the NaCl concentration increases (the slope of the curve corresponds to the stiffness of the charged membrane in pN nm^{-1}); (b) experimental histograms established using 10 different facetted objects and corresponding Gaussian fits (straight line) of the stiffness distribution.

deformation was on the order of the membrane thickness (Figure 2a).[20] However, Figure 2a indicates that addition of sodium chloride leads to a significant decrease of the slope of the reversible linear part of the force versus distance curves. Statistical measurements of this slope, which can be ascribed to the membrane stiffness, were performed on several polyhedrons ($\sim$10) and for different salt concentrations. The obtained Gaussian distributions (Figure 2b) allow extracting the mean membrane stiffness (k_{mean}) and the standard deviation for each salt concentration.

With the use of an appropriate mechanical model[14,21] based on shell theory, the Young's modulus (E) of the membrane can be directly extracted from the AFM experiment: $E = (0.75(1 - \nu^2))^{0.5} \times (k_{mean}R/h^2)$, where E denotes the Young's modulus, ν the Poisson ratio ($\nu = 0.33$), k_{mean} the mean membrane stiffness, R the radius of curvature of the polyhedron face (determined from the AFM topography image), and h the membrane thickness (4.8 nm).[14] The bending rigidity (K_C) can be derived from: $K_C = Eh^3/12(1 - \nu^2)$.[22] Figure 3 shows the evolution of the bending rigidity of the catanionic membrane
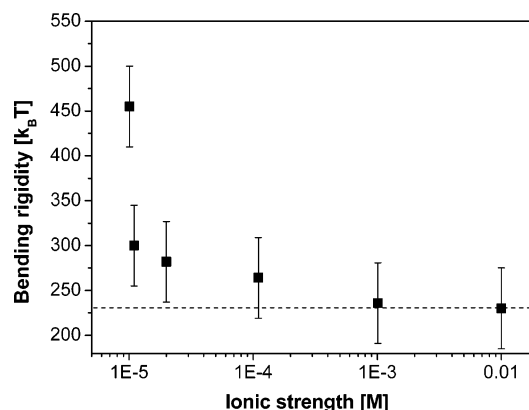

Figure 3. Evolution of the bending rigidity determined from AFM force−distance measurements as function of the ionic strength tuned by addition of sodium chloride (NaCl).
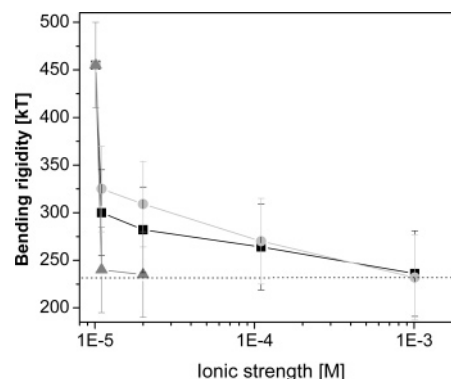

Figure 4. Bending rigidity of the catanionic membranes as function of the ionic strength by addition of NaCl (■), CsCl (●), and $NaClO_4$ (▲).

as function of the ionic strength tuned by addition of sodium chloride. Without added salt, the bending rigidity of the catanionic membrane is very high ($K_C = 450 \pm 38\ k_BT$) if compared to a lipid bilayer.[23] When salt is added to the solution, an important decrease of the bending rigidity is observed. This result could be explained by a progressive salt-screening of the electrostatic contribution ($K_C^{el.}$) to the bending rigidity.[1,10,16,17] This assumption is reinforced by the presence of a plateau value ($K_C \sim 230\ k_BT$) at high salt concentration, where screening of the electrostatic effects is expected. Indeed, comparable values were reported for the bending rigidity of an uncharged lipid bilayer in the gel state and with similar chain length.[24] The plateau value can thus be assigned to the nonelectrostatic contribution of the bending rigidity (K_C^{nel}).

Influence of ion hydration was also investigated by studying the effect of different added salts on the rigidity of the charged catanionic membrane. Force-versus-deformation measurements were performed on catanionic polyhedrons in presence of sodium chloride (NaCl), cesium chloride (CsCl), or sodium perchlorate ($NaClO_4$). As shown in Figure 4, independently of the nature of the added salt, the increase of the ionic strength leads to a decrease of the bending rigidity until it reaches the same plateau value (K_C^{nel}). This result confirms the existence of an electrostatic contribution to the bending rigidity of a charged membrane which vanishes at high salt concentration. The difference in bending rigidity between the effect of NaCl and CsCl is within the error of the measurement. Thus, despite the highest ability to hydrate the cation Na^+ compare to Cs^+,[25] no effect larger than uncertainty in measurement derived from statistical average as could be established here of the nature of the cation of the added salt could be evidenced.

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Contrary to cations, a clear effect is observed when perchlorate anion (ClO_4^-) is used instead of Cl^-. Indeed, the addition of $NaClO_4$ leads to a strong decrease of the bending rigidity which reaches the plateau value corresponding to K_C^{nel} for a very low $NaClO_4$ concentration ($\sim 10^{-6}$ M). Moreover, when the concentration of $NaClO_4$ increases above 10^{-5} M, a destruction of the polyhedrons is observed by AFM. This destruction of the membrane, characteristic of a strong interaction with the perchlorate anion, can be attributed to the properties of the perchlorate anion which is known to be less hydrated than Cl^- [26] and thus supposed to interact more strongly with the positively charged membrane.[27] These experiments seem to indicate that the nature of the anion has a stronger effect than the nature of the cation on the rigidity of the catanionic membrane. From purely electrostatic considerations, water is better at stabilizing cations than anions because the partial negative charge on oxygen is twice the positive charge on the two hydrogen and thus anions are more free to interact with charged membranes.[25] Moreover, since the catanionic membrane is positively charged, a stronger interaction with anions compared to cations is also expected.

In conclusion, we have shown that AFM-force microscopy is a versatile method to study directly the electrostatic effects on the bending rigidity of a charged membrane. Our experiments confirm previous theoretical assumptions concerning the existence of an electrostatic contribution to the bending rigidity. Moreover the sensitivity of this method allows exploring the effect of the nature of the salt onto the properties of charged membranes. Numerical calculations using the Poisson−Boltzmann mean field theory are at present performed in order to confirm the existence of an electrostatic contribution to the bending rigidity of a charged membrane.

References and Notes

(1) Lipowsky, R.; Sackmann, E. *Structure and Dynamics of Membranes*; Elsevier Science: Amsterdam, 1995.

(2) Liang, X.; Mao, G.; Ng, K. Y. S. *J. Colloid Interface Sci.* **2004**, *278*, 53−62.

(3) Dubois, M.; Zemb, T. *Curr. Opin. Colloid. Intererface Sci.* **2000**, *5*, 27−37.

(4) Leikin, S.; Parsegian, V. A.; Rau, D. C.; Rand, R. P. *Annu. Rev. Phys. Chem.* **1993**, *44*, 369−395.

(5) Deme, B.; Dubois, M.; Zemb, T. *Langmuir* **2002**, *18*, 1005−1013.

(6) Dubois, M.; Zemb, T.; Fuller, N.; Rand, R. P.; Parsegian, V. A. *J. Chem. Phys.* **1998**, *108*, 7855−7869.

(7) Ricoul, F.; Dubois, M.; Belloni, L.; Zemb, T. *Langmuir* **1998**, *14*, 2645−2655.

(8) Jokela, P.; Jonsson, B.; Eichmuller, B.; Fontell, K. *Langmuir* **1988**, *4*, 187−192.

(9) Parsegian, V. A.; Rand, R. P.; Fuller, N. L. *J. Phys. Chem.* **1991**, *95*, 4777−4782.

(10) Brotons, G.; Dubois, M.; Belloni, L.; Grillo, I.; Narayanan, T.; Zemb, T. *J. Chem. Phys.* **2005**, *123*, 024704.

(11) Coldren, B.; Warriner, H.; van Zanten, R.; Zasadzinski, J. A.; Sirota, E. B. *Langmuir* **2006**, *22*, 2465−2473.

(12) Coldren, B.; Warriner, H.; van Zanten, R.; Zasadzinski, J. A.; Sirota, E. B. *Langmuir* **2006**, *22*, 2474−2481.

(13) Dubois, M.; Demé, B.; Gulik-Krzywicki, T.; Dedieu, J.-C.; Vautrin, C.; Désert, S.; Perez, E.; Zemb, T. *Nature* **2001**, *411*, 672−675.

(14) Delorme, N.; Dubois, M.; Garnier, S.; Laschewsky, A.; Weinkamer, R.; Zemb, T.; Fery, A. *J. Phys. Chem. B* **2006**, *110*, 1752−1758.

(15) Daicic, J.; Fogden, A.; Carlsson, I.; Wennerström, H.; Jonsson, B. *Phys. Rev. E* **1996**, *54*, 3984−3998.

(16) Fogden, A.; Daicic, J.; Mitchell, D. J.; Ninham, B. W. *Physica A* **1996**, *234* (1−2), 167−188.

(17) Fogden, A.; Ninham, B. W. *Adv. Colloid Interface Sci.* **1999**, *83* (1−3), 85−110.

(18) Delorme, N.; Bardeau, J.-F.; Bulou, A.; Poncin-Epaillard, F. *Thin Solid Films* **2006**, *496*, 612−618.

(19) Zemb, T.; Dubois, M. *Aust. J. Chem.* **2003**, *56* (10), 971−979.

(20) Vliegenthart, G. A.; Gompper, G. *Biophys. J.* **2006**, *91*, 834−841.

(21) Reissner, E. *Bull. Am. Math. Soc.* **1949**, *55* (3), 295−295.

(22) Landau, L. D.; Lifshitz, E. M., *Theory of Elasticity*; Pergamon Press: New York, 1986.

(23) Boal, D., *Mechanics of the Cell*; Cambridge University Press: Cambridge, 2002.

(24) Daillant, J.; Bellet-Amalric, E.; Braslau, A.; Charitat, T.; Fragneto, G.; Graner, F.; Mora, S.; Rieutord, F.; Stidder, B. *PNAS* **2005**, *102*, 11639−11644.

(25) Zavitsas, A. A. *J. Phys. Chem. B* **2001**, *105* (32), 7805−7817.

(26) Gurau, M. C.; Lim, S.-M.; Castellana, E. T.; Albertorio, F.; Kataoka, S.; Cremer, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 10522.

(27) Claessens, M. M. A. E.; van Oort, B. F.; Leermakers, F. A. M.; Hoekstra, F. A.; Cohen, Stuart, M. A. *Biophys. J.* **2004**, *87*, 3882−3893.