

Experimental evidence of the electrostatic contribution to membrane bending rigidity

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(received 25 November 2003; accepted in final form 28 April 2004)

PACS. 87.16.Dg – Membranes, bilayers, and vesicles.

PACS. 41.20.Cv – Electrostatics; Poisson and Laplace equations, boundary-value problems.

PACS. 62.20.Dc – Elasticity, elastic constants.

Abstract. – We have investigated the thermal fluctuations of giant unilamellar dimyristoylphosphatidylcholine vesicles in the presence of both non-ionic and ionic surfactants (peptides) with identical apolar chains. Using vesicle fluctuation analysis, the effects of ionic and non-ionic surfactants upon membrane bending rigidity in the case of no added salt have been determined and the electrostatic contribution thereby isolated. We interpret these experimental findings in terms of a mean-field free-energy model for the adsorption of charged surfactants to a lipid bilayer and couple these results to describe the electrostatic contribution to membrane bending rigidity. This experimental study demonstrates how electrostatics affect the elastic properties of unilamellar bilayers.

Membranes are complex and dynamic entities which serve not only to compartmentalize life but also as the site of many important targeting and signalling events. The membrane is thus an active port of biological function. For example, the localization of proteins to membrane interfaces is sensitive to membrane composition, pH , as well as charge effects. How membrane shape and mechanical properties may be altered by these factors is still not clear.

Embedded in Helfrich's curvature free energy is a description of the mechanical and conformational properties of membranes [1]:

$$\mathcal{F} = \sigma A + \frac{k_c}{2} \int_A dA \left(\frac{1}{r_1} + \frac{1}{r_2} - 2H_0 \right)^2. \quad (1)$$

Here σ defines the effective surface tension which depends upon the total area (A) and volume of the vesicle; r_1 and r_2 are local curvature radii of the membrane; H_0 is the spontaneous curvature; and k_c is the bending elastic modulus, typically of the order 10^{-19} J ($\sim 25 k_B T$) for lipid membranes.

The effect of electrostatics upon k_c has been approached from various theoretical perspectives [2–6] (for a review, see [7]). Experiments, however, have provided less direct evidence of electrostatic effects. Studies of the stability and structure of surfactant-water microemulsions [8–10] have in some cases yielded an indirect measure of k_c which cannot be decoupled from the Gaussian curvature [11–14]. The bending rigidity of such systems has also been

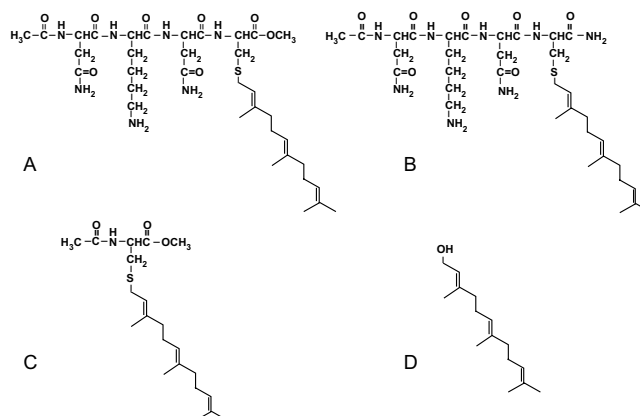


Fig. 1 – Structures of the synthesized farnesylated peptides. The ionic surfactants, (A) Ac-NKNC-(farnesyl)-OMe and (B) Ac-NKNC-(farnesyl)-NH₂. The non-ionic surfactants, (C) Ac-C-(farnesyl)-OMe and (D) farnesol.

determined at a flat interface by ellipsometry (for planar surfactant films) [12,13]. Others have studied electrostatic effects in stacks of membranes, an analysis which is complicated due to the multitude of interactions between layers [15–20]. The bending rigidity of SOPC bilayers with increasing concentrations of the charged lipid POPS has been investigated via tether formation but an electrostatic contribution to k_c could not be detected [21]. A vesicle fluctuation analysis study of phosphatidic acid-lipid mixtures revealed a modest charge effect but other effects of incorporating phosphatidic acid into the membrane are unclear [22].

In this letter, we present evidence of the electrostatic correction to membrane bending rigidity. As thermal fluctuations of membranes are on the order of $k_B T$, they can be observed and analyzed to characterize membrane elasticity (k_c). Using the non-invasive method of vesicle fluctuation analysis (VFA), we demonstrate the effects of both non-ionic and ionic surfactants (fig. 1) upon the bending rigidity of unilamellar dimyristoyl phosphatidylcholine (DMPC) vesicles. As the system is free of added salt, the surface charge density resulting from the adsorption of ionic surfactants is screened only by the non-adsorbed surfactants and surface-inactive counterions. In this minimal, two-component system, surfactant-membrane adsorption and the influence upon membrane elastic properties is thus a delicate interplay between entropic and electrostatic effects. Comparing how both uncharged and monovalent cationic⁽¹⁾ surfactants modify the bending rigidity of large unilamellar vesicles facilitates the determination of the electrostatic contribution to the bending rigidity, k_{el} : $k_c = k_0 + k_{el}$.

Experimental techniques have been described in more detail [23]. In brief, DMPC was obtained from Avanti Polar Lipids (Alabama, USA). Organic solvents and sugars were products of Sigma-Aldrich (Denmark). All materials were used without further purification. Farnesylated peptides were obtained by a combination of solid-phase and solution synthesis methods [23]. Giant unilamellar vesicles (GUVs) were cultivated by swelling dried DMPC-peptide/farnesol films in a ~ 75 mM sucrose solution [24] and were resuspended in a ~ 76 mM glucose solution to enhance phase contrast and gravitationally stabilize the vesicles [25]. Solution osmolarities were regulated using a freezing-point osmometer (Model 3D3, Advanced Instruments Inc., Norwood, Mass., USA). Partitioning of farnesylated peptides into model membranes has been experimentally quantified [26].

⁽¹⁾Without loss of generality.

Vesicle fluctuation analysis (VFA) was used to analyze the thermal fluctuations in the contour of the GUVs at 37 °C. Visualizing the vesicle's two-dimensional contour in the focal plane of the objective by phase contrast microscopy (Zeiss Axiovert S100, Göttingen, Germany), a series of 4000 contours was captured and analyzed in real time. A single snapshot required 4 ms to obtain and the time interval between consecutive images was 40 ms. Based on a procedure as described in Henriksen *et al.* [27], values for the bending rigidity, k_c , were extracted where k_c and the effective reduced tension, $\frac{\sigma R^2}{k_c}$, are fitting parameters. Each determined value for k_c is an average of between 3–10 different vesicles of diameter 15–25 μm . Error bars represent the standard deviation amongst a population of vesicles.

Both uncharged species, farnesol and Ac-C-(fsl)-OMe, are found to modestly decrease k_c . Concentrations of 5.0 mol% of the uncharged peptide and farnesol are observed to slightly reduce k_c to $1.32 \pm 0.07 \times 10^{-19}$ J and $1.34 \pm 0.04 \times 10^{-19}$ J from $1.40 \pm 0.03 \times 10^{-19}$ J for pure DMPC. Accounting for experimental error, this represents no significant change. Even larger concentrations of farnesol (25 mol%) only modestly decrease k_c to $1.30 \pm 0.05 \times 10^{-19}$ J. As farnesol and Ac-C-(fsl)-OMe have a very high affinity for the membrane [23], it can be concluded that these non-ionic surfactants have a very small effect upon k_c .

The charged surfactants, on the other hand, induce a significant *increase* in k_c : with 5.0 mol% added, Ac-NKNC-(farnesyl)-OMe increases k_c to $1.59 \pm 0.03 \times 10^{-19}$ J and Ac-NKNC-(farnesyl)-NH₂ to $1.72 \pm 0.03 \times 10^{-19}$ J. After the series of dilutions required for VFA, the concentration of adsorbed charged peptides is estimated to be ≤ 1 mol% [23]. (This estimate emerges from the bulk peptide concentration and the approximate peptide-DMPC partition coefficient as determined in [23].) This change in k_c is thus quite dramatic, especially in contrast to how a much higher concentration of farnesol has a very small *diminishing* effect upon k_c . Having determined the effects of the farnesyl chain upon membrane elastic properties in this way allows us to isolate the electrostatic contribution to membrane bending rigidity.

Variations in peptide concentration (2.5 mol% *vs.* 5.0 mol% Ac-NKNC-(fsl)-OMe) reveal that this increase in k_c is not very sensitive to the bulk concentration of peptide — a reflection of the self-regulating nature of this no-added-salt system of surfactants and their counterions.

The slight variation between the two peptide species, Ac-NKNC-(farnesyl)-OMe and Ac-NKNC-(farnesyl)-NH₂, may be attributed to a small difference in the partitioning coefficient due to the presence of the carboxyl methyl group (-OMe).

Theories on the electrostatic correction to bending rigidity have approached the problem from various perspectives [2–6] (for a review see [7]). Mindful that our system has no added salt and that the surfactants can contribute to both interfacial charge density and electrostatic screening, we consider the effects of charged lipopeptide adsorption upon membrane bending rigidity in terms of the dimensional estimate of Pincus *et al.* [4]. As determinations of the electrostatic contribution to bending rigidity for various systems show that the scale of k_{el} is independent of geometry [2, 28] and boundary conditions [7], this scaling form is thus representative of the basic form of k_{el} :

$$k_c = k_0 + k_{el}, \quad (2)$$

$$\frac{k_{el}}{k_B T} = c \cdot \frac{(n_0^+)^2 l_B}{\kappa^3}, \quad (3)$$

where $l_B = \frac{e^2}{\epsilon k_B T}$ is the Bjerrum length⁽²⁾, $\kappa^{-1} = \left(\frac{8\pi e^2 n_b^+}{\epsilon k_B T} \right)^{-1/2}$ the Debye screening length, n_0^+ the surface density of charged surfactants, n_b^+ the concentration of surfactants in bulk

⁽²⁾Expressed in c.g.s. units.

TABLE I – *Bending rigidity, k_c , of DMPC GUVs containing uncharged and charged surfactants at 37°C. Some data has been published in [23]. Due to a series of dilutions required for VFA experiments, the concentration of the charged peptide adsorbed to the membrane is estimated to be lower than the concentration added.*

	Surfactant	k_c ($\times 10^{-19}$ J)
	0.0 (DMPC)	1.40 ± 0.03
Uncharged	Ac-C-(farnesyl)-OMe (5.0 mol%)	1.32 ± 0.07
	farnesol (2.5 mol%)	1.34 ± 0.03
	farnesol (5.0 mol%)	1.34 ± 0.04
	farnesol (25 mol%)	1.30 ± 0.05
Charged	Ac-NKNC-(farnesyl)-OMe (2.5 mol% added)	1.60 ± 0.08
	Ac-NKNC-(farnesyl)-OMe (5.0 mol% added)	1.59 ± 0.03
	Ac-NKNC-(farnesyl)-NH ₂ (5.0 mol% added)	1.72 ± 0.03

(*i.e.* those not adsorbed to the surface), c a prefactor of order unity, and $k_c = k_0$ for the case of uncharged surfactants. The electrostatic contribution, k_{el} , is sensitive to very small concentrations of surfactant at the interface (small n_0^+). We are unable to precisely quantify the fraction of adsorbed ionic surfactants (n_0^+). However, this value can be approximated using the Davies adsorption isotherm which accounts for electrostatic effects [29,30] together with experimental evidence of the binding energy of the farnesyl chain to membranes (*i.e.* the non-electrostatic binding energy) [26]. Estimations of n_0^+ using this theory in combination with eq. (3) predict k_{el} to be several $k_B T$ for surface concentrations of ≤ 1 mol%⁽³⁾.

With increasing bulk charge density, k_{el} attains a plateau value which remains consistent over a large concentration regime (10^{-5} – 10^{-6} M). This robustness is reflected in the experimental results which show little variation of the electrostatic contribution despite variations in concentration of the peptides (table I). The significant increase in k_c due to k_{el} is in accordance with the experimental findings. (At high bulk concentrations, k_{el} begins to decrease due to the screening effect of the non-adsorbed surfactants.)

Considering our experimental parameters, this mean-field theory provides an accurate description of charged surfactant-membrane interactions in the low concentration limit investigated. The bulk surfactant concentration is well below that of the critical micelle concentration [31] ensuring the surfactants are in monomer form. Interactions between peptides—and thus fluctuations in charge density—are minimal [32]. The surfactants can, however, exchange between the membrane and aqueous phases [26]. As the charges are adsorbing, mobile, and can redistribute on the flexible membrane surface, this implies that the system is charge regulating with boundary conditions that interpolate between those of Dirichlet (constant potential) and Neumann (constant surface charge) [33]. In the linear regime, however, it has been shown that both boundary conditions yield the same scaling form (eq. (3)) where only c differs [6,28].

A number of theoretical approximations for k_{el} challenge a detailed comparison between experiment and theory. Most calculations of k_{el} (including eq. (3)) are performed in the Debye-Hückel approximation. Accounting for the Gouy-Chapman layer results in a slower decay of the electric potential and thicker electric double layer; k_{el} thus likely represents a lower limit of the electrostatic contribution to bending. This makes a calculation of k_c in the linear regime an unrealistic approximation. Considering that the thickness of the membrane is much less

⁽³⁾Values of $\alpha = 10 k_B T$, the peptide's dimension, $a^+ = 6 \text{ \AA}$, $n_0^+(a^+)^2 \sim 0.001$, and $c = \pi$ yields $k_{el} \sim 3 k_B T$ [6,30].

than that of the Debye screening length, the two monolayers are in reality coupled. (The case of coupled monolayers has been considered [34].) Image charges should also be considered. Furthermore, the Davies adsorption isotherm assumes the adsorption of surfactants to be independent of membrane curvature. To be completely rigorous, the experiments will be refined using peptides whose partitioning into membranes can be precisely quantified by, for example, fluorescence. In conjunction with a systematic study over a more varied concentration regime, a deeper quantitative correlation between theory and experiment will be developed.

In the present study we have established a significant enhancement of the bending rigidity of the membrane due to charge effects. We find good harmony between the theoretical estimate and experimental observations ($k_{el} \sim 3-5 k_B T$). This result is of the same order of k_{el} observed in weakly charged lamellar phases (k_{el} up to $2 k_B T$) [15]. A similar order of k_c increase was observed in lipid-phosphatidic acid mixtures but no attempt was made to extract k_{el} [22]. This study is distinct in that we compare ionic *vs.* non-ionic surfactant effects upon membrane bending rigidity and are thus able to isolate the electrostatic contribution, k_{el} . Such a self-regulating charged surfactant-membrane system can provide a more accessible platform than previously chosen systems upon which future studies of electrostatics and membrane elasticity can be based.

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We are grateful to J. BRASK and K. J. JENSEN for synthesizing the peptides. Thanks are also due to P. A. PINCUS and R. PODGORNİK for insightful discussions and J. R. HENRIKSEN for software production. ACR is supported by a Julie Payette Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) and MEMPHYS - Centre for Membrane Biophysics by the Danish National Research Foundation.

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