

Opposing Effects of Cation Binding and Hydration on the Bending Rigidity of Anionic Lipid Bilayers

M. M. A. E. Claessens,[†] F. A. M. Leermakers,^{*,†} F. A. Hoekstra,[‡] and M. A. Cohen Stuart[†]

Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, the Netherlands, and Laboratory of Plant Physiology, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, the Netherlands

Received: January 4, 2007; In Final Form: March 28, 2007

We correlate the molecularly realistic self-consistent field predictions for the mean bending modulus k_c of charged lipid vesicles with experimental observations of the size R of corresponding vesicles that are produced by the freeze–thaw method. We elaborate on the Ansatz that the bending modulus is related to the membrane persistence length and that this length scale sets the radius of the vesicles. Alkali cations have a remarkable effect on the mean bending modulus and thus on the equilibrium radius of negatively charged entropically stabilized dioleoylphosphatidylglycerol (DOPG) vesicles. Where cation hydration typically results in thicker and thus stiffer membranes, specific adsorption to the bilayer surface results in a decrease of the surface charge density and the thickness of the membrane-associated electric double layer. As a result of these opposing effects on k_c and R , the largest DOPG vesicles are found in the presence of K^+ , which combines an intermediate hydration enthalpy and PG-binding affinity.

Introduction

Biological membranes are typically negatively charged. This is mainly because approximately 5–20% of the lipids in the membranes are anionic phospholipids. The negative surface charge and the resulting negative membrane potential make the membrane very sensitive to interactions with cations. This is probably one of the reasons cations are involved in so many membrane-related processes. An increase in the intracellular concentration of Ca^{2+} , for example, is thought to be a requirement for intracellular fusion and a signal for exocytosis in many secretory cells.^{1,2}

In order to obtain electroneutrality in the solution close to the negatively charged surface, the membrane potential ψ_0 is accompanied by a diffuse layer of cations in solution. As a result, the electrostatic potential drops with the distance x from the membrane surface. At low potentials ($ze\psi_0/(k_B T) < 1$), the potential profile $\psi(x)$ can be written as $\psi(x) = \psi_0 e^{-\kappa x}$ in which κ^{-1} is the Debye length given by $\kappa^2 = 2Qc z^2$. In this equation, c is the salt concentration, z the valency of the ions, and the Bjerrum length Q is given by $e^2/(4\pi\epsilon_0\epsilon_r k_B T)$, in which e is the elementary charge and ϵ_0 is the dielectric permittivity. From the expressions, it is clear that the double layer extends further into the bulk solutions at low salt concentration and low valency.

The surface charge and presence of the electric double layer will influence the mechanical properties of the membrane. The electrostatic part of the mean bending modulus k_c of the charged bilayer follows from the properties of the electric double layer that is associated with the membrane surface. Within a mean field approximation, the electrostatic contribution to k_c , k_c^{el} can be calculated on the basis of the Poisson–Boltzmann equation.^{3,4} In this analysis, the electrical free energy for a charged cylindrical surface is expanded in the inverse powers of the radius of curvature of the cylinder, R . The k_c^{el} can subsequently

be obtained from the coefficient of the quadratic term in this expansion. For low surface charge densities, this results in^{3,4} $k_c^{\text{el}} = \frac{3}{2}\pi(k_B T)Q\sigma^2/\kappa^3 e^2$ in which σ is the surface charge density. The mean bending modulus k_c is therefore predicted to decrease with increasing salt concentration and valency, when the surface charge density is assumed to be fixed $k_c^{\text{el}} \propto c_s^{-3/2}$. However, screening of the headgroup charges by the counterions will promote a closer packing of the phospholipids. This gives rise to an increase of σ and therefore an increase in k_c^{el} . Moreover, closer packing of the phospholipids leads to thicker bilayers and therefore to an increase of the overall (nonelectrostatic) k_c . Both aspects thus counteract the κ^{-3} effect.

There is yet another way in which cations can affect k_c^{el} . By binding to negatively charged membrane, cations decrease the surface charge density σ , which results in a decrease in k_c^{el} . The binding of ions to model phospholipid bilayers has been studied extensively. It has been well-established that cations bind to the phosphate group of the phospholipids. Although there is still no consensus on the exact values of the binding constants, the effectiveness of cation binding decreases in the order $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$.^{5–9} This already indicates that the effective surface charge and thus k_c of a phospholipid bilayer will be affected by the cation present in solution.

The dependence of k_c (and k_c^{el}) on the concentration and type of cations is expected to influence the equilibrium radius of charged phospholipid vesicles. For entropic reasons, the preferred aggregate structure of a charged phospholipid bilayer is the vesicle. The radius R of entropically stabilized vesicles depends to a large extent on the persistence length of the bilayer ξ_p . This can be understood in the following way: the translational entropy will favor many small and thus strongly curved vesicles, but bending bilayers on a length scale much smaller than ξ_p will cost energy because the bilayer will lose undulation entropy.^{10,11} As R follows ξ_p and ξ_p is an exponential function of k_c , it seems plausible that, in first order: $R \propto \xi_p \propto e^{k_c/k_B T}$. A

[†] Laboratory of Physical Chemistry and Colloid Science.

[‡] Laboratory of Plant Physiology.

previous study, in which experiments on the radius of DOPG vesicles were correlated with self-consistent field predictions on k_c of a charged bilayer membrane, revealed that the experimentally found R and the corresponding theoretical predictions of k_c have very similar ionic strength dependencies.^{10,11}

The stability of vesicles is not only determined by the mean bending modulus, but we argued before^{10,11} that there is also a very important role for the Gaussian bending modulus \bar{k} . Thermodynamically stable vesicles only exist when the bending energy $E_{\text{ves}} = 4\pi(2k_c + \bar{k})$ is compensated by entropy. A negative Gaussian bending modulus stabilizes the vesicles. Experimentally, not much is known about the value of the Gaussian bending modulus. From theoretical evaluations, however, we generally find that \bar{k} is sufficiently negative such that the bending energy E_{ves} can be small (order $10k_B T$). Such a value can easily be compensated by, e.g., translational entropy, undulation entropy, or entropy associated with the polydispersity, and the vesicles are thermodynamically stable. Note that we do not claim that all vesicle systems are automatically near their equilibrium size. We will return to this below. In our theoretical consideration discussed below, we will focus on the mean bending modulus, because in our opinion this parameter is more directly linked to the equilibrium size of the vesicles.

Whereas anions adsorb to neutral lipids and determine the membrane interactions,^{11,12} they do not specifically interact with anionic phospholipids. As a result, the influence of a series of anions on the equilibrium radius of negatively charged vesicles is relatively simple and can be understood from the hydration characteristics of the anions.¹⁰ Strongly hydrated anions decrease the solvent quality for the lipid tails and therefore tend to dehydrate the membrane. This dehydration leads to thicker membranes with an increased k_c . Differences in the equilibrium radius of DOPG vesicles in NaCl, NaBr, and NaI solutions could be attributed to differences in the hydration of Cl^- , Br^- , and I^- . In the case of cations, hydration is therefore also expected to influence k_c and thus R of negatively charged phospholipid vesicles.

Here, we will report on the effect of alkali cations on the equilibrium radius of entropically stabilized negatively charged dioleoylphosphatidylglycerol (DOPG) vesicles. Because of ion hydration and specific binding to the PG headgroup, cations are, on top of the generic electrostatic screening effects, expected to affect the equilibrium radius of negatively charged vesicles in a nontrivial way. Self-consistent field predictions will be used to understand the physics underlying the experimental findings.

Materials and Methods

Experiments. The anionic phospholipid dioleoylphosphatidylglycerol (DOPG) is purchased as a sodium salt from Avanti Polar Lipids (Birmingham, AL), and used without further purification. Analytical grade NaCl, KCl, and CsCl are obtained from Merck (Darmstadt, Germany), LiCl from J. T. Baker Chemicals (Deventer, Holland), and RbCl from Fluka.

The experimental procedure to obtain equilibrium vesicles has been described in detail elsewhere.¹⁰ Briefly, DOPG is dried from a chloroform solution under a stream of nitrogen. Traces of solvent are removed from the lipid film by storing it for at least 2 h under vacuum. The dry lipid film is then rehydrated with a salt solution of the desired ionic strength to a concentration of 2.5 mM lipid. This solution is subjected to repetitive cycles of freezing in liquid nitrogen and thawing. The freeze–thaw cycling results in fragmentation and resealing of the vesicles.¹³ After approximately 15 freeze–thaw cycles, the

vesicle radius no longer changes with increasing number of cycles. Apparently, subsequent cycles have no effect on the redistribution of material between the vesicles. At low ionic strength, the lipids have a relatively high solubility in water, and the vesicles spontaneously evolve to this radius by a kind of Ostwald ripening.¹⁰ Moreover, the dependence of the radius R on the lipid concentration agrees with what literature predicts for vesicles stabilized by undulations and translational entropy.^{10,11,14,15} We therefore assume that the vesicles produced by the freeze–thaw method are close to thermodynamic equilibrium, so that their radius is a thermodynamically regulated quantity.

Electrophoretic mobility measurements are performed on a Malvern Zetasizer 3 (Malvern Instruments, U.K.). The vesicles that are used in these experiments are obtained by extrusion of a rehydrated DOPG solution of 25 mM through a 100 nm polycarbonate filter. In this way, a rather monodisperse solution of vesicles with a radius of approximately 60 nm is obtained for all salt solutions used. This makes the comparison between the different cations more easy. Note that these vesicles typically are not at their equilibrium size. The relaxation of these systems toward equilibrium is, however, so slow that this does not interfere with the electrophoretic mobility measurements.

Vesicle radii are determined using dynamic light scattering. The instrumental setup consists of an ALV-5000 correlator, a scattering device with an ALV-125 goniometer, and a multiline Lexel AR laser source. The data are collected at an angle of 90° at a wavelength of 513 nm. During the measurements, the temperature was kept constant at 293 K. Before each measurement, the samples are diluted with electrolyte solution. A cumulant analysis is used to determine the average hydrodynamic vesicle radius from the intensity autocorrelation function. Minor differences in medium viscosity between the salts and changes in viscosity with ionic strength are not accounted for. Although this does weakly affect the evaluation of the absolute values of the hydrodynamic radii, it has no influence on the observed trends.

Calculations. The self-assembly of lipidlike molecules into bilayers in both flat and homogeneously curved geometries are routinely studied in a self-consistent field (SCF) framework. As in our previous work,¹⁰ we use a discretization scheme of Scheutjens and Fleer¹⁶ to solve for the equilibrium structure (in terms of total volume fraction profiles) of the (curved) bilayers. Besides structural information, we obtain corresponding data on thermodynamic and mechanical properties of these bilayers. Here, we focus on the mechanical parameters. Even though details can be found in various places, e.g., see refs 10 and 16, it is useful to briefly mention the main premises and assumptions.

Fundamental to our SCF theory is that freely jointed chain conformations of the lipid molecules are generated on a lattice. In the lattice, the lattice sites are arranged in layers. The geometry of these layers has to be prefixed. When the layers are flat, we can evaluate the properties of flat bilayers; in cylindrical or spherical lattice geometries, we find corresponding properties of curved bilayers. The equilibrium distribution of the molecules is found from combining the statistical weights of all possible and allowed conformations. The statistical weight of each conformation is given by the Boltzmann weight, basically the exponent of (minus) the self-consistent potential of this conformation normalized by the thermal energy. The self-consistent potential contains terms due to the charge of the molecules (as in the Poisson–Boltzmann theory of charged interfaces) and terms due to the volume of the segments. Short-

range nearest-neighbor interactions are estimated by the Bragg–Williams approximation wherein the probability of a contact with some segment is given by a three-layer average of the volume fraction of that segment. The interactions are parameterized by Flory–Huggins χ parameters. As the system is assumed to be incompressible (all lattice sites are occupied), it turns out that one needs to define interaction parameters for unlike contacts only (the values that are used are discussed below). One can show that, when the segment potential profiles are consistent with the volume fraction profiles, the underlying free energy is optimized. This optimized free energy, which is a functional of both the segment potentials as well as the volume fractions, is used to extract the grand potential for the system. The tensionless bilayers (grand potential per unit area vanishes) are the reference point for further investigations. Spherically as well as cylindrically curved bilayers with well-defined radii are generated in a corresponding coordinate system. For a given number of lipids in the system, the area of the membrane can adjust freely such that the bilayers found are automatically tensionless. As a consequence, the grand potential is fully attributed to the curvature energy.

The radius R of a vesicle is found by the first moment over the radial volume fraction profiles. The mean and Gaussian curvatures follow trivially from this. The mechanical parameters of the lipid bilayer can be obtained unambiguously¹⁷ from fitting the Helfrich equation to the grand potential of both spherically and cylindrically curved vesicles. The Helfrich equation¹⁸ describes the surface tension γ of a bilayer as a second-order expansion in the mean curvature $J = 1/R_1 + 1/R_2$ and the Gaussian curvature $K = 1/R_1R_2$, in which R_1 and R_2 are the two radii of curvature that locally describe the bilayer. The flat

$$\gamma(J, K) = \gamma_0 + \frac{1}{2}k_c(J - J_0)^2 + \bar{k}K \quad (1)$$

equilibrium membrane is tensionless, and thus we set $\gamma(0, 0) = 0$. As a consequence, eq 1 reduces to

$$\gamma(J, K) = -k_cJ_0J + \frac{1}{2}k_cJ^2 + \bar{k}K \quad (2)$$

The spontaneous curvature J_0 is thought to be the curvature that minimizes the curvature free energy of a cylindrical (tube) vesicle. In all systems discussed below, it is found that $J_0 = 0$. This result is easily justified because the spontaneous curvatures of the two monolayers J_0^m cancel in the bilayer configuration. The Gaussian bending modulus \bar{k} determines the topology of the interface, whereas its rigidity is determined by the mean bending modulus k_c . Again, as told above, for negatively charged lipids we often find \bar{k} to be much smaller than zero. This negative value lowers the curvature energy of the vesicles to such low values that entropic contributions (translation, undulation) can balance it. Here, we will not go into these details.

Even though the \bar{k} is routinely calculated, we will focus below on the trends found for the mean bending modulus. The procedure that is followed is rather simple. We consider a cylindrically curved bilayer $K = 0$, $J = 1/R$, the surface area per unit length of the cylinder is given by $A = 2\pi R$. For this case, eq 2 simplifies to

$$\gamma A = \pi k_c J \quad (3)$$

As a result, k_c can be obtained directly from the slope of the γA vs J plots.

We consider noninteracting (dilute) lipidlike bilayers in an aqueous solution with a given ionic strength. Special attention

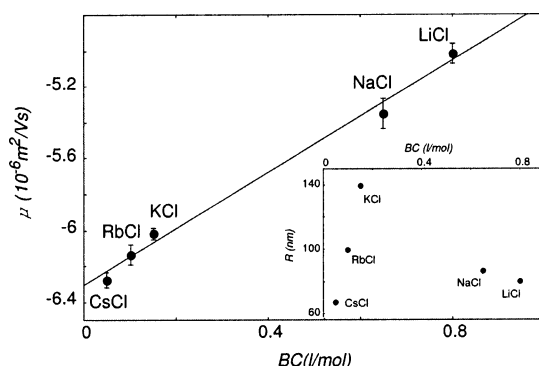


Figure 1. The electrophoretic mobility μ of DOPG vesicles in 100 mM of an alkali chloride as a function of the tabulated binding constant⁹ (BC) of the cation with the phosphatidylglycerol headgroup. Inset: The hydrodynamic radius of DOPG vesicles, obtained using the freeze–thaw method, in 100 mM alkali chloride solution as a function of the BC.

is paid to the parameters that specify the interactions of the cations with the lipids. The lipid molecule is modeled, as in previous studies, as a chain molecule $C_{18}X_2C_2X_2C_{18}$. Here, the C_{18} segments stand for the hydrophobic phospholipid tail, while the $X_2C_2X_2$ represents the hydrophilic headgroup. Each segment X carries a charge of $-1/4$ e, making the total charge per lipid -1 e. The water molecules are modeled as a simple monomer solvent W . The system further contains monomeric cations and anions to account for the salt present. These monomers carry a charge of $+1$ e and -1 e, respectively. The hydration of ions is taken into account by the choice of the Flory–Huggins interaction parameters, $\chi_{anion-W}$ and $\chi_{cation-W}$, where the stronger hydrogen bonding properties are represented by the more negative interaction parameters. The binding of cations to the anionic lipid headgroup is achieved by choosing negative values for $\chi_{cation-X}$. The headgroup of the lipid molecule is made more soluble in water by choosing $\chi_{X-W} = -2$. In all calculations, the following segment-type dielectric constants are used: $\epsilon_C = 2$, $\epsilon_X = \epsilon_{cation} = \epsilon_{anion} = 5$, $\epsilon_W = 80$. The local dielectric constants are computed from a volume fraction weighted average.

Results

Experiments. By binding to a negatively charged DOPG bilayer, cations lower the surface charge on the DOPG vesicle. To check whether the surface charge on the vesicles is in agreement with the binding constants (BC) tabulated in the literature,⁹ the electrophoretic mobility, μ , of extruded vesicles with a radius of approximately 60 nm in 100 mM of an alkali chloride solution is measured. It is found that the electrophoretic mobility of the DOPG vesicles is almost linearly related to the binding constants of the alkali cations (Figure 1). The stronger the cations bind to the phospholipid headgroup, the lower the surface charge and therefore the electrophoretic mobility are. The charge density on the DOPG vesicles therefore decreases in the presence of alkali cations in the order $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$.

The binding of the ions is expected to influence the equilibrium radius of DOPG vesicles. Literature predictions indicate that the lowering of the surface charge will result in a decrease in k_c .^{3,4,19} To observe this effect, solutions of 2.5 mM DOPG in a series of alkali chloride concentrations were subjected to freeze–thaw cycles to obtain vesicles that are close to thermodynamic equilibrium. The hydrodynamic radius of the resulting vesicles is a nontrivial function of the ionic strength for all monovalent salts used, as can be seen in Figure 2.

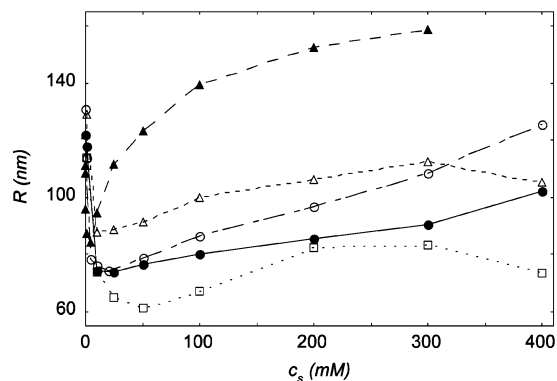


Figure 2. Effect of the alkali metal concentration on the equilibrium radius of DOPG vesicles. The graph shows the radius of DOPG vesicles after 15 freeze–thaw cycles in (●) LiCl, (○) NaCl, (▲) KCl, (△) RbCl, and (□) CsCl.

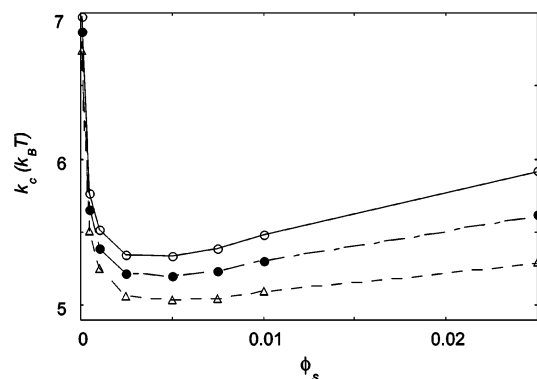


Figure 3. The mean bending modulus as a function of the ionic strength. Cation hydration has a profound influence on the mean bending modulus k_c predicted by SCF theory. Data are shown for $\chi_{\text{cation-W}} = -3$ (○), $\chi_{\text{cation-W}} = -2$ (●), and $\chi_{\text{cation-W}} = -1$ (△); $\chi_{\text{anion-W}}$ was kept constant at -2 .

The overall behavior of R as a function of ionic strength appears to be the same for all salts tested. At low ionic strength, up to approximately 10–50 mM, the vesicle radius decreases, while at higher salt concentrations, R increases with ionic strength. When the vesicle radii in 100 mM salt are compared, those produced in CsCl are the smallest. The sizes of the vesicles increase when they are made in LiCl, NaCl, RbCl, and KCl, respectively. Figure 1 shows that, when the vesicle radius is plotted as a function of the binding constants of the alkali cations to the phosphatidylglycerol headgroup,⁹ this gives rise to a maximum in R for K^+ ions.

Calculations. Our working hypothesis is that the radius of the entropically stabilized vesicles is controlled by k_c . To determine the effects of cation hydration and binding on k_c , k_c was determined from SCF calculations for cylindrically shaped vesicles ($K = 0$). The general behavior of k_c as a function of ionic strength was approximately the same for all parameter sets tested. At low ionic strength, k_c was observed to decrease, whereas at high ionic strength, k_c increased with the volume fraction of salt in the system. The effects of cation hydration and binding on k_c were first studied separately.

Cation hydration was modeled by changing $\chi_{\text{cation-W}}$, while keeping $\chi_{\text{anion-W}}$ constant at -2 . In these calculations, the cations were assumed not to bind specifically to the anionic headgroup; $\chi_{\text{cation-X}} = 0$. The results are comparable to those found earlier for changing anion hydration.¹⁰ At all ionic strengths tested, the most negative values for $\chi_{\text{cation-W}}$ gave rise to the largest values for k_c (Figure 3).

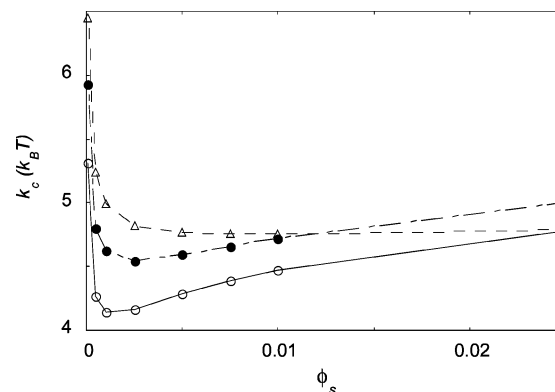


Figure 4. SCF predictions for the effect of the binding of cations to the segments X in the lipid headgroup on the mean bending modulus k_c of a charged lipid bilayer. Data are shown for $\chi_{\text{cation-X}} = -5$ (○), $\chi_{\text{cation-X}} = -15$ (●), and $\chi_{\text{cation-X}} = -25$ (△).

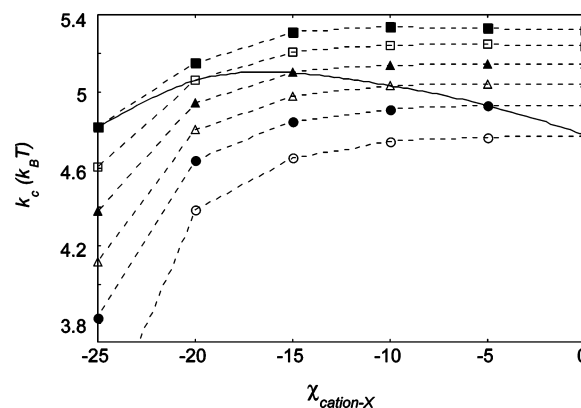


Figure 5. The mean bending modulus k_c as a function of $\chi_{\text{cation-X}}$. Data are shown for $\chi_{\text{cation-W}} = 0$ (○), $\chi_{\text{cation-W}} = -0.5$ (●), $\chi_{\text{cation-W}} = -1$ (△), $\chi_{\text{cation-W}} = -1.5$ (▲), $\chi_{\text{cation-W}} = -2$ (□), and $\chi_{\text{cation-W}} = -2.5$ (■). The solid line exemplifies the expected behavior of realistic cations.

The influence of the binding of cations to the lipid headgroup on k_c is more complex. Cation binding was mimicked by choosing a negative χ -parameter for the interaction between the cations and the segments X in the model lipid. Due to the low density of X segments in the bilayer surface, the values that have to be chosen for $\chi_{\text{cation-X}}$ are rather high before some effect is observed. This is a natural artifact of the (Bragg–Williams) mean-field approximation; in Figure 4, we show the typical result. At low and intermediate salt concentrations, strong binding leads to decreased values of k_c . At high ionic strength, the k_c curves belonging to different values of $\chi_{\text{cation-X}}$ can cross, and the behavior of k_c with $\chi_{\text{cation-X}}$ becomes more complicated.

The results of varying $\chi_{\text{cation-W}}$ and $\chi_{\text{cation-X}}$ simultaneously can be seen in Figure 5. In this figure, we present predictions for the overall mean bending modulus k_c for several values of degrees of cation hydration ($\chi_{\text{cation-W}}$) as a function of the specific binding of the cation on the lipid headgroup ($\chi_{\text{cation-X}}$). Stronger binding of ions to the lipid headgroup, and thus more negative values of $\chi_{\text{cation-X}}$, lead to a decrease in k_c for all $\chi_{\text{cation-W}}$ values tested. Further, the more negative values of $\chi_{\text{cation-W}}$ give rise to higher values of k_c .

In reality, the most strongly hydrated alkali cations have the largest binding affinity for the DOPG headgroup. As a result, cation hydration and cation binding have antagonistic effects on k_c of the lipid membrane. The solid line in Figure 5 exemplifies the expected behavior of real cations; it combines large $\chi_{\text{cation-W}}$ with $\chi_{\text{cation-X}}$ values, intermediate $\chi_{\text{cation-W}}$ with

$\chi_{\text{cation-X}}$, and $\chi_{\text{cation-W}}$ with $\chi_{\text{cation-X}}$ values. When the hydration and binding properties of real ions are considered, the combinations of high $\chi_{\text{cation-W}}$ with high $\chi_{\text{cation-X}}$ and low $\chi_{\text{cation-W}}$ with low $\chi_{\text{cation-X}}$ give rise to a maximum in k_c as a function of $\chi_{\text{cation-X}}$ (Figure 5). The exact position of the maximum is difficult to predict, as it is hard to determine the values of the χ -parameters for realistic cations.

Discussion

The general behavior of and correlation between the measured radius R and predicted k_c of charged lipid bilayers as a function of the ionic strength has been discussed before.^{10,11} For the alkali salts tested here, the same trends were observed. At low ionic strength, the vesicle radius decreases, whereas at high ionic strength, a slight increase in R is observed with increasing salt concentration. Similar behavior is seen for k_c as a function of the ionic strength for all parameter settings.

The initial decrease in k_c and R at low salt concentration is mainly a result of the decrease in electric double layer thickness. The electric double layer has to bend with the lipid bilayer; its presence therefore results in an increase of k_c . Upon addition of more salt, the thickness of the electric double layer decreases, and therefore k_c decreases. This decrease is partly counteracted by closer lipid packing when the headgroup charges get screened by counterions. Closer packing of the lipids leads to thicker and stiffer membranes, which results in an increased k_c . As analytical theoretical predictions generally neglect these changes in lipid packing, the observed changes in k_c and R with ionic strength do not follow theoretical predictions on k_c^{el} as a function salt concentration.^{3,4}

At a certain ionic strength, the effect of a decrease in electric double layer thickness becomes negligible compared to the increase in lipid bilayer thickness. This increase in the thickness of the lipid bilayer is not only caused by the effects of charge screening; differences in hydration between the cations also appear to play a role. Strong ion hydration and therefore more negative values of $\chi_{\text{cation-W}}$ give rise to higher values of k_c and, thus, larger vesicles. This effect is general for hydrated ions in the presence of a lipid bilayer; it was previously observed for a series of anions.¹⁰ In the presence of the more strongly hydrated Cl^- ions, the freeze-thaw method gave larger vesicles compared to the radii found in the presence of Br^- and I^- . Calculations showed that when hydration is taken into account the bilayers become thicker with increasing anion hydration.¹⁰ It is however not yet clear whether the ions directly dehydrate the membrane or if the effect is due to differences in solubility of the hydrated ions in the apolar part of the bilayer. The increase in the gel-to-liquid-crystalline phase transition temperature observed for the zwitterionic phospholipid dipalmitoylphosphatidylcholine in the presence of alkali chlorides²⁰ supports the idea that the ions dehydrate the bilayer membrane and cause the lipids to pack into thicker membranes.

At very high ionic strength, the vesicle radius passes through a maximum (Figure 2). This maximum is probably related to a maximum in the bilayer thickness and is comparable to the maximum in the equilibrium thickness found for free non-ionic film as a function of the salt concentration.²¹ Although we do not fully understand this behavior, it is probably related to the structure of the salt solution at high ionic strength.

The data on alkali chlorides presented here cannot be explained by ion hydration alone. In contrast to the data on equilibrium vesicles in the presence of different anions,¹⁰ the radii of DOPG vesicles in the presence of the alkali cations do not simply increase with their hydration enthalpies ($\text{Li}^+ > \text{Na}^+$

$> \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$). Specific binding of the cations to the anionic DOPG headgroup is probably responsible for this anomaly. Unlike cations, anions are not expected to bind to similarly charged membranes. This makes their behavior apparently easier to interpret.

By adsorbing to a negatively charged phospholipid bilayer, cations change the surface charge of the membrane. With the BC of the cations taken into consideration, the surface charge of phosphatidylglycerol vesicles is expected to decrease in the order $\text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$. Measurements on the electrophoretic mobility of extruded vesicles in 100 mM salt support this assumption (Figure 1). k_c^{el} is predicted to scale with the surface charge density as $k_c^{\text{el}} \propto \sigma^2$,^{3,4,19} cation binding is therefore expected to decrease k_c . As mentioned before, however, these theoretical analytical predictions do not account for changes in lipid packing, which also changes k_c . When the binding of ions to the lipid headgroup is included in the SCF calculations, which do allow for changes in lipid packing, k_c is still observed to decrease with increasing $\chi_{\text{cation-X}}$ (Figure 4). The decrease in k_c at low ionic strength is largest for the cation that binds most strongly to the lipid headgroups, i.e., with the most negative $\chi_{\text{cation-X}}$. This means that cations do not only affect k_c by decreasing the double layer thickness, but also by directly lowering σ .

So far, the effects of cation hydration and binding were considered separately. Strong hydration tends to increase k_c , whereas strong binding leads to a decrease in k_c . In reality, the most strongly hydrated alkali cation, Li^+ , also has the highest affinity for the DOPG headgroup, while the weakly hydrated ions Cs^+ has a much lower binding affinity for the PG headgroup. The opposing effects of ion hydration and cation binding are probably responsible for the observed dependence of R on the presence of the alkali cations. Calculations in which $\chi_{\text{anion-W}}$ and $\chi_{\text{anion-X}}$ data are combined show that, when combinations of low $\chi_{\text{anion-W}}$ and $\chi_{\text{anion-X}}$, intermediate $\chi_{\text{anion-W}}$ and $\chi_{\text{anion-X}}$, and high $\chi_{\text{anion-W}}$ and $\chi_{\text{anion-X}}$ values are compared, negatively charged bilayers have the highest k_c in the presence of a cation with intermediate binding and hydration properties (Figure 5). This is in agreement with the finding that equilibrium DOPG vesicles are the largest in the presence of KCl (Figure 1).

Conclusions

In this study, we combined predictions of a molecularly realistic self-consistent field theory with experimental observations. The target of the calculations is the membrane bending rigidity. The bending rigidity sets the membrane persistence length. We expect that this length scale is closely related to the expected equilibrium radius R of lipid vesicles. Vesicles that result from repetitive freeze-thaw cycles converge to a well-defined radius, which was measured by dynamic light scattering. We correlated the experimental observations with our theoretical predictions. The bending rigidity of anionic membranes is typically larger than that of equivalent neutral ones.^{10,22} This is a result of the electric double layer associated with the charged surface and of changes in lipid packing due to surface charge screening. Besides these generic salt effects, cation-specific effects on both the theoretically predicted k_c and the experimentally found R are observed. The thickness of a negatively charged membrane increases with cation hydration, which results in larger k_c and R values. Specific adsorption of cations decreases the surface charge density and affects in this way both the electric double layer thickness and lipid packing. The most strongly hydrated cations also have the largest affinity for the

lipid headgroups, which results in largest values of k_c and R for cations with intermediate binding and hydration properties.

Acknowledgment. The authors thank J. van Male and J. van den Oever for their help with the SCF calculations. This work was financially supported by the Dutch Science and Technology Foundation (STW).

References and Notes

- (1) Arnold, K. In *Handbook of Biological Physics*; Lipowsky, R., Sackmann, E., Eds.; Elsevier Science B.V.: Amsterdam, 1995; Vol. 1, Chapter 19; pp 904–957.
- (2) Mayer, A. *Annu. Rev. Cell Dev. Biol.* **2002**, *18*, 289.
- (3) Winterhalter, M.; Helfrich, W. *J. Phys. Chem.* **1988**, *92*, 6865.
- (4) Lekkerkerker, H. N. W. *Physica A* **1989**, *159*, 319.
- (5) Eisenberg, M.; Gresalfi, T.; Riccio, T.; McLaughlin, S. *Biochemistry* **1979**, *23*, 5213.
- (6) Tatulian, S. A. *Biochim. Biophys. Acta* **1983**, *736*, 189.
- (7) Tatulian, S. A. *Eur. Biochem. J.* **1987**, *170*, 413.
- (8) Kraayenhof, R.; Sterk, G. J.; Wong Fong Sang, H. W.; Krab, K.; Epand, R. M. *Biochim. Biophys. Acta* **1996**, *1282*, 293.
- (9) Cevc, G. *Biochim. Biophys. Acta* **1990**, *1031*, 311.
- (10) 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.
- (11) Claessens, M. M. A. E.; Leermakers, F. A. M.; Hoekstra, F. A.; Cohen Stuart, M. A. *Langmuir*, accepted for publication, 2007.
- (12) Petrache, H. I.; Zemb, T.; Belloni, L.; Parsegian, V. A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 7982.
- (13) Macdonald, R. C.; Jones, F. D.; Qiu, R. Z. *Biochim. Biophys. Acta* **1994**, *1191*, 362.
- (14) Simons, B. D.; Cates, M. E. *J. Phys. II* **1992**, *2*, 1439.
- (15) Herve, P.; Roux, D.; Bellocq, A. M.; Nallet, F.; Gulikkrzywicki, T. *J. Phys. II* **1993**, *3*, 1255.
- (16) Evers, O. A.; Scheutjens, J. M. H. M.; Fleer, G. J. *Macromolecules* **1990**, *23*, 5221.
- (17) Oversteegen S. M.; Leermakers, F. A. M. *Phys. Rev. E* **2000**, *62*, 8453.
- (18) Helfrich, W. *Z. Naturforsch.* **1973**, *C 28*, 693.
- (19) Kumaran, V. *Phys. Rev. E* **2001**, *64*, 1.
- (20) Koynova, R.; Caffrey, M. *Biochim. Biophys. Acta* **1998**, *1376*, 91.
- (21) Boomgaard van den, T.; Lyklema, J. *Langmuir* **1989**, *245*, 245.
- (22) Petrache, H. I.; Tristram-Nagle, S.; Gawrisch, K.; Harries, D.; Parsegian, V. A.; Nagle, J. F. *Biophys. J.* **2004**, *86*, 1574.