Phase evolution of lamellar cationic lipid-DNA complex: Steric effect of an electrolyte

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The complexation isotherms of DNA plus lipids of a symmetric lamellar cationic lipid-DNA system were determined within a mean field free energy. The free energy incorporates the ion's finite size of NaCl simple electrolyte in solution and makes use of known structure data on this complex. The results for the predicted isotherms are in qualitative agreement with the trends of the experimental data for this property. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1809597]

I. INTRODUCTION

Cationic lipid-DNA (CL-DNA) complexes are promising vectors to be used in gene therapy.[1–8] In recent years their internal structure was determined with high resolution x-ray small angle diffraction experiments.[9–11] It was shown that these complexes present an equilibrium lamellar stack of lipid layers with intercalated DNA macromolecules distributed in a two-dimensional crystalline array. Experiments show that the internal structure of these systems depends on cationic to neutral lipids composition molar fraction on bilayers and the overall cationic lipid to anionic DNA molecular weight ratio ρ.[9] It is also affected by the addition of the simple electrolyte salt NaCl in solution.[10] For a given lipid composition the phase evolution of its structure manifests through complexation isotherms of the DNA's average separation distance R as a function of ρ, displaying three characteristic regions. The following is observed a region where complexes coexist with excess DNA, then a phase of neutral complexes where all positive charge of lipids is compensated by DNA negative charge, and finally a phase where complexes coexist with cationic liposomes, for increasing ρ. The first detailed theoretical explanation of the complex phase evolution was given by Harries *et al.*[12] who developed a complete theoretical framework to study the structural isotherms with a nonlinear Poisson-Boltzmann (PB) mean field energy. In their work Harries *et al.* considered the presence of NaCl electrolyte, which was modeled as a pointlike salt. They demonstrated that the main mechanism underlaying DNA-lipid self-assembly was driven by counterion release into bulk solution of the previously condensed counterions on single DNA and cationic liposomes.[13] An important effect taken into account in their theory was the mobility of the charged head groups forming the bilayers, which leads to a novel boundary condition on the system's electric field within the framework of the PB theory.[12] Also coupling of bilayer curvature with its charge density modulations were studied by Harries *et al.*,[14] and the full phase diagram of complex overall structure as a function of bilayers lipid composition was studied with their theory.[15] Moreover, a recent work by Fleck *et al.*[16] made an extension of the work by Harries *et al.* to incorporate the solution's pH and charge group dissociation effects, and its coupling to lipid's mobility degree of freedom. This way, they generalized the approach of Harries *et al.* to a new boundary condition on PB equation. Yet, an analytical study of the PB free energy for low lipid's density and salt free solution case was undertaken by Bruinsma.[17]

In this paper we extended the work of Harries *et al.*[12] and generalized the PB free energy of their theory to include steric effects, due to finite size of salt ions, on the equilibrium R values of CL-DNA self-assembled complexes. Our extension is based on the work of Borukhov *et al.*,[18] who developed a modified PB free energy that takes into account effects of short-range excluded volume interactions of ions in the inhomogeneous ion's density close to highly charged surfaces and bulk electrolytes. Borukhov *et al.* showed that such short-range interactions can become comparable to the Coulomb ones at high salt concentration. A similar PB free energy incorporating ion's excluded volume interactions was put forward by Iglic and Iglic.[19]

In this work we followed the same philosophy as the pioneering work of Harries *et al.*[12] and utilized their approach to determine thermodynamic equilibrium properties such as the mean field energy of complex and interaxis DNA distance as a function of ρ for given bilayer lipid composition. Using reported experimental data by Koltover *et al.*[10] on the complex structural parameters and a model of equal ions size and case of highly charged lipid layers, we find that ion's size effects lead to small corrections to the predicted isotherms as a function of ρ and membrane charge, with respect to those obtained from a pointlike ion's model.

II. MEAN FIELD FREE ENERGY OF CATIONIC LIPID-DNA SELF-ASSEMBLY

Due to the internal symmetry of the complex, we consider the unit cell depicted in Fig. 1, which is formed by two halves of DNA strands separated by the mean distance R and two flat lipid bilayers with stack repeat distance d and unit cell depth h. The membranes have a surface charge density $\sigma(x)$ of cationic and σ_T-$\sigma(x)$ of neutral lipids, with σ_T being the total surface density of lipids and surface area per head group $a=70$ Å^2.[10] They are made of ϕ_{TAB} mole frac-

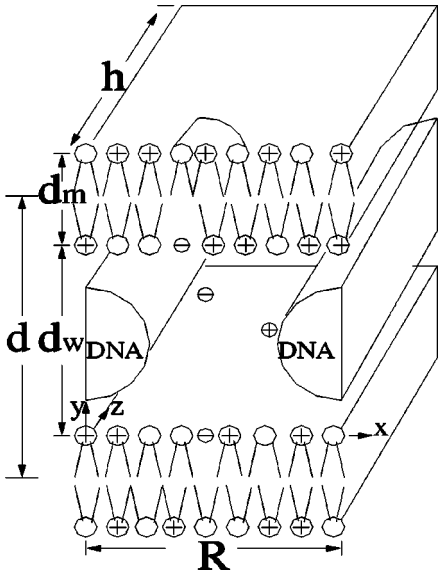

FIG. 1. Unit cell of lamellar CL-DNA complex with NaCl in solution.

tion of the dioleoyltrimethylammonium propane DOTAP (TAP) positively charged lipid and $\phi_{PC}=1-\phi_{TAB}$ molar fraction of dioleoylphosphatidylcholin DOPC (PC) neutral component. There is an intervening symmetric 1:1 NaCl electrolyte at bulk concentration $c^*=150$ mM in water of dielectric constant $\varepsilon=78$. It is assumed that both ionic species are of same diameter d_i. DNA molecule is modeled as a cylinder of $r_D=10$ Å radius with constant negative charge of density per unit lenght $\lambda=-e/1.7$ Å with e being the magnitude of the elementary charge. Following the approach of Harries *et al.*,[12] the free energy of the complex is given as

$$F_c=(f_{field}+f_{ion}+f_{lipid})h, \tag{1}$$

where

$$f_{field}=\frac{\varepsilon}{8\pi}\int_0^R dx\int_0^{d_w} dy(\nabla\psi)^2 \tag{2}$$

is the electrostatic energy of the complex. In Eq. (1), f_{ion} is the entropy of mixing of ions and water molecules, as proposed by Borukhov *et al.*[18] for finite ion's size

$$\begin{aligned} f_{ion}=\frac{k_BT}{d_i^3}\int_0^R dx\int_0^{d_w} dy\Bigg\{ & c^+d_i^3\ln\left[\frac{c^+d_i^3-2c^*c^+d_i^6}{c^*d_i^3}\right] \\ & +c^-d_i^3\ln\left[\frac{c^-d_i^3-2c^*c^-d_i^6}{c^*d_i^3}\right] \\ & +(1-c^+d_i^3-c^-d_i^3)\ln(1-c^+d_i^3-c^-d_i^3) \\ & -(1-2c^*d_i^3)\ln(1-2c^*d_i^3)\Bigg\}, \end{aligned} \tag{3}$$

where we incorporated the term $-(1-2c^*d_i^3)\ln(1-2c^*d_i^3)$ for $d_i\neq 0$ due to the difference in ions concentration inside complex and bulk solution in the same way as in the approach of Harries *et al.*[12] Equation (3) extends the theory of Harries *et al.*,[12] first proposed for point-like ions. It reduces to their original result in the limit of negligible ion's size d_i or low concentration c^*. The last term in Eq. (1), f_{lipid}, was first introduced in the theory of Harries *et al.* to include in a mean field level the lipid's size and entropy of mixing of mobile DOTAP and DOPC molecules in the two charged bilayers:

$$f_{lipid}=2k_BT\int_0^R dx\left\{\sigma\ln\left[\frac{\sigma}{\sigma_T}\right]+(\sigma_T-\sigma)\ln\left[\frac{\sigma_T-\sigma}{\sigma_T}\right]\right\}, \tag{4}$$

with k_B being the Boltzmann constant and T being the temperature. Functional minimization of Eq. (1) with respect to $c^{\pm}$ leads to the known result of Borukhov *et al.*[18] for the profile concentration of positive (Na^+) and negative (Cl^-) ions given by

$$c^{\pm}=\frac{c^*e^{\mp ze\psi/k_BT}}{1-2c^*d_i^3+2c^*d_i^3\cosh(ze\psi/k_BT)}. \tag{5}$$

The electric potential $\psi(x,y)$ satisfies the Poisson equation

$$\nabla^2\psi=-\frac{4\pi}{\varepsilon}[+zec^+-zec^-]. \tag{6}$$

By Gauss's law, ψ also fulfills the boundary conditions

$$\nabla\psi\cdot\mathbf{n}=\begin{cases}\dfrac{4\lambda}{\varepsilon r_D}, & \text{on DNA surface}\\ \dfrac{4\pi\sigma(x)}{\varepsilon}, & \text{on lipid membrane.}\end{cases} \tag{7}$$

It is assumed that inside DNA and lipid bilayer the dielectric constant is zero. $\mathbf{n}$ is a unitary vector pointing outward the dielectric boundaries.

Taking the variation of Eq. (1) with $\sigma(x)$ for the given constraint of mobile charge density $\sigma(x)$ on membrane, second part of Eq. (7), leads to the novel boundary condition first derived by Harries *et al.* for the surfactant profile

$$\sigma(x)=\frac{e^{-(\psi+\lambda)}}{a[(1-\phi_{TAP})/\phi_{TAP}+e^{-(\psi+\lambda)}]}. \tag{8}$$

Since complexes are formed from cationic liposomes of surface charge $e\phi_{TAB}/a$, the surface density of cationic lipid $\sigma(x)$ in a layer forming the complex satisfies for λ the conservation of charge equation

$$e\int_0^R dx\sigma(x)=\frac{e\phi_{TAB}}{a}. \tag{9}$$

We assume the valence of ions is $z=1$. We will describe the thermodynamic phase evolution of the CL-DNA system in equilibrium with excess DNA as obtained by the equality of DNA chemical potential in the complex with that in bulk solution, F_{DNA}. This equilibrium condition leads to the average separation R_1 between DNA strands that is according to the theory of Harries *et al.*,[12] set by

$$F_c-R\frac{\partial F_c}{\partial R}=F_{DNA}. \tag{10}$$

The resulting equilibrium R_1 is constant as the parameter ρ increases up to a maximun value

$$\rho_1=\phi_{TAB}R_1\frac{l}{a}, \tag{11}$$

with $\rho_1 < 2.2$ where all liposomes are already involved into complex formation (negatively overcharged complex) and R_1 being the average interhelical equilibrium separation. Here $l = 1.7$ Å is the separation of elementary charge on DNA backbone. $\rho_1 := 2.2$ corresponds to the definition of isoelectric point of stoichiometrically charge-neutral complex where the number of DOTAP molecules and DNA bases are equal. Thus, (R_1, ρ_1) gives the lower bound of isotherm of one phase complexes where all DNA and cationic lipids participate in complex formation. In order to know the precise value of R_1, we determined numerically the charging energy $F_{DNA} = (f_{field} + f_{ion})h$ of a single DNA of length $h = 1$ Å in bulk electrolyte solution by means of Eqs. (2)–(9) (in all our calculations updated solutions of ψ were obtained with a precision of 0.01% difference). We use a unit square cell of volume size $d_w \times d_w \times h$ and salt concentration c^*. Thereafter, the free energy of complex formation F_c was determined using Eqs. (1)–(9) and a $R \times d_w \times h$ cell size (due to the symmetry of the system, equilibrium thermodynamic properties R and ρ do not depend on h). Equation (10) is satisfied for R_1. The next region of the phase diagram corresponds to R that is inversely proportional to charge on membrane through $R = (e/a)(\rho/\phi_{TAB})$ up to $\rho = \rho_2$, value where a new R_2 is obtained. Reaching of R_2 happens when the complex coexists in equilibrium with excess liposomes and all DNA molecules are inside complexes (positively overcharged complex). In this case there are two processes of complex formation; if cationic lipids cannot be exchanged between complex and liposomes, then the equation of number conservation of lipid molecules $N_{lipid} = L^2/a$ in the CL-DNA system is given in Harries *et al.*[12] theory by

$$\frac{\partial F_c}{\partial R} = \frac{F_B(\phi_{TAB})}{R}, \tag{12}$$

which provides the value R_2 at equilibrium where L is the total lateral size of free liposomal membrane. ρ_2 is obtained from Eq. (11) with the change $R_2 \to R_1$. F_B is the chemical potential or charging energy of a flat charged, excess liposomal membrane in aqueous solution.

Let us consider N^c chains in the complex. The mole fractions of cationic lipids in the complex ϕ_c and in free liposomes ϕ_B satisfy the constraint to equal ϕ_{TAB}, the overall mole fraction of cationic lipids,

$$\left(\frac{RN^c}{L}\right)\phi_c + \left(1 - \frac{RN^c}{L}\right)\phi_B = \phi_{TAB}. \tag{13}$$

Here RN^c/L is the fraction of lateral (volume) space occupied by N^c DNA molecules in complex with respect to the total lateral size L of free lipid layers. On the other hand, if cationic lipids are soluble, the condition that lipids must have the same electrochemical potential both inside complex and liposomes leads to the equation derived by Harries *et al.*,[12]

$$\frac{\partial F_c(\phi_c)}{\partial \phi_c} = \frac{dF_B(\phi_{TAB})}{d\phi_B}. \tag{14}$$

In addition, the theory of Harries *et al.* states that it must fulfill the osmotic pressurelike equation of a compresible system,

$$\frac{\partial F_c}{\partial R} - \frac{F_B(\phi_{TAB})}{R} = \frac{(\phi_c - \phi_B)}{R}\frac{dF_B(\phi_B)}{d\phi_B}. \tag{15}$$

For any of the two processes mentioned above, if one were to determine the equilibrium value R_2 of the DNA-DNA isotherm (region of complex + excess liposome) either from Eq. (12), or using Eqs. (13)–(15), then both R_2 and ρ_2 will denote the starting point of isotherm for this region, respectively. However, they will lead to different numerical predictions for R_2 since they correspond to different approximations. Thus, R_2 will still be the upper bound of one phase complex region.

III. RESULTS AND DISCUSSION

The excess free energies of naked DNA, F_{DNA}, and of charged lipid layer, F_B, in a concentrated electrolyte solution are not known thermodynamic properties experimentally or from an exact theoretical model. Therefore, we have to resort to their full numerical calculation in a mean field level.[20] We performed this task with a PB equation valid for equal size ions and symmetric $z{:}z$ electrolyte model. In fact both ionic species have different diameters; 1.9 Å for Na^+ and 3.6 Å, for Cl^-; however, PB equation is not known for size asymmetry. We consider the effective diameter d_i for both ion types and calculate first the excess energies $(f_{field} + f_{ion})h$ of charging a DNA molecule, then of a cationic lipid membrane in $c^* = 150$ mM aqueous solution of $l_D = 7.866$ Å Debye screening length as a function of ion's diameter d_i, $[l_D = \sqrt{(\epsilon k_B T/4\pi c^* e^2)}]$. Therefore, for the case of DNA we solved numerically such equations using a unit square cell of lateral size $d_w = 5l_D$ and used the same method for the case of charged liposomal membrane in aqueous solution. The boundary conditions on the electric field, $\psi(x) \to 0$ as $x \to \infty$ and constant $d\psi(x)/dx$ on dielectric boundaries [Eqs. (7)] were imposed. The numerical results show that the excess free energy of DNA is approximately cell size independent at this salt concentration. This fact can be seen, for instance, when pointlike ions are considered, yielding $\beta(f_{field} + f_{ion})h = 1.17$, 1.0, and 1.3 with d_w

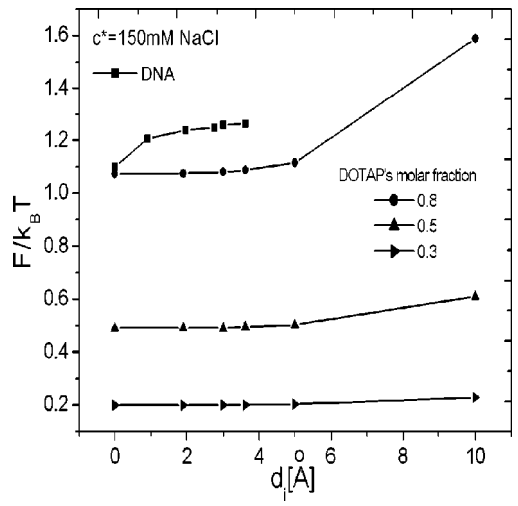

FIG. 2. Charging energies of naked DNA and flat cationic lipid bilayer at fixed molar fraction ϕ_{TAP} of DOTAP as a function of ion's diameter d_i.

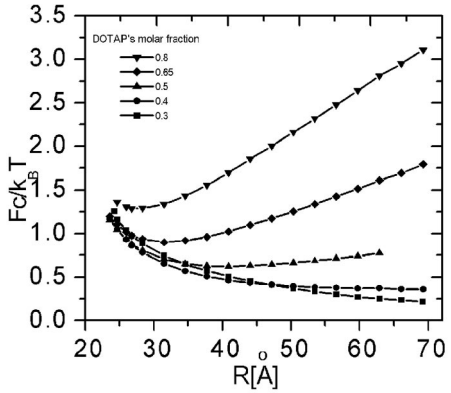

FIG. 3. CL-DNA complex free energy at five fixed molar fractions ϕ_{TAP} of DOTAP as a function of DNA-DNA inter-helical separation R.

$=2.3l_D$, $5l_D$, and $7l_D$, respectively ($\beta=1/k_BT$). Likewise, for membranes $\beta(f_{field}+f_{ion})h=1.92$, 1.66, and 2.4 which are all about the same order of magnitude for $d_w=0.5l_D$, $1.5l_D$, and $2.5l_D$, respectively. It should be noted that for all results given here the electrochemical potentials, μ^+ of Na^+ and μ^- of Cl^- are assumed to be equal, that is, $\exp[\beta\mu^+]=\exp[\beta\mu^-]=c^*d_i^3/(1-2c^*d_i^3)$ (expression first obtained in Ref. 18), which implies their bulk concentration c^* are the same. Nevertheless, we outline here how this restriction can be avoided in order to take into account rigorously the real chemical potentials, which turn out to be different. They are given correctly by $\mu^+=\mu_\pm^{\text{bulk}}+\chi$ and $\mu^-=\mu_\pm^{\text{bulk}}-\chi$ with $\exp[\beta\mu_\pm^{\text{bulk}}]=c^*d_i^3\gamma_\pm/(1-2c^*d_i^3)$ and $\gamma_\pm=0.754$ the experimentally observed mean activity coefficient.[21] The constant χ is obtained self-consistently from the overall electroneutrality condition

$$\int_0^R dx\int_0^{d_w} dy[+zec^+(x,y)-zec^-(x,y)]+2e\int_0^R dx\sigma(x)=|\lambda|. \tag{16}$$

In this case Eq. (5) for c^+ and c^- no longer holds.[22] However, as said above, we have assumed $\mu^+=\mu^-$ for all results presented in this paper. In a following paper[22] this restriction will be relaxed in order to obtain with higher accuracy all thermodynamic properties such as R versus ρ. In Fig. 2 the excess energy for charging a DNA molecule as a function of ion diameter d_i is depicted. Also in this figure are plotted the graphics of excess energy for charging a flat surfactant membrane and three cases of $\phi_{TAB}=0.8$ (highly charged membrane), 0.5, and 0.3 (almost neutral membrane), obtained using a lateral squared cell size $d_w=5l_D$ and $c^*=150$ mM. It can be noticed that in all cases of Fig. 2, charging energies enhance due to the increase in magnitude of steric effects [f_{ion} term in Eq. (1)] with respect to zero size ions.

In order to find the DNA-DNA average separation distance R_1 we analyzed first the case of complex at equilibrium with excess DNA molecules in solution. Therefore, we use Eq. (10) that requires as an input the determination of the complex free energy $F_c(R)$ and $F_{DNA}=(f_{field}+f_{ion})h$, DNA charging energy. Following the work by Harries *et al.*[12] we depict the complex free energy F_c; in Fig. 3 are plotted

TABLE I. Experimental values of isoelectric CL-DNA system cell size at $c^*=0.0$ mM of NaCl.

ϕ_{TAB}	d(Å)[a]	d_m(Å)[a]	d_w(Å)[b]
0.3	71.33	42.0	29.33
0.4	68.0	39.97	28.03
0.5	66.38[c]	38.2	28.18
0.6	64.89	37.39[c]	27.5
0.7	63.42	36.67[c]	26.75
0.8	61.91[c]	35.9[c]	26.01
1	59.12	34.5	24.62

[a]Taken from Ref. 10.
[b]From $d_m=d-d_w$.
[c]Extrapolated.

F_c for five molar fractions of cationic lipids $\phi_{TAP}=0.3$, 0.4, 0.5, 0.65, 0.8 as a function of R, at $c^*=150$ mM and effective ion size $d_i=2.75$ Å $=(1.9$ Å $+3.62$ Å$)/2$. The calculations we made of all $F_c(R)$, some of which are plotted in Fig. 3, rely on experimental structure data reported by Koltover *et al.* in Ref. 10. They found important changes both in, R due to the presence of the electrolyte solution, as well as in the structural parameter values characterizing the stability of the complex, namely, d_w as a function of bilayer charge density. Therefore, we know the actual DNA+water gap d_w in the complex interior at different molar fraction ϕ_{TAP}, information that we use in our geometry defining the unit cell volume $R\times d_w\times h$ of the complex. In Table I are summarized the bilayer stack separation distances $d=d_m+d_w$ as a function of ϕ_{TAB}, with d_m being the bilayer thickness. In Fig. 4 are shown typical calculated complex free energies $F_c(\phi_{TAB})$ as a function of ϕ_{TAB} for different cells with lateral sizes $R=1.4l_D$, and $3.2l_D$, with d_w taken from Table I, and two effective ion diameters $d_i=0.0$ Å, and $d_i=2.75$ Å, respectively. Finally for $R=1.7l_D$, $d_i=2.75$ Å, and $d_i=0.0$ Å, results that lead to Table II below.

In second column of Table II are provided numerical values of F_{DNA} using a square cell size $d_w=5l_D$, as a function of d_i. It is now possible with the help of Eq. (10) to find the values of R_1 which are given in column 4, whereas Eq. (11) provides us column 5 that contains the predictions of the ratio ρ_1 of cationic lipid/DNA, normalized to its isoelectric value 2.2.

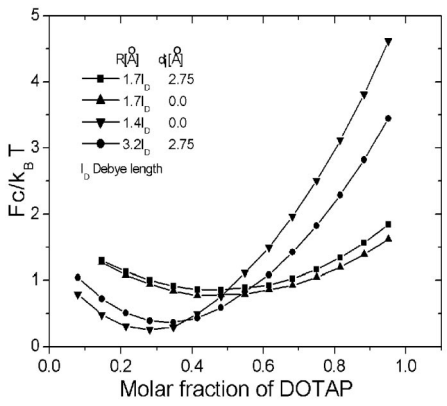

FIG. 4. Free energy of CL-DNA complex for three lateral sizes R of unit cell and fixed d_i, as a function of molar fraction ϕ_{TAP} of charge on bilayers.

TABLE II. Theoretical results of points $(R_1,\rho_1/2.2)$ and $(R_2,\rho_2/2.2)$ defining the CL-DNA complex phase evolution as a function of d_i and ϕ_{TAB} in $c^*=150$ mM concentrated aqueous solution of NaCl.

						No lipid demixing		With demixing	
d_i(Å)	βF_{DNA}	ϕ_{TAB}	R_1(Å)	$\rho_1/2.2$	βF_B	R_2(Å)	$\rho_2/2.2$	R_2(Å)	$\rho_2/2.2$
0	1.1013	0.3	36.05	0.5778	0.3983	55.75	0.8935	46.09	0.7352
2.75	1.2493	0.3	40.98	0.6568	0.4004	61.0	0.9777	44.0	1.1453
0	1.1013	0.5	29.72	0.7939	0.9821	51.43	1.3739	42.47	1.0986
1.9	1.2400	0.5	32.25	0.8615	0.9832	54.68	1.4607	[a]	[a]
2.75	1.2493	0.5	33.58	0.8970	0.9868	55.75	1.4893	42.0	1.1909
3.62	1.2645	0.5	34.58	0.9238	0.9909	59.56	1.5911	[a]	[a]
0	1.1013	0.8	25.81	1.1032	2.1470	40.0	1.7097	41.15	1.5622
2.75	1.2493	0.8	27.76	1.1865	2.1589	40.71	1.7828	41.5	1.9064

[a]Not determined. $\beta=1/k_BT$.

The onset of positively overcharged complexes is defined by the set (R_2,ρ_2) that is determined from column 6 of βF_B, and from Eqs. (2)–(9) and Eq. (12), resulting in columns 7 and 8 under the condition of no lipid exchange. Similarly using Eqs. (13)–(15) are obtained columns 9 and 10 when there is lipid solubility. In order to verify the importance of ion size in the present model we give in Table II calculations performed with ion sizes $d_i=1.9$ Å and 3.62 Å. Table II contains our main results since it compares the prediction of the model outlined above, Eq. (1), with the reported experimental data of Koltover *et al.*[10] for the phase evolution of CL-DNA system. Such comparison is made in Fig. 5 where are plotted experimental complexation isotherms of DNA and charged lipids at different ϕ_{TAP} are plotted. Also, in this figure are depicted the predicted value of (R_1,ρ_1) and (R_2,ρ_2) using only the lipid demixing conditions of Eqs. (13)–(15) with $d_i=2.75$ Å. Qualitatively, we can see they are in better agreement with the experimentally observed data than a pointlike ion model (see Table II). Yet, the set (R_2,ρ_2) is better determined with lipid demixing rather than using the condition of no solubility. Particularly, equilibrium distances of separation between DNA strands, R_1 and R_2, are better predicted for highly charged membranes ($\phi_{TAB}=0.8$), being closer to the average experimental values ($\phi_{TAB}=1$) than for the less charged cases ($\phi_{TAB}=0.5, \phi_{TAB}=0.3$) where theory worsen. We find there is in general good qualitative agreement among theories with the prevailing trend of the experimental isotherms. However, we cannot assert whether ion finite size effects are an important quantitative correction to the complexation isotherms as compared to calculations for the same property when these effects are neglected. The experimental data for the isotherms contain large error bars (see Refs. 9–11), therefore, they do not allow a quantitative comparison between both the models considered. Further calculations are being carried out to allow for exact match of salt ions chemical potentials, and the introduction of hydration effects of the solvent molecules on bilayers and DNA-DNA interactions at high salt strength.

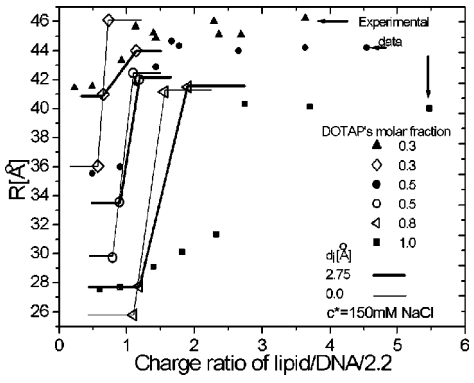

FIG. 5. Equilibrium DNA-DNA separation R as a function of lipid/DNA's charge ratio ρ at fixed molar fraction ϕ_{TAP} of charge on bilayers. The solid and dashed lines are guides to the eye. Lower open symbols: ◇, ○, and ◁, are theory predictions using 2.75 Å ion size (thick line) for $(R_1,\rho_1/2.2)$ of complexes coexisting with excess DNA (thin line for $d_i=0.0$ Å). Coexistence with lipids are given by $(R_2,\rho_2/2.2)$, the upper open symbols connected by lines. Experimental data taken from Ref. 10.

IV. CONCLUSION

In this paper we have made a theoretical study of short-range excluded volume interaction of an electrolyte on the complexation isotherms of DNA-lipid complexes which exhibit the lamellar symmetry. We found that such effects lead to small corrections to the predicted isotherms (see for instance Fig. 5) with respect to the case where they are not taken into account. Nevertheless, both models considered (with $d_i=0.0$ Å and $d_i=2.75$ Å) lead to qualitative agreement with the general trends of the experimental isotherms. Our research was based on a generalization we made of the PB free energy of the theoretical framework developed by Harries *et al.*[12] to investigate the complex phase evolution thermodynamics. The resulting extended version of their PB free energy incorporates a modification of the ion's entropy of mixing to include steric effects. In the limit of low ion concentration or negligible ion size, it reproduces the known result of the mean field energy of the theory of Harries *et al.*

Aside from the lipids, DNA, and ion's electrostatic and excluded volume interactions, it would be also interesting to include the hydration interaction of solvent molecules which turns out to be relevant on the complex stability at high salt content (see Fig. 6 of Ref. 10). This should further help our comprehension on the role of different sources of molecular interactions among all complex's constituents and drive the mechanisms of stability of CL-DNA complexes.

ACKNOWLEDGMENT

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