DNA-Lipid Complexes: Stability of Honeycomb-Like and Spaghetti-Like Structures

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ABSTRACT A molecular level theory is presented for the thermodynamic stability of two (similar) types of structural complexes formed by (either single strand or supercoiled) DNA and cationic liposomes, both involving a monolayer-coated DNA as the central structural unit. In the "spaghetti" complex the central unit is surrounded by another, oppositely curved, monolayer, thus forming a bilayer mantle. The "honeycomb" complex is a bundle of hexagonally packed DNA-monolayer units. The formation free energy of these complexes, starting from a planar cationic/neutral lipid bilayer and bare DNA, is expressed as a sum of electrostatic, bending, mixing, and (for the honeycomb) chain frustration contributions. The electrostatic free energy is calculated using the Poisson-Boltzmann equation. The bending energy of the mixed lipid layers is treated in the quadratic curvature approximation with composition-dependent bending rigidity and spontaneous curvature. Ideal lipid mixing is assumed within each lipid monolayer. We found that the most stable monolayer-coated DNA units are formed when the charged/neutral lipid composition corresponds (nearly) to charge neutralization; the optimal monolayer radius corresponds to close DNA-monolayer contact. These conclusions are also valid for the honeycomb complex, as the chain frustration energy is found to be negligible. Typically, the stabilization energies for these structures are on the order of 1 k_BT/Å of DNA length, reflecting mainly the balance between the electrostatic and bending energies. The spaghetti complexes are less stable due to the additional bending energy of the external monolayer. A thermodynamic analysis is presented for calculating the equilibrium lipid compositions when the complexes coexist with excess bilayer.

INTRODUCTION

The use of positively charged liposomes as potential carriers of DNA molecules into target cells has been suggested by a number of authors (Felgner et al., 1987, 1996; Felgner and Ringold, 1989; Gao and Huang, 1991; Lasic, 1997). However, the transfection efficiency of the aggregation complexes formed by DNA and cationic liposomes in aqueous solution is still unpredictable. Furthermore, only a few very recent studies provide direct experimental evidence concerning the morphology of these complexes (Gershon et al., 1993; Tarahovsky et al., 1996; Sternberg et al., 1994; Sternberg, 1996; Rädler et al., 1997; Lasic et al., 1997; Gustafsson et al., 1995; Eastman et al., 1997; Zuidam and Barenholz, unpublished observations). From these experiments, which use different mixtures of liposomal lipids, it appears that at least three structural types of DNA/membrane complexes are possible. In one study, based on synchrotron x-ray diffraction and optical microscopy experiments, it was suggested that the complexes formed upon adding λ- or plasmid-DNA to a solution of mixed cationic/neutral ("helper") liposomes consist of a multilayered array of alternating lipid bilayers and DNA monolayers. The DNA strands intercalated between the lipid bilayers are parallel to each other, forming a one-dimensional lattice with a repeat distance that depends upon the lipid-to-DNA ratio and the cationic lipid mole fraction (hence charge density) (Rädler et al., 1997). The cationic lipid used in these experiments was DOTAP {*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*,trimethylammonium methylsulfate} and the helper lipid was either DOPC (dioleoyl-phosphatidylcholine) or DOPE (dioleoyl-phosphatidyl ethanolamine). Similar complex structures were suggested by other authors (Lasic et al., 1997; Gustafsson et al., 1995). Theoretical studies pertaining to some aspects of these complexes were published recently (Dan, 1996).

Several other experimental studies suggest another possible class of DNA/lipid complex morphologies, in which the common structural motif is a single DNA strand (or supercoiled DNA double-strand) coated by a highly ("negatively") curved and positively charged lipid monolayer (see Fig. 1) (Felgner et al., 1996; Sternberg et al., 1994; Tarahovsky et al., 1996). To avoid exposure of the hydrophobic lipid tails to water, the structure can be completed in one of two ways. One possibility is to surround the inner monolayer with another (oppositely bent) monolayer to form a bilayer-coated DNA (as shown in Fig. 1 *b*); this structure was named *spaghetti-like* after its visual appearance in freeze-fracture electron micrographs. The other option is to associate many monolayer-coated DNA units into an inverted hexagonal (hereafter called *honeycomb*) array (see Fig. 1 *a*). The lipid arrangement in this complex corresponds to the symmetry of the H_{II} phase. Interestingly, the frequently used helper lipid DOPE is known to form a stable H_{II} phase even without DNA.

The formation of honeycomb complexes was suggested by Felgner et al. (1996) based on molecular packing considerations and experimental results from several laboratories. Tarahovsky et al. (1996), who studied aqueous solu-

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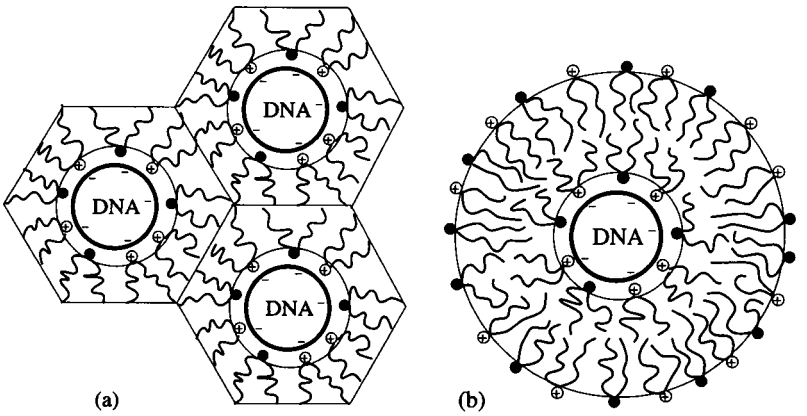

FIGURE 1 Two models of DNA-lipid complexes utilizing a DNA rod coated by a mixed (cationic/nonionic) lipid monolayer as the central structural element. The figure shows, schematically, a two-dimensional cross section of the complexes, through a plane perpendicular to the DNA axis. (*a*) A honeycomb-like complex composed of a hexagonally packed bundle of monolayer-coated DNA units. (*b*) A spaghetti-like complex, composed of a bilayer-coated DNA.

tions containing DNA, lecithin, and Ca^{2+} ions, interpreted the fibrillar DNA-lipid structures in their freeze-fracture micrographs as bundles of DNA molecules arranged in finite honeycomb clusters (Fig. 1 *a*), surrounded by a positively bent monolayer with its headgroups facing the water.

The spaghetti-like structure was suggested by Sternberg et al. (1994) and Sternberg (1996), based on electron microscopy studies [see also Felgner et al. (1996)]. The system studied involved a mixture of (mostly supercoiled) plasmid DNA and DC-Chol {3β-[N-(*N'*,*N'*-dimethylaminoethane)carbamoyl]cholesterol}/DOPE liposomes. Spaghetti-like structures were found at molar ratios ranging from 1:4 to 3:2 (DC-Chol:DOPE). For higher DOPE contents nonbilayer, presumably honeycomb, structures were found. Other *monovalent* cationic lipids such as DMRIE (1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide) (Eastman et al., 1997), DOTAP, and DOTMA {*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride} also seem to favor the formation of monolayer-coated DNA complexes. However, spaghetti formation was not observed upon using *polyvalent* cationic amphiphiles {such as DOSPA (2,3-dioleoyloxy-*N*-[2(sperminecarboxamido)ethyl]-*N*,*N*-dimethyl-1-propanaminum trifluoroacetate)} nor with single-strand, short (15-mer) oligonucleotides (Sternberg, 1996). Another notable observation is that the spaghetti complexes were mostly found to be still connected to compact liposomal aggregates, presumably cationic liposome/DNA complexes, [named *meatballs* (Sternberg et al., 1994)]. Thus it is not entirely clear whether these complexes are thermodynamically stable or metastable intermediates.

Upon approaching each other the negatively charged DNA and the positively charged membrane are partly neutralizing their charge, concomitantly releasing the partly bound counterions from the diffuse screening layer into the bulk solution. This process, which lowers the electrostatic free energy of the system, is the thermodynamic driving force for complex formation. On the other hand, the adaptation of the lipid membrane to the complex geometry may involve unfavorable free energy contributions. In the multilayered lipid/DNA complex, for instance, the cationic lipids will tend to segregate in the vicinity of the DNA strands, thus inflicting a "demixing" entropy penalty. In the tight binding complexes illustrated in Fig. 1 another factor plays an important role, namely, the elastic deformation energy associated with coating the DNA by a highly (negatively) curved monolayer (or bilayer), especially if the monolayer spontaneous curvature corresponds, even approximately, to the planar bilayer geometry. By using an appropriate lipid mixture, e.g., by adding DOPE, which helps the monolayer conforming to the DNA curvature, the elastic deformation energy may be largely reduced, yet it appears quite difficult to find lipid mixtures that will simultaneously reduce both the electrostatic and the bending free energies. On the other hand, it is not obvious whether the most stable complex also provides the ideal choice for a transfection agent. Clearly then, it is of great interest to understand the factors governing the structure and stability of DNA/lipid complexes.

Our goal in this paper is to study, theoretically, the interplay between the various factors that determine the thermodynamic stability and structural details of the two types of complexes shown in Fig. 1. In particular we shall be interested in calculating the electrostatic, bending, and mixing contributions to the complex free energy, and their dependence on the cationic/helper lipid composition (and hence, the membrane charge density, spontaneous curvature, and bending rigidity) and lipid-to-DNA concentration ratio. We shall also consider the coexistence thermodynamics between the complexes and an excess bilayer or DNA phase, and the ensuing distribution of the cationic and nonionic lipids between these structures.

The theoretical model presented in the next section involves several (quite common) assumptions and approximations. The electrostatic free energy will be calculated using the diffuse double layer theory based on the solutions of the Poisson-Boltzmann equation. For the membrane elastic energy we shall use the quadratic curvature expression (Helfrich, 1973), with bending rigidity and spontaneous curvature depending linearly on the lipid composition (Andelman et al., 1994; May and Ben-Shaul, 1995). The DNA, either single-stranded or supercoiled, will be treated as a uniformly charged rigid rod. Finally, the cationic and nonionic lipids will be assumed to mix ideally within a given mono-

layer. In the next sections we shall see that this theoretical framework provides significant insights into the mechanisms governing complex formation, as well as numerical estimates pertaining to the thermodynamic stability of DNA/lipid complexes, at least those of the kind depicted in Fig. 1.

The process of complex formation from separated DNA and lipid liposomes involves drastic changes in the membrane structure. It is reasonable to assume that after these changes the lipid compositions in the complex and the bilayer will not remain equal. If the complexes are prepared using either excess DNA or excess bilayer, then after a while they will coexist with either a bare DNA or free bilayer phase. In the latter case one must account for the fact that the lipid compositions in the two coexisting structures are generally different. Assuming that the system reaches true thermodynamic equilibrium [corresponding to free lipid exchange (Gershon et al., 1993)] we shall formulate the thermodynamic conditions governing complex/bilayer coexistence and present numerical results for several representative cases.

As in the schematic illustration of the lipid-DNA complexes in Fig. 1, we treat these structures as being uniform along the DNA axis (perpendicular to the plane of the drawing). In other words, the DNA molecules are regarded as (essentially infinite) rigid rods. This assumption is consistent with the fact that the (lateral) linear dimension of the lipid molecules surrounding the DNA ($\leq$1 nm) is much smaller than the DNA persistence length, ($\xi \sim 50$ nm). Of course, DNA molecules are not infinitely rigid and long and do undergo one-dimensional (1D) bending undulations, on a length scale larger than ξ. Such undulation forces play a major role in determining the structure and osmotic pressure of columnar liquid crystalline phases of DNA in (lipid-free) aqueous solutions (Strey et al., 1997). However, their effect on the thermodynamic stability of the spaghetti and honeycomb complexes are expected to be small, for the following reasons. From the free energy calculations reported in subsequent sections it follows that the complex stabilization energies are on the order of 1 k_BT per 1 Å of DNA length, and hence $\sim 10^2$–10^3 k_BT per persistence length. For the spaghetti complex, which consists of a single bilayer-coated DNA strand, this implies that upon a bending deformation the tubular bilayer envelope will bend together with the DNA skeleton, without changing the local lipid packing geometry. (Recall that the free energy cost of a 1D curvature deformation on the order of $1/\xi$ of a DNA strand of length ξ is on the order of one k_BT.) In fact, it is not difficult to show that the 1D bending rigidity of a bilayer coated DNA is about twice as large as that of bare DNA, implying a similar increase in the persistence length. (The electron micrographs of Sternberg et al. (1994) indeed suggest a persistence length of $\sim$100 nm for the spaghetti complex.) The symmetry of the honeycomb complex resembles the symmetry of the columnar phase of DNA in lipid-free aqueous solutions, where undulation forces strongly affect the spacing between DNA strands. However, the presence of the intervening lipid monolayers in the honeycomb complex implies a drastic difference between these two structures. Unlike in the lipid-free phase, the monolayer coated units are held together by strong attractive forces between the lipid tails. The cohesive "hydrophobic" energy is larger than 10^3 k_BT per DNA persistence length. The persistence length of a honeycomb-like bundle of such units is considerably larger than that of a single DNA strand, indicating that a collective bending deformation of the complex is rather unlikely. Individual DNA strands may undergo 1D bending undulations within their own lipid "tubes". However, because of the strong electrostatic and elastic restoring forces the amplitudes of these fluctuations are expected to be small and the effects on the local packing geometry should be minor.

One of our qualitative findings is that the electrostatic and bending energy contributions to the complex formation free energy are generally comparable. This can be illustrated using a highly simplified, yet instructive, structural model for the DNA/monolayer complex. It is reasonable to assume that the surface charge density of the lipid monolayer coating the DNA strand will be adjusted so as to neutralize the DNA charge. Neglecting the presence of either co- or counterions in the gap between the DNA and the surrounding monolayer, we can treat this system as a capacitor composed of two concentric cylindrical surfaces. The inner surface, of radius R^D, is that of the DNA, and the outer surface, of radius $R^I > R^D$, represents the charged interface of the monolayer. We shall use L to denote the length of the DNA rod and σ^D its surface charge density. Charge neutrality implies that the surface charge density of the monolayer is $\sigma^I = \sigma^D R^D/R^I$. The electrostatic energy U_e of the complex is now that of a cylindrical capacitor, namely, $U_e/L = \ln(R^I/R^D)\pi(R^D \sigma^D)^2/\epsilon$, where ϵ is the permittivity of water. Using the Bjerrum length $Q_B = e^2/(4\pi\epsilon k_BT)$ (Q_B = 7.14 Å for $T = 298K$), and noting that for the DNA $\sigma^D = e/(2\pi R^D l)$ with e denoting the elementary charge, and l = 1.7 Å the length per unit charge along the DNA, we find $U_e/(L\, k_BT) = (Q_B/l^2) \ln(R^I/R^D)$. This attractive electrostatic contribution is counterbalanced by the bending energy, U_b, of the monolayer. This energy can be calculated by the familiar expression (Helfrich, 1973) $U_b/A = (k/2)(c - c_o)^2$ with $c = 1/R^I$ denoting the monolayer curvature, k the bending rigidity, and $A = 2\pi R^I L$ the inner monolayer area. Assuming vanishing spontaneous curvature, $c_o = 0$, we find $U_b/L = \pi k/R^I$. Adding the electrostatic and bending energies, $U = U_e + U_b$, we get

$$\frac{U}{L} = \frac{Q_B}{l^2} k_BT \ln \frac{R^I}{R^D} + \frac{\pi k}{R^I}. \tag{1}$$

Minimizing U with respect to R^I yields

$$R^I = \left(\frac{\pi k}{k_BTQ_B}\right) l^2. \tag{2}$$

Using, say, a bending rigidity $k = 10\ k_BT$ we find an optimal monolayer radius of R^I = 12.7 Å, which is just

barely larger than the DNA radius $R^D = 12$ Å (for B-DNA). Our more detailed calculations confirm that the optimal monolayer radius is, indeed, very close to that of the DNA rod and that the bending and electrostatic energies are often comparable.

FREE ENERGY

In this section we describe our model for calculating the free energies of the spaghetti and honeycomb complexes shown in Fig. 1. A common structural element of both structures is the monolayer-coated DNA. It is convenient to start our description with the spaghetti complex, i.e., the bilayer-coated DNA, and then proceed to the monolayer-coated DNA, from which expressions for the free energy of the honeycomb complex can easily be derived.

The spaghetti complex

Consider a charged (DNA) rod of length L and radius R^D, surrounded by a lipid membrane of radius R, measured at the bilayer midplane. The membrane is composed of two monolayers; an external monolayer of radius $R^E = R + d$, measured at the surface containing the lipid polar headgroups, and an inner monolayer of radius $R^I = R - d$, with $2d$ denoting the bilayer thickness. The bilayer is composed of two components, a cationic lipid, "L" and a neutral ("helper") lipid, "S". We shall use N_L and N_S to denote the number of these molecules in the bilayer; $N = N_L + N_S$ is the total number of molecules constituting the membrane. Also, $N = N^E + N^I$ where N^E and N^I are the numbers of lipids in the external and internal monolayers, respectively. Using N_L^E and N_L^I, (N_S^E and N_S^I), to denote the numbers of L (S) lipids in the outer and inner monolayer, respectively, we also have $N_L^E + N_S^E = N^E$, $N_L^I + N_S^I = N^I$, and $N_L^E + N_L^I = N_L$, $N_S^E + N_S^I = N_S$. Throughout this work we shall assume a fixed area per molecule, a, for both components. Then we have $4\pi RL = a\,N$, $2\pi(R + d)L = aN^E$, and $2\pi(R - d)L = aN^I$.

The complex free energy, per unit length of DNA, is determined by the bilayer radius R (or, equivalently, the total number of lipids N), and by the lipid compositions (neutral lipid mol fraction) in the two monolayers; $\phi^E = N_S^E/N^E$ and $\phi^I = N_S^I/N^I$. Of course, any other three independent variables can be used; for instance, the fraction, $\alpha = N^E/N = (R + d)/(2R)$, of molecules in the external monolayer, the overall mol fraction, $m = N_S/N$, of neutral lipid in the bilayer and, say, ϕ^I. Clearly,

$$m = \alpha\phi^E + (1 - \alpha)\phi^I. \tag{3}$$

Instead of the free energy per unit length, $\hat{f} = F/L$, we find it convenient to use another intensive quantity: the free energy per lipid molecule in the complex $f = f^c(R, m, \phi^I) = F/N$, which can be expressed as a weighted sum of contributions from the lipids in the inner and outer monolayers,

$$f = \alpha f^E + (1 - \alpha)f^I. \tag{4}$$

The free energy of the complex, and hence f, can be expressed as a sum of electrostatic, bending, and mixing terms,

$$f = f_e + f_b + f_m. \tag{5}$$

We next outline our model for each of these three contributions.

Bending free energy

The elastic bending energy of a lipid monolayer (or bilayer) of cylindrical curvature c can be expressed using the familiar Helfrich (Helfrich, 1973) expression $f_b/a = k\,(c - c_o)^2/2$, which involves the splay constant, k, and the spontaneous curvature, c_o. Although the bending of, say, a planar monolayer into the highly curved monolayer in a tight DNA/monolayer complex implies a severe curvature deformation, we shall keep using the quadratic approximation for f_b; an approximation that was shown to be valid in other, highly curved, systems as well (Szleifer et al., 1988; May and Ben-Shaul, 1995; Andelman et al., 1994).

Both k and c_o depend on the monolayer composition. As proved appropriate for certain lipid mixtures (Andelman et al., 1994; May and Ben-Shaul, 1995), we shall assume that these quantities vary linearly with composition,

$$\begin{aligned} c_o(\phi) &= c_o^L + \phi(c_o^S - c_o^L), \\ k(\phi) &= k_L + \phi(k_S - k_L), \end{aligned} \tag{6}$$

where k_L, k_S, c_o^L, and c_o^S are the bending rigidities and spontaneous curvatures of the pure L and S monolayers. Noting that the curvatures in the two monolayers are of opposite signs we find

$$\begin{aligned} f_b^E &= \frac{a}{2}k(\phi^E)\left[\frac{c}{1 + d/R} - c_o(\phi^E)\right]^2, \\ f_b^I &= \frac{a}{2}k(\phi^I)\left[\frac{c}{1 - d/R} + c_o(\phi^I)\right]^2. \end{aligned} \tag{7}$$

Mixing free energy

Assuming ideal lateral mixing of the cationic and neutral lipids in each monolayer, we write

$$f_m(\phi) = k_B T[\phi \ln \phi + (1 - \phi)\ln(1 - \phi)]. \tag{8}$$

Electrostatic free energy

The electrostatic free energy will be calculated using the Gouy-Chapman model of the diffuse double layer, which is based on solutions of the Poisson-Boltzmann (PB) equation. For a 1:1 electrolyte the PB equation is

$$\Delta\Psi = \kappa^2 \sinh \Psi, \tag{9}$$

where Δ is the Laplacian, $\Psi = e\Phi/(k_B T)$ is the reduced electrostatic potential, Φ the electrostatic potential, $1/\kappa =$

$(8\pi Q_B n_0)^{-1/2}$ is the Debye length, and n_0 the total ionic bulk density. For the present problem we have to solve the PB equation in both the inner (between the charged rod and the inner monolayer) and outer (from the outer monolayer to infinity) regions of the complex. Because of the cylindrical symmetry of the system the PB equation is one-dimensional, that is, $\Psi''(r) + \Psi'(r)/r = \kappa^2 \sinh \Psi(r)$. We shall assume that the two monolayers are electrically decoupled. (This assumption is fulfilled in the limit $\epsilon_L/(\epsilon\kappa d) \ll 1$, where ϵ_L is the dielectric constant of the bilayer interior.) Our assumption that the areas per headgroup of both lipids do not change in the course of bending implies that the surface charge densities are constant, depending only on the lipid composition, ϕ. Note that this assumption, which is valid when the areas per headgroup are dictated by the balance between headgroup repulsions and the hydrocarbon-water surface tension, also implies that the neutral surface (with respect to bending deformations) coincides with that of the headgroups.

The boundary conditions for solving the PB equation in the inner region are $\Psi'(R^D) = 2Q_B/(R^D l)$ and $\Psi'(R^I) = 4\pi Q_B(1 - \phi^I)/a$. In the outer region we have $\Psi'(R^E) = -4\pi Q_B(1 - \phi^E)/a$ and $\Psi'(\infty) = 0$.

The free energy of double layer formation is given as a sum of the electrostatic energy and the mixing entropy of the (ideal) electrolyte solution (Verwey and Overbeek, 1948),

$$F_e = \frac{\epsilon}{2}\int_V dv(\nabla\Phi)^2 + k_B T \int_V dv\left[n_+ \ln\frac{n_+}{n_0} + n_- \ln\frac{n_-}{n_0} - (n_+ + n_- - 2n_0)\right], \tag{10}$$

where n_+ and n_- are the local number densities of the positively and negatively charged ions, respectively. The integration has to be carried out over the whole space. Using the PB-equation and the boundary conditions gives for F_e in both the inner and outer regions of the spaghetti complex

$$\frac{F_e^I}{k_B T L} = \pi\left(\frac{R^I}{a^I}\Psi^I - \frac{R^D}{a^D}\Psi^D\right) + \frac{\kappa^2}{4Q_B}\int_{R^D}^{R^I} dr r(\Psi \sinh \Psi - 2\cosh \Psi + 2),$$

$$\frac{F_e^E}{k_B T L} = \pi\frac{R^E}{a^E}\Psi^E + \frac{\kappa^2}{4Q_B}\int_{R^E}^{\infty} dr\, r(\Psi \sinh \Psi - 2\cosh \Psi + 2), \tag{11}$$

with $a^I = a/(1 - \phi^I)$, $a^E = a/(1 - \phi^E)$, $a^D = 2\pi R^D l$, and $F_e = F_e^E + F_e^I$, also $\Psi^I = \Psi(R^I)$ etc. The electrostatic free energy per molecule is now given by $f_e = (F_e^E + F_e^I)a/(4\pi RL)$.

Planar symmetric bilayer

In the limit $\kappa R \gg 1$ the bilayer is essentially flat and well separated from the DNA. By symmetry the compositions of the two monolayers must be equal. We use this limit, of noninteracting DNA and planar membrane, as a reference state for calculating the free energy of the complex, f.

Let $f^{pl} = F^{pl}/N = F(c = 0, \phi^E = \phi^I = m)/N$ denote the free energy per molecule in the planar bilayer. The bending and mixing contributions to f^{pl} are given by

$$f_b^{pl} = \frac{a}{2}k(m)c_0^2(m), \qquad f_m^{pl} = k_B T[m \ln m + (1 - m)\ln(1 - m)]. \tag{12}$$

The electrostatic energy per molecule for the planar symmetric membrane is (Lekkerkerker, 1989)

$$\frac{f_e^{pl}}{k_B T} = 2(1 - m)\left[\frac{1 - q}{p} + \ln(p + q)\right] \tag{13}$$

with $p = 2(1 - m)Q_B\pi/(\kappa a)$ and $q = \sqrt{p^2 + 1}$.

We can use the expressions for the free energy per molecule in the bent and flat bilayer to define a molecular *formation* free energy of the complex from the separated DNA and lipid bilayer,

$$\Delta f = f - \left(f^{pl} + \frac{\hat{f}^D a}{4\pi R}\right), \tag{14}$$

where $\hat{f}^D = F_e^D/L$ is the electrostatic energy per unit length of the isolated charged rod and $F_e^D = F_e^I(R^I \to \infty, \phi^I = 1)$.

As in Eq. 4 the formation free energy of the spaghetti complex can be expressed as a sum of inner and outer monolayer contributions,

$$\Delta f = \alpha\Delta f^E + (1 - \alpha)\Delta f^I, \tag{15}$$

where $\Delta f^E = \Sigma_{i=\{e,m,b\}}\Delta f_i^E$ and $\Delta f_i^E = f_i^E - f_i^{pl}$; $i = \{e, m, b\}$. Δf^I is defined analogously. Note that the last term from Eq. 14 appears only in the electrostatic contribution,

$$\Delta f_e^I = f_e^I - \left(f_e^{pl} + \frac{\hat{f}^D a}{2\pi R^I}\right). \tag{16}$$

Finally, note that in the separated state the bilayer is flat. Thus, Δf_e contains not only the electrostatic interaction between the rod and the membrane but also the electrostatic energy needed to bend the membrane from the flat state to one having a curvature $c = 1/R$. This means that the bending elastic energy, f_b, does not include the electrostatic contribution; for a fixed headgroup area (as we assume here) this contribution is small compared to the chain contribution (Lekkerkerker, 1989).

The honeycomb complex

The honeycomb complex can be regarded as an array of monolayer-coated DNA units. The lipid monolayer corresponds to the inner monolayer of the spaghetti complex. The free energy of forming a monolayer-coated DNA, starting from a planar monolayer of the same composition, involves only the bending and electrostatic contributions,

$$\Delta f^{\mathrm{I}} = \Delta f^{\mathrm{I}}_{\mathrm{b}} + \Delta f^{\mathrm{I}}_{\mathrm{e}}. \tag{17}$$

The monolayers coating the DNA strands in the honeycomb complex are not exactly identical to the inner monolayers in the spaghetti complex, because some of the lipid chains must be stretched out into the "corners" of the hexagonal lattice. This involves an additional free energy penalty known as the frustration energy, Δf_{f} (Seddon, 1990). Thus, the free energy per lipid in the honeycomb complex is given by

$$\Delta f^{\mathrm{hon}} = \Delta f^{\mathrm{I}} + \Delta f_{\mathrm{f}}. \tag{18}$$

The frustration energy is an important factor in determining the stability of the H_{II} phase. Clearly, if the distance $\bar{l}$ from the interface to the hexagonal corners (see Fig. 2) exceeds the maximal, fully stretched, chain length, l_{m}, the frustration energy will be intolerably high, and the hexagonal phase cannot exist. For $\bar{l} < l_{\mathrm{m}}$ the frustration energy can be calculated using a simple spring model (Duesing et al., 1997). According to this model the excess free energy of a chain of length $l(\phi)$, (see Fig. 2), is given by

$$g(\phi) = \tau\left(\frac{l(\phi) - d}{d}\right)^2, \tag{19}$$

where τ is the spring constant and d is the equilibrium chain length in a planar bilayer. The *surface* averaged frustration energy is then $\Delta f_{\mathrm{f}} = \int da\, g / \int da$, where the integration is carried out over the interface. According to Fig. 2

$$l(\phi) = \frac{(R^{\mathrm{I}} + \bar{l})\sqrt{3}}{2\cos\phi} - R^{\mathrm{I}}, \tag{20}$$

which leads to

$$\Delta f_{\mathrm{f}} = \frac{6\tau}{d^2\pi}\left[\frac{\sqrt{3}}{4}(R^{\mathrm{I}} + \bar{l})^2 - \sqrt{3}\ln\sqrt{3}(R^{\mathrm{I}} + \bar{l})(R^{\mathrm{I}} + d) + \frac{\pi}{6}(R^{\mathrm{I}} + d)^2\right]. \tag{21}$$

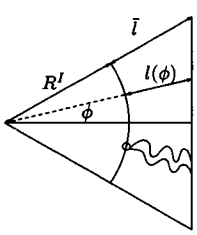

FIGURE 2 Schematic representation of a segment of the inverted hexagonal phase. The chain length at position ϕ is denoted by $l(\phi)$.

The most stable honeycomb lattice corresponds to the minimum of Δf_{f} with respect to the lattice constant $\bar{l}$. Minimization of Δf_{f} yields $\bar{l}^{\mathrm{eq}} = 2\ln\sqrt{3}\,(R^{\mathrm{I}} + d) - R^{\mathrm{I}}$ which, upon insertion into Eq. 21, leads to

$$\Delta f_{\mathrm{f}} = \frac{6\tau}{\pi}\left(\frac{R}{d}\right)^2\left(\frac{\pi}{6} - \sqrt{3}(\ln\sqrt{3})^2\right). \tag{22}$$

To estimate the frustration energy suppose $d = 15$ Å and $R^{\mathrm{I}} = 14$ Å. This implies $\Delta f_{\mathrm{f}}/\tau = 0.0070$ and $\bar{l}^{\mathrm{eq}} = 17.9$ Å. If the honeycomb consisted of double stranded (supercoiled) DNA and hence an inner monolayer of, say, $R^{\mathrm{I}} = 26$ Å, we would find $\Delta f_{\mathrm{f}}/\tau = 0.0139$ and $\bar{l}^{\mathrm{eq}} = 19.0$ Å. It should be noted that much larger values of R^{I} would not allow the formation of a hexagonal lattice as the interstitial voids in the H_{II} structure cannot be reached by the lipid chains.

The surface averaged frustration energy is an approximation. Given the uniform distribution of chain segments in the hydrophobic volume it neglects the change in the molecular surface area for molecules whose chain length is not the optimal one. One may take this into account by replacing the *surface* averaged frustration energy by a *volume* averaging via $\Delta f_{\mathrm{f}} = \int dv\, f / \int dv$. Determination of f_{f} in this case does not lead to a simple analytical expression. However, numerically we find for $d = 15$ Å and $R^{\mathrm{I}} = 14$ Å a frustration energy of $\Delta f_{\mathrm{f}}/\tau = 0.0074$ and $\bar{l}^{\mathrm{eq}} = 17.7$ Å, whereas $R^{\mathrm{I}} = 26$ Å yields $\Delta f_{\mathrm{f}}/\tau = 0.0149$ and $\bar{l}^{\mathrm{eq}} = 18.8$ Å. Thus, the area averaged Δf_{f} is a good and convenient approximation.

An estimate of τ may be obtained using experimental values for the lateral compression modulus of bilayer membranes, K_{A}, defined by

$$2\frac{f}{a_0} = \frac{K_{\mathrm{A}}}{2}\left(\frac{a}{a_0} - 1\right)^2. \tag{23}$$

Here f is the stretching energy per molecule, i.e., the energy needed to change the molecular area from the equilibrium value a_0 to a. (The factor of two on the left-hand side accounts for the fact that the bilayer membrane consists of two monolayers.) Since the hydrophobic core is incompressible the membrane thickness l is related to the area per molecule via $a = v/l$, where v is the molecular volume. This leads, for double-chained lipids, to $\tau = a_0 K_{\mathrm{A}}/8$. Using the typical values $K_{\mathrm{A}} = 500$ mN/m^{-1} $= 1.2\ k_{\mathrm{B}}T$ Å^{-2} (Evans and Needham, 1987) and $a_0 = 65$ Å^2 (Parsegian and Rand, 1995) we find $\tau \approx 10\ k_{\mathrm{B}}T$. Thus, for example, for $d = 15$ Å and $R^{\mathrm{I}} = 14$ Å we get $\Delta f_{\mathrm{f}} \approx 0.07\ k_{\mathrm{B}}T$.

Bilayer-complex coexistence

The theoretical model presented above can be used to calculate the free energy $F^{\mathrm{c}}(N^{\mathrm{c}}_{\mathrm{L}}, N^{\mathrm{c}}_{\mathrm{S}}; L^{\mathrm{c}})$, of a complex composed of $N^{\mathrm{c}}_{\mathrm{L}}$ cationic lipids, $N^{\mathrm{c}}_{\mathrm{S}}$ helper lipids, and DNA of length L^{c}. For both the honeycomb and spaghetti structures shown in Fig. 1, $N^{\mathrm{c}}/L^{\mathrm{c}} = (N^{\mathrm{c}}_{\mathrm{L}} + N^{\mathrm{c}}_{\mathrm{S}})/L^{\mathrm{c}}$ dictates the radius, R, of the complex. For the honeycomb complex $N^{\mathrm{c}}/L^{\mathrm{c}} = (2\pi/a)R$, where $R = R^{\mathrm{I}}$ is the monolayer radius at the

headgroup surface; $N^c/L^c = (4\pi/a)R$ for the spaghetti structure, with R denoting the radius of the bilayer midplane and with $a = a_L = a_S$ being the average area per lipid headgroup. Thus, for both structures we can write $N^c = \alpha(L^c)R$. (Interestingly, $N^c \sim R$ holds also for the multilayered DNA-membrane complex reported by Rädler et al. (1997) and Lasic et al. (1997), with R denoting the average distance between DNA strands within a given layer.) Thus, the stability of the complex can be conveniently characterized in terms of the intensive quantity, $f^c(R, \phi^c) = F^c(N_L^c, N_S^c; L^c)/N^c$.

Thermodynamically, the most stable complex configuration corresponds to the global minimum of f^c with respect to R and ϕ^c. However, attaining this minimum is not always possible. Suppose, for instance, that a large amount of DNA molecules (of total length L) is added to an aqueous solution of liposomes containing $N = N_L + N_S$ lipid molecules. Suppose further that for this lipid composition, $m = N_S/N$, the honeycomb structure is the most stable complex geometry. If $N < (2\pi L/a)R^D$ (with $R^D < R$ being the DNA radius) then, most likely, all the liposomal lipids will be consumed in forming complexes of some optimal radius $R = \tilde{R}(m) > R^D$ and composition $\phi = m$, which need not correspond to the minimum of $f^c(R, \phi)$. The solution will also contain non-complexed DNA molecules.

The opposite limit is more interesting. Namely, suppose the system contains a large excess of lipid molecules. In this case, the complexes formed may adjust their lipid composition and radius so as to minimize $f^c(R, \phi)$, with the excess bilayer serving as a lipid reservoir; ϕ^c may be very different from m. More generally, assuming that the system can reach thermodynamic equilibrium, the lipid compositions in the complex and bilayer, as well as the complex radius, will be determined by the usual conditions for phase coexistence, i.e., by the equality of the chemical potentials of the L and S lipids in the two structures or, equivalently, by the minimum of the total free energy of the system.

To establish the equilibrium conditions in the DNA-(mixed) lipid system let us assume that one packing geometry, say the honeycomb structure, is more stable than other possible geometries for all lipid compositions. (Thus, we shall not be concerned here with phase transitions between complexes of different symmetries.) We first consider the case of excess lipid in the system and assume that all the DNA available is involved in complex formation, that is, $L^c = L$. We ignore the translational entropies of the complexes and the liposomes, as well as interaction free energies between these species. Thus, the total free energy of the system is a sum $F = F^c(N_L^c, N_S^c; L) + F^b(N_L^b, N_S^b)$ of the internal (packing) free energies of the lipids in the complex and bilayer phases. The total numbers $N_L = N_L^c + N_L^b$ and $N_S = N_S^c + N_S^b$ of cationic and helper lipids are constant. Hence $N = N_S + N_L$ and the overall lipid composition $m = N_L/N$ are also constant. We treat the lipid layers in the complex and the free membrane as incompressible, with fixed $a_S = a_L = a$ in both phases. Then, the system is in thermodynamic equilibrium when

$$F = F^c(N_L^c, N_S^c; L) + F^b(N_L^b, N_S^b)$$
$$= N^c f^c(R, \phi^c) + (N - N^c) f^b(\phi^b) \quad (24)$$

is minimal with respect to variations in N_L^c and N_S^c, or equivalently, R and ϕ^c. Note that $N^c = (2\pi L/a)R \equiv \gamma R$ is a function of the complex radius, R, and ϕ^b is related to ϕ^c by the conservation condition (lever rule).

$$\chi\phi^c + (1 - \chi)\phi^b = m, \quad (25)$$

where $\chi = N^c/N = (\gamma/N)R$ is the fraction of lipids associated in complexes; $f^b(\phi^b)$ is the free energy per molecule in a lipid bilayer of composition $\phi^b = N_S^b/N^b = N_S^b/(N - N^c)$.

The minimum conditions, $\partial F/\partial N_L^c = \partial F^c/\partial N_L^c - \partial F^b/\partial N_L^c = \mu_L^c - \mu_L^b = 0$ and $\partial F/\partial N_S^c = \mu_S^c - \mu_S^b = 0$ are the familiar requirements for the equality of chemical potentials in the complex and bilayer phases. [Note that $\mu_L^c = (\partial F^c/\partial N_L^c)_{N_S^c}$, etc., are not the derivatives of the Helmholtz free energy at constant volume. This is the usual definition of the chemical potential in incompressible phases, see Hill (1960).] More convenient for our purposes here is to minimize F, as given by the second equality in Eq. 24, with respect to R and ϕ^c. The result is

$$\left(\frac{\partial f^c}{\partial \phi^c}\right)_R = \frac{df^b}{d\phi^b}, \quad (26)$$

$$f^b - \left[f^c + R\left(\frac{\partial f^c}{\partial R}\right)_{\phi^c}\right] = (\phi^b - \phi^c)\frac{df^b}{d\phi^b}. \quad (27)$$

If f^c were independent of R, these two equations would describe the familiar, common tangent, condition for phase equilibrium in a two component system. The coexistence conditions are modified here because of the complex ability to adjust R so as to minimize F. On the other hand, if the complex radius were fixed then minimization of F would only yield the single (equal tangent) condition (Eq. 26). Using this condition and the lever rule, Eq. 25, would then yield both ϕ^b and ϕ^c for the given radius R and composition m. In the next section we shall see that $f^c(R, \phi^c)$ has a deep minimum at $R \approx R^D$ and hence that the complex radius is, indeed, nearly constant for most relevant values for χ and m.

The other limit of interest is that of excess DNA. In this case, assuming that all the lipids are involved in complex formation (i.e., no free bilayer), the total free energy of the system can be written as

$$F = N^c f^c(R, m) + L\left(1 - \frac{aN}{2\pi RL}\right)\hat{f}^D, \quad (28)$$

where $\hat{f}^D$ is the free energy per unit length of bare (uncoated) DNA; $f^c(R, m)$ is the free energy per lipid molecule in a complex of radius R, and composition $\phi = m$ corresponding to the overall mol fraction of charged lipid in the solution. L is the total length of DNA in the system, of which a fraction $(aN)/(2\pi RL)$ is coated by the lipid layer.

Minimization of F with respect to R yields the equilibrium condition

$$R^2 \frac{\partial f^c}{\partial R} + \frac{a}{2\pi} \hat{f}^D = 0 \qquad (29)$$

which in terms of the formation free energy defined in Eq. 14 reads $\partial \Delta f^c / \partial R = 0$.

In the most general case we should allow for three phase equilibria: bare DNA, bilayer of composition ϕ^b, and complex of composition ϕ^c and radius R. The calculations reported in the next section reveal that in practically all cases of interest, either all the DNA or all the lipids are involved in complex formation.

RESULTS AND DISCUSSION

Coating the DNA by a monolayer

In this section we calculate the free energy of a DNA strand surrounded by a mixed, cationic/neutral, lipid monolayer. The free energy of this complex will be calculated as a function of the monolayer radius, $R = R^I$, and lipid composition $\phi = \phi^I$. It will be interesting, for example, to find out for which values of R and ϕ the free energy of forming the complex from separated, bare DNA and planar lipid bilayer, is minimal. We are not concerned yet with the question of how the monolayer would protect its hydrophobic tails from contact with the aqueous environment. This will be treated later.

According to Eq. 17 the free energy of forming the monolayer-coated DNA complex (starting from a planar bilayer of the same composition, $m = \phi^I$) is a sum of electrostatic and bending contributions; $\Delta f^I = \Delta f^I_b + \Delta f^I_e$. The electrostatic contribution is expected to be negative, at least for a range of compositions ϕ and complex radii R. In the numerical calculations of Δf^I_e we shall use $R^D = 12$ Å for the DNA radius and $l = 1.7$ Å for the DNA length per unit (i.e., one elementary) charge; both corresponding to B-DNA. We shall assume a Debye length of $l_D = 1/\kappa = 10$ Å, which dictates the bulk concentration of free ions ($n_o \approx 0.06\ \mathrm{nm}^{-3} = 0.1\ M$). Clearly the monolayer radius R^I must be larger than R^D. Even if the DNA and cationic lipid charges are only partially hydrated it is expected that strong, short-range, excluded volume interactions will prevent direct contact between the DNA and the monolayer mantle, implying $R^I > R^D$. Thus, in all the calculations presented below we shall, somewhat arbitrarily, restrict the monolayer radius to $R^D > 14$ Å. Later on, in the DNA-bilayer coexistence section, we will add a short-range repulsive potential to the complex free energy. Our numerical results are not sensitive to these ramifications of the model.

The calculation of the bending energy of the mixed monolayer, Δf^I_b, requires the bending rigidities and spontaneous curvatures of the pure, cationic and neutral, monolayers; see Eqs. 6 and 7. In all the calculations reported in this work we have used the following set of elastic constants: $k_L = 13\ k_BT$, $k_S = 2.5\ k_BT$, $c^L_o = 0$, and $c^S_o = -1/20$ Å^{-1}. Recall also that we use $a = 65$ Å^2 for the interfacial area per lipid molecule. There are no direct measurements, so far, of the elastic properties of cationic liposomes neither of the composition dependence of $k(\phi)$ and $c_0(\phi)$. In the absence of exact information the following findings have guided us in choosing the elastic constants. The elastic properties of DOPE, which often serves as a helper lipid, were measured using the osmotic stress method by Gruner et al. (1986) who found $2\ k_S \approx 5\ k_BT$, $c^S_o \approx 1/20$ Å. These values were determined for the inverted hexagonal phase (H_{II}) where the monolayer curvatures are similar to those in the spaghetti and honeycomb complexes. In choosing $k_L = 13\ k_BT$ we have in mind the cationic component DC-Chol. Being a cholesterol derivative it has a rather rigid hydrophobic backbone. It is known that adding, say, 20% cholesterol to a PC bilayer increases the bending modulus of the membrane by, roughly, a factor of two (Duwe et al., 1990). Therefore we may expect a similar increase in the elastic contribution to the bending modulus of a DOPE membrane containing DC-Chol; hence the choice $k_L = 13\ k_BT$. (More generally, for most lipids k is in the range of 10–20 k_BT; DOPE has a particularly low bending rigidity.) Because of their strong inter-headgroup repulsions cationic lipids tend to increase the monolayer spontaneous curvature, as compared to that of neutral lipids; i.e., $c^L_o > c^S_o$. Consistent with this notion, yet somewhat arbitrarily, we assume here $c^L_o = 0$.

The free energy of DNA/monolayer complex formation, Δf^I, as well as its electrostatic and elastic components, calculated for the above set of molecular properties, is

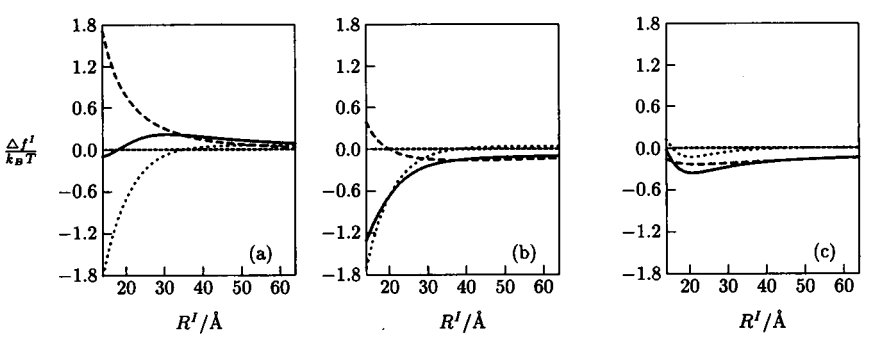

FIGURE 3 The formation free energy, per molecule, Δf^I (*solid line*). Also shown are the bending, Δf^I_b, (*dashed line*), and electrostatic Δf^I_e, (*dotted line*), components of Δf^I. The lipid compositions (neutral lipid mol fractions) are $\phi^I = 0.1$ (*a*), 0.5 (*b*), 0.9 (*c*).

shown in Fig. 3 as a function of the complex radius, R^I. Δf^I is shown for three representative compositions, $\phi^I = 0.1$, 0.5, and 0.9, ranging from high to low contents of the cationic lipid.

Consider first the electrostatic contribution, Δf_e^I. Its determination is based on Eq. 16 and numerical solutions of the PB equation for cylindrical symmetry (see Eqs. 11). In the separated state ($R^I \to \infty$), f_e^I reduces to the electrostatic energy of the bare DNA and a planar monolayer. The electrostatic energy per unit length $\hat{f}^D = F_e^D/L$ of a bare DNA is derived by integrating the PB equation $\Psi'' + \Psi'/r = \kappa^2 \sinh \Psi$, with the boundary conditions $\Psi'(R^D) = 2Q_B/(R^D\ l)$ and $\Psi'(\infty) = 0$ (Stigter, 1975; LeBret and Zimm, 1984). For $R^D = 12$ Å, $L = l = 1.7$ Å and $l_D = 10$ Å one obtains the free energy per unit DNA *charge* $\tilde{f}_e^D = l\ \hat{f}^D = 2.04\ k_BT$. Note that the numerical solution of the PB equation also accounts for the phenomenon of counterion condensation (Manning, 1978).

It is interesting to compare the electrostatic free energy of a (bare) planar lipid layer with the same surface charge density as that of a bare DNA rod, namely, $\sigma^D = e/(2\pi R^D l) = 0.125\ \mathrm{Cm}^{-2}$. The monolayer electrostatic energy is given by Eq. 13, yielding $\tilde{f}_e^{pl} = 2\pi R^D l f_e^{pl}/a = 2.39\ k_BT$. Thus, $\tilde{f}_e^{pl} - \tilde{f}_e^D = 0.35\ k_BT > 0$, i.e., the diffuse double layer is of higher free energy for the planar membrane, as expected, due to the larger translational entropy of the ion clouds around the cylindrical surface. The values obtained for $\tilde{f}_e^{pl}$ and $\tilde{f}_e^D$ can also be used to calculate the maximal gain in the electrostatic free energy of complex formation. This limit corresponds to bringing the DNA rod and an oppositely charged monolayer of the same charge density to close contact (i.e., $R^D = R^I = 12$ Å). In this hypothetical limit, which corresponds to complete charge neutralization, $f_e = 0$, and the gain in electrostatic energy, (per lipid molecule), i.e., the electrostatic contribution to the complex formation energy, is: $\Delta f_e^I = -(\tilde{f}_e^{pl} + \tilde{f}_e^D)[a/(2\pi R^D l)] = -2.22\ k_BT$; the factor in square brackets is the number of unit charges on the DNA surface corresponding to the area of one lipid molecule, $a = 65$ Å^2. The surface area per unit (DNA or cationic lipid) charge at close DNA-monolayer contact is 128 Å^2, implying $1 - \phi^I = 65/128 \approx 0.51$ for the mol fraction of the cationic lipids in the monolayer. The formation free energy for (nearly) this value of ϕ^I is displayed in Fig. 3 *b*. Thus, the value of Δf_e^I at $R^I = 12$ Å will be (nearly) $-2.22\ k_BT$, corresponding to complete disappearance of the diffuse double layers.

More generally, the monolayer surface charge density is given by $\sigma^I = e(1 - \phi^I)/a = 0.246\ (1 - \phi^I)\mathrm{Cm}^{-2}$. If ϕ^I is small, say $\phi^I = 0.1$ as in Fig. 3 *a*, the membrane is highly charged. In this case, the R^I dependence of Δf_e^I, for the range of R^I shown is very similar to that for $\phi^I = 0.5$; in both cases similar amounts of counterions are released into the bulk solution, leading to a similar decrease in the electrostatic free energy. At extremely small monolayer-DNA separations, i.e., as $R^I - R^D \to 0$, the counterions in the "gap" are highly confined, leading to a sharp increase of Δf^I. However, according to the PB equation, this happens when $R^I - R^D < 0.3$ Å (not shown in Fig. 3) where the PB equation is not applicable. Furthermore, at such short distances one must take into account excluded volume repulsions (see below). A qualitatively different R dependence is observed for large ϕ^I (e.g., $\phi^I = 0.9$ as displayed in Fig. 3 *c*), which corresponds to a weakly charged monolayer. Here, Δf_e^I first decreases as R decreases, again due to the partial release of counterions from the gap between the DNA and the monolayer. The decrease in Δf_e^I is slow because the number of counterions released (per lipid molecule) is small. At shorter distances, the confinement of the remaining counterions leads to an increase in Δf_e^I, resulting in the appearance of a shallow minimum in the electrostatic free energy.

Consider now the bending contribution, Δf_b^I, to the complex formation energy (Fig. 3). Recall that small ϕ^I means a large fraction of the charged component in the monolayer. This, in turn, implies large bending rigidity and nearly vanishing spontaneous curvature. Both factors contribute to the high elastic bending energy associated with wrapping the DNA by a (highly charged) lipid monolayer, as clearly seen in Fig. 3 *a*. In this case, the elastic energy cost nearly compensates the gain in electrostatic energy. Thus, a highly charged but rather rigid membrane would not serve as a good DNA mantle.

In the other limit, i.e., large ϕ^I, the monolayer spontaneous curvature nearly matches that of the (oppositely curved) DNA surface, implying favorable bending energy upon complex formation (starting from a planar geometry). However, the magnitude of this free energy gain is low, because of the low value of $k(\phi^I) \approx k_S$. Since for small R^I the electrostatic contribution to Δf^I was found to be repulsive, weakly charged (including soft) monolayers are also not expected to form stable DNA complexes.

For intermediate values of ϕ^I the elastic energy gives only a small contribution to Δf^I. This is because $c_o(\phi^I)$ is moderately negative, implying a similar bending free energy cost for either wrapping the monolayer around the DNA or adopting a planar configuration. Since for intermediate ϕ^I the gain in electrostatic energy is nearly as large as for small ϕ^I, this composition range is the most suitable one for complex formation.

In conclusion, the uncharged helper lipid appears to play an important role in the formation of a monolayer-coated DNA complex. First, it enables adjustment of the charge on the monolayer surface to ensure charge neutrality. Second, it lowers the cost of elastic energy associated with bending the monolayer around the DNA. The interplay between the electrostatic and elastic energy contributions is favorable for a range of (intermediate) compositions. In the presence of an excess lipid bilayer phase the complex can more easily reach this favorable region, as we shall show later in this section.

A monolayer-coated DNA complex cannot exist free in solution. However, it can coat itself with another monolayer to form the spaghetti-like complex. Alternatively, a bundle of such complexes may associate to form the tubular hon-

eycomb array. This bundle of hexagonally packed DNA/monolayer units can coat itself by one external monolayer, to avoid exposure of the outermost hydrophobic chains to the aqueous solvent.

Wrapping the monolayer-coated DNA with another oppositely curved monolayer to form the spaghetti complex is expected to involve an additional bending energy price, thus (partially or fully) destabilizing the complex formed. Similarly, the formation of the honeycomb complex involves the frustration free energy discussed in the previous section. The calculations presented below reveal that, in general, the frustration free energy associated with forming the honeycomb structure is lower than the bending energy penalty inflicted by the formation of the spaghetti complex. In other words, between these two structures, the honeycomb complex is the more favorable option.

The free energy calculations presented below require specification of the (relaxed) monolayer thickness, d. We shall use $d = 15$ Å, corresponding to the length of a lipid composed of a double chain C-14 tail of length 12 Å and headgroups of size 3 Å and a lipid area per headgroup of 65 Å^2.

The spaghetti structure

Suppose first that the lipid compositions of the inner and outer monolayers constituting the bilayer mantle of the spaghetti complex are equal; $\phi^I = \phi^E = m$ with m denoting the overall composition of the bilayer. The complex formation free energy, Δf, is the weighted sum of the formation free energies (per lipid molecule) in the internal and external monolayers, $\Delta f = (1 - \alpha)\Delta f^I + \alpha\Delta f^E$, with $\alpha = (R + d)/(2R)$, R being the radius of curvature of the bilayer midplane, (see Eq. 15).

In Fig. 4 we show Δf, Δf^I, and Δf^E as a function of m for $R = R^I + d = 14 + 15 = 29$ Å, corresponding to the minimal value which we have allowed for the distance between the DNA and the surface of the inner monolayer. Note that for this value of R, most of the lipid molecules are packed in the external monolayer, $\alpha = 0.76$. Fig. 4 reveals qualitatively different behaviors of Δf^I and Δf^E. While Δf^I is generally negative, with a minimum value at $m \approx 0.5$, Δf^E is positive for all compositions and shows a maximum at nearly the same composition where Δf^I is minimal. The positive contribution of Δf^E to the complex energy is due to the unfavorable bending energy of the external monolayer. Although relatively small in magnitude, the contribution of this term to Δf is amplified by the fact that most of the lipids constituting the complex belong to the external monolayer. Consequently, as we see in Fig. 4, the formation free energy of the complex stays negative (and small) only for a narrow range of compositions. This range becomes narrower as R increases, and completely disappears for $R > 34$ Å.

The results in Fig. 4 were obtained for a complex in which the lipid compositions in both monolayers were artificially chosen to be equal. It is much more reasonable to assume, for any given m, that in the course of complex formation the bilayer will adjust ϕ^I and ϕ^E, so as to minimize the total free energy of the system. This repartitioning of the lipids between the two monolayers involves a certain ("demixing") free energy cost, (see Eq. 8), which the system may or may not choose to pay. The optimal compositions in the inner and outer monolayers, for a given total composition m and bilayer radius R, are determined by the condition

$$\left(\frac{\partial \Delta f}{\partial \phi^I}\right) = 0. \tag{30}$$

In Fig. 5 we show the results obtained for Δf by allowing the compositions to optimize according to the last condition. (Again we use $R = 29$ Å.) For comparison we also show the results for the case $\phi^I = \phi^E = m$. We see that this extra compositional degree of freedom leads to a much broader

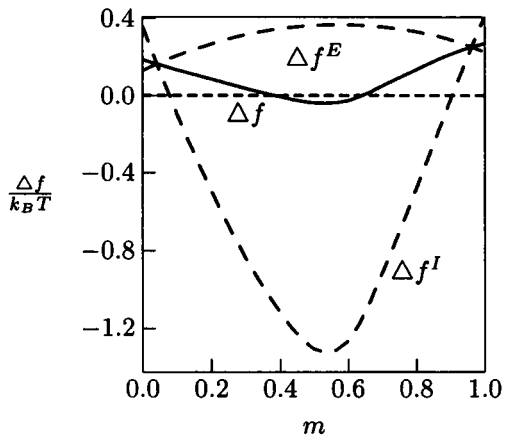

FIGURE 4 The formation free energy Δf of a bilayer coated charged rod (spaghetti) as a function of the lipid composition m. Also shown are the formation free energies of the inner and outer monolayers $\Delta f^I(\phi^I = m)$ and $\Delta f^E(\phi^E = m)$.

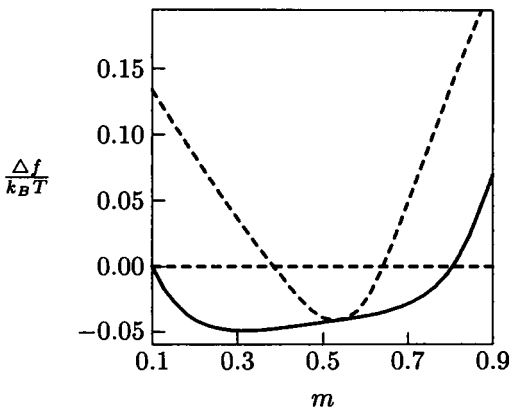

FIGURE 5 The formation free energy of the spaghetti complex Δf, as a function of the composition m, for close bilayer-DNA contact, $R = 29$ Å. The solid curve is obtained by allowing the compositions in the inner (ϕ^I) and outer (ϕ^E) layers to minimize the complex free energy according to Eq. 30 subject to $\alpha\,\phi^E + (1 - \alpha)\phi^I = m$; $\alpha = (R + d)/(2R)$. The dashed curve, corresponding to a bilayer where $\phi^E = \phi^I = m$, is shown for comparison.

range of overall compositions m over which the complex is stable, ($\Delta f < 0$). Furthermore, the minimum in the formation free energy becomes a little deeper and shifts to a lower value of the total bilayer composition, $m \approx 0.3$. The location of the minimum is easily explained. If there were no demixing free energy penalty, then according to the results shown in Fig. 3, the optimal compositions would be $\phi^I \approx 0.5$ and $\phi^E = 0$, (the minimum of Δf^E at $\phi^E = 1$ is higher). For $\alpha = 0.76$ this implies a minimum at $m = (1 - \alpha)\phi^I + \alpha\phi^E \approx 0.25$. The demixing penalty slightly changes this prediction; the minimum is slightly below $m = 0.3$ and the inner and outer monolayer compositions are $\phi^I = 0.48$ and $\phi^E = 0.24$.

The existence of a stable complex requires that Δf will have a minimum at a finite value of R. Fig. 6 shows the complex formation free energy as a function of R for $m = 0.3$ and $\phi^I = 0.48$ (where Δf is minimal with respect to the composition variables). We see that Δf is minimal when the DNA and the surrounding bilayer are nearly touching each other. As R increases Δf goes through a shallow maximum before approaching its asymptotic value, suggesting that complex formation may involve a small activation barrier. It should be noted that the rather weak minimum (at close contact) results from a delicate balance between two large contributions: the electrostatic free energy that stabilizes the complex and the bending energy that acts in the opposite direction. Since the bending energy depends sensitively on the type of lipids constituting the membrane, it should be possible, at least in principle, to control the stability of the complex by chemical modifications.

Of course, all our quantitative conclusions concerning the stability of the spaghetti complex are only valid for the particular choice of elastic constants that we have used in the calculations. However, our finding that this complex is only marginally stable (or unstable) appears more general. In the next section we shall see that the honeycomb complex is considerably more stable, implying that our conclusions with respect to this complex are more robust. Before turning to this system it should be pointed out that in a very recent paper Sternberg (1996) suggested (based on electron microscopy studies) that the DNA "rod" inside the spaghetti complex is not a single double-stranded helix but, rather, a supercoiled double helix whose hard core radius is $R^D \approx 24$ Å, i.e., about twice the radius for which the results in Figs. 4–6 were calculated.

Although a supercoiled DNA is not exactly a charged rod of radius $R^D = 24$ Å, it would be interesting to use this model to obtain an estimate for the formation free energy of the supercoiled DNA/bilayer complex. In Fig. 7 we show Δf as a function of the bilayer composition m, for a negatively charged rod of radius 24 Å. The surface charge density of the supercoiled DNA is assumed to be the same as that of B-DNA. The results shown in Fig. 7 are for a bilayer whose radius of curvature is $R = R^D + d + 2 = 41$ Å, with the last 2 Å representing the (minimally allowed) distance between the surface of the DNA and that of the inner monolayer. The major conclusion from this calculation is that Δf is considerably lower than that of the simple (single double helix) DNA complex. Because of the lower bending free energy involved in forming this complex Δf^I becomes more negative and Δf^E becomes less positive. Furthermore, because of the larger value of R, the fraction of molecules in the external monolayer decreases (from $\alpha = 0.76$ to $\alpha = 0.68$), thus emphasizing the favorable contribution from the internal monolayer.

Finally we note that the "thicker" spaghetti complex is stable for a wider range of lipid compositions. The formation free energy is generally minimal for the lowest possible value of R.

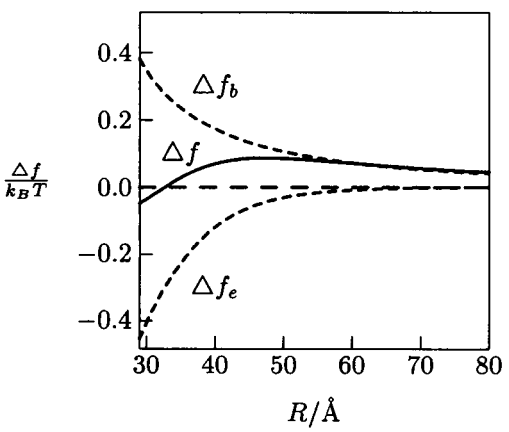

FIGURE 6 The formation free energy of a spaghetti complex, Δf, and its electrostatic and bending components, Δf_e and Δf_b, as a function of R for $m = 0.3$. For every R the compositions of the inner and outer monolayers are free to adjust so as to minimize the complex free energy.

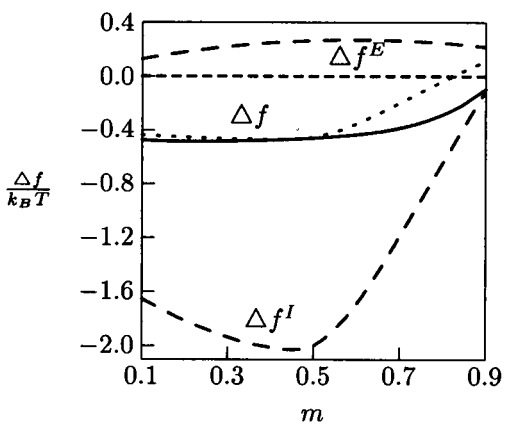

FIGURE 7 The formation free energy, Δf, of a bilayer coated charged rod of radius $R^D = 24$ Å as a function of the lipid composition of the bilayer m (*solid line*). This system serves as a model of a spaghetti complex composed of supercoiled DNA. Also shown are the formation free energies of the inner and outer monolayer Δf^I and Δf^E. The bilayer radius, corresponding to a tight complex, is $R = 41$ Å. The compositions of the inner and outer monolayers, ϕ^I and ϕ^E, ensure that $\Delta f(R, m, \phi^I)$ is minimal (*solid line*); the dotted curve represents Δf for $\phi^I = \phi^E = m$.

The honeycomb structure

The hexagonal arrangement of the honeycomb complex protects the hydrocarbon chains from contact with water, but at the expense of a certain chain stretching (frustration) energy Δf_f. We have used Eq. 22 to estimate Δf_f. For R^I = 14 Å, $\tau = 10\ k_BT$, and d = 15 Å this equation yields Δf_f = 0.07 k_BT, which according to Fig. 4 is just a negligible correction to the formation free energy of the monolayer-coated DNA complex. Hence $\Delta f^{hon} = \Delta f^I + \Delta f_f \approx \Delta f^I$. The formation free energy of a honeycomb complex containing DNA of radius R^D = 12 Å is thus given by the Δf^I curve in Fig. 4.

Similar calculations were carried out for a honeycomb complex involving supercoiled DNA, R^D = 24 Å. In this case we expect a higher frustration energy Δf_f as the chains must be more strongly stretched to reach the hexagonal corners. Using R^I = 26 Å, which implies $\bar{l}^{eq}$ = 19.0 Å, we find $\Delta f_f = 0.14\ k_BT$. Again, the frustration energy is negligible compared to the formation free energy of the monolayer coated DNA complex. The formation free energy of this "thicker" honeycomb complex is given by the Δf^I curve in Fig. 7.

From Fig. 4 it follows that, typically, the formation free energy of a honeycomb complex consisting of single DNA strands is $\Delta f^I \approx 1\ k_BT$/lipid molecule, implying $\Delta \hat{f}^I = (2\pi R^I/a)\ 1\ k_BT$/Å of DNA length. Thus, for a DNA strand of, say, length L = 100 Å the complex stabilization energy is on the order of 100 k_BT, comparable to the energy of one ordinary chemical bond. A similar calculation for a complex of supercoiled DNAs would yield, for L = 100 Å, a fourfold higher stabilization (see Fig. 7).

Bilayer-complex coexistence

As discussed in the Free Energy section, the lipid composition, ϕ^c, in a DNA/lipid complex, coexisting with an excess bilayer phase, is generally different from that in the bilayer, ϕ^b. The compositions in these two phases as well as the complex radius R are determined by Eqs. 26 and 27, which ensure that the system free energy is minimal subject to the material conservation (lever) rules. In this section we shall demonstrate how ϕ^c, ϕ^b, and R vary as a function of the total lipid composition in the system, m, for a system in which the complexes formed have a honeycomb structure. Similar calculations can be performed for the spaghetti complexes.

The solutions of Eqs. 26 and 27 are determined, of course, by the functional dependence of the complex free energy (per lipid molecule), $f^c(R, \phi^c)$, on the complex radius and composition, as well as of the bilayer free energy, $f^b(\phi^b)$, on its lipid composition. In Fig. 8 we show how $f^c(R, \phi^c)$ varies with the monolayer radius R for several lipid compositions ϕ^c. As already noted in previous sections (see Figs. 3 and 6), for most compositions of interest, f^c, which consists of electrostatic, bending, and mixing contributions, decreases nearly monotonically as R decreases toward the DNA radius R^D = 12 Å. Clearly, however, f^c must increase steeply at very small monolayer-DNA separations $r = R - R^D$, due either to excluded volume or hydration forces. To account for this short-range repulsion we may define a modified complex free energy $\tilde{f}^c(R, \phi) = f^c(R, \phi) + f^h(R)$, which includes a short range repulsive component $f^h(R)$. We model this term by the exponential form $f^h(r) = e^{-r/\xi}$ with a decay length ξ = 1 Å. The choice of the functional form of $f^h(R)$ and the value of ξ are somewhat arbitrary, yet the results of the calculated compositions, ϕ^c, ϕ^b are insensitive to these choices, as will be shown and explained shortly. In fact, the results obtained using either f^c or $\tilde{f}^c$ are very similar. The plots of $\tilde{f}^c(R, \phi)$ in Fig. 8 clearly show that for most compositions this function obtains its minimum roughly at the same value of R; $R = \tilde{R}$ = 15 ± 2 Å. In Fig. 9 we show how $f^b(\phi)$ and $f^c(R, \phi)$ vary with ϕ; $f^c(R, \phi)$ is shown for R = 14 Å, corresponding to the minimum value of this function for intermediate ϕ. Note that f^c obtains a pronounced minimum at $\phi^c = \tilde{\phi}^c$ = 0.61, whereas f^b shows a relatively shallow minimum at ϕ^b =

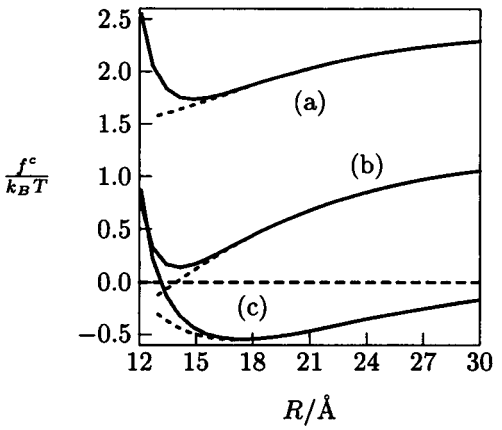

FIGURE 8 Variations of $\tilde{f}^c(R, \phi^c)$ (*solid lines*) and $f^c(R, \phi^c)$ (*dashed lines*) with the monolayer radius R for ϕ^c = 0.2 (*a*), ϕ^c = 0.4 (*b*), and ϕ^c = 0.8 (*c*).

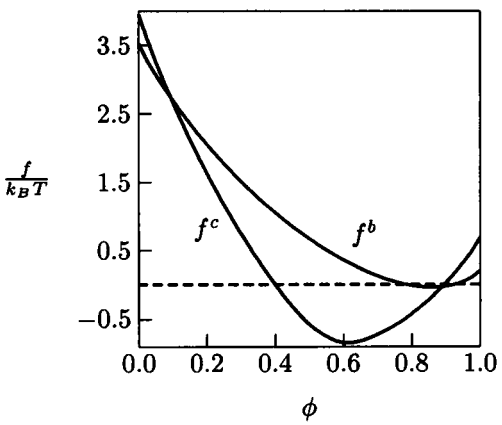

FIGURE 9 The bilayer and complex free energies, $f^b(\phi)$ and $f^c(R, \phi)$, as a function of composition for R = 14 Å.

$\tilde{\phi}^b = 0.86$. These minima reflect the balance of electrostatic, bending, and mixing terms. Fig. 10 *a* shows the bilayer and complex compositions, at coexistence, as a function of the total lipid composition m. Two sets of solutions are shown for a given number of lipids per DNA charge; $Nl/L = 4.6$. One corresponds to the full numerical solution of Eqs. 25–27 for ϕ^b, ϕ^c, and R. The other solution is obtained using only the equal tangent condition, Eq. 26 (i.e., disregarding Eq. 27) subject to the lever rule, Eq. 25, with R fixed at 14 Å. The two sets of solutions are obviously quite similar. The equilibrium values of the complex radius, R, derived from Eqs. 25–27 are shown in Fig. 10 *b*. Also shown in this figure are the values $\tilde{R}$ corresponding to the minimum of $\tilde{f}^c$. This would be the equilibrium radius of the complex in a system containing excess DNA.

A qualitative explanation of the results shown in Fig. 10 can be given as follows. First, because of the sharp minimum of $f^c(R, \phi)$ as a function of R (for most ϕ) the complex will strongly resist deviations of R from $\tilde{R}$. Alternatively put, since just outside $R = \tilde{R}$, $|\partial f^c/\partial R|$ is large, Eq. 27 is easily satisfied for essentially all values of f^b, f^c, ϕ^b, and ϕ^c, including those ϕ^b and ϕ^c derived from the equal tangent condition, Eq. 26. Thus, this latter condition is the decisive one for the determination of ϕ^b and ϕ^c; the radius R need not deviate significantly from $\tilde{R}$ except for very high values of m as confirmed in Fig. 10 *b*. This explains why Eq. 26 combined with $R \approx \tilde{R}$ provides reasonable estimates for the coexisting compositions ϕ^b and ϕ^c.

Since the minimum of $f^c(R, \phi)$ is considerably steeper than that of $f^b(\phi)$ (Fig. 9) the equal tangent condition, Eq. 26, will be satisfied over a relatively narrow region of ϕ^c values, around $\tilde{\phi}^c$, whereas the deviation of ϕ^b from $\tilde{\phi}^b$ can be quite large. In other words, whenever the lever rule and the equal tangent condition can be simultaneously met the complex composition will be close to its optimal value $\tilde{\phi}^c$ with ϕ^b adjusting so as to satisfy these requirements. This behavior is reflected by the rapid approach of ϕ^c to its optimal value at low m and its slow departure from $\tilde{\phi}^c$ at higher m's, with ϕ^b adjusting according to the lever rule (see Fig. 10 *a*).

CONCLUSIONS

Using a molecular-level model that includes the electrostatic, curvature elasticity, and lipid mixing entropy contributions to the free energy of a DNA/lipid mixture, we have calculated the thermodynamic stability of two closely related structural models of DNA/lipid complexes. We found that the spaghetti-like bilayer-coated DNA complex is only marginally stable. On the other hand, the densely packed honeycomb complex was found to be stable over a wide range of compositions. These conclusions refer to complexes involving either single (double-stranded helix) DNA molecules or supercoiled DNA. We also found that the electrostatic and bending energy contributions to the formation free energy are comparable, and generally of opposite signs.

Quantitatively our results involve some uncertainties associated with the choice of molecular constants, such as the bending moduli. They are also affected by the approximate nature of our free energy expressions, e.g., the use of the PB equation for calculating the electrostatic free energy. Nevertheless, we believe that our major qualitative conclusions are correct; in particular, the comparable roles of the bending and electrostatic free energies in complex stabilization.

We have not considered in this paper other possible complex geometries. Of particular interest would be to calculate the thermodynamic stability of the multilayered, bilayer-DNA complex mentioned in the introduction. In this system, unlike for the cylindrical geometries analyzed in the present work, the complex stability is expected to be governed, primarily, by the interplay between the electrostatic and lipid mixing contributions to the free energy. Since in this type of complexes the DNA strands are not fully surrounded by lipid monolayers, the electrostatic interaction may be somewhat less favorable than in the spaghetti or honeycomb complexes. Yet, since the bending energy is also small, it is possible that the net formation energy is more favorable than that of the cylindrical geometries. In this complex geometry 1D bending undulations of the DNA strands intercalated between the lipid bilayers may affect

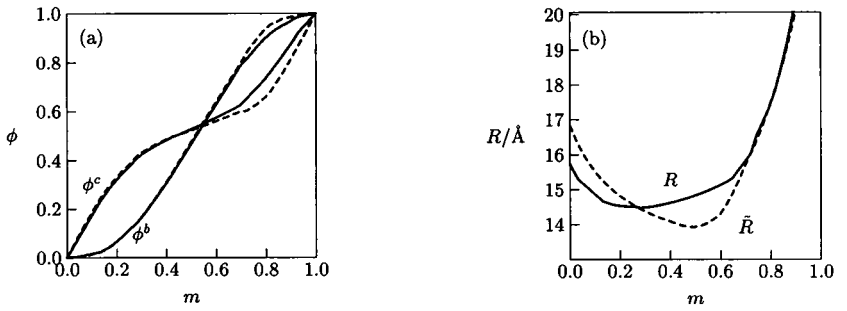

FIGURE 10 (*a*) The equilibrium compositions ϕ^b and ϕ^c in a planar bilayer and honeycomb complex when both species coexist in solution, as a function of the total lipid composition in the system m. The dashed lines are calculated for a fixed complex radius, $R = 14$ Å, using the equal tangent condition Eq. 26, and the lever rule Eq. 25 for $\chi = 0.5$, i.e., the total number of lipids in the two phases are equal. The solid lines correspond to the full numerical solution of Eqs. 25–27, in which case the complex radius R (and hence χ) is allowed to vary so as to minimize the total free energy. (*b*) The optimal complex radius $\tilde{R}$ as derived from the solution of the Eqs. 25–27, as a function of m (*solid line*). For comparison we also show the optimal radius, $\tilde{R}$, of the complex as determined by the minimum of $\tilde{f}^c$ (*dashed line*).

both the DNA-DNA spacing and the inter-bilayer distance. Other factors such as bilayer curvature modulations around the DNA molecules, membrane undulations, and electrostatic forces also play a role in determining the stability of the multilayered complexes. The application of a molecular level theory in the spirit of the present paper to study these complexes is underway.

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