Multilamellar Structures of DNA Complexes with Cationic Liposomes

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ABSTRACT Studies of DNA complexes with cationic liposomes are prompted by the search for nonviral DNA carriers for gene therapy. Recent experiments have identified a stable multilamellar phase in which ordered smectic layers of DNA alternate with cationic bilayers. In this paper we identify the forces governing DNA adsorption on cationic lamellae, including a membrane-induced attraction between the adsorbed DNA. Calculating the DNA interhelical spacing as a function of system composition, the model successfully explains recent surprising observations.

INTRODUCTION

Interest in wide-scale implementation of gene therapy has led to an extensive search for versatile DNA carriers. Complexes formed by DNA with cationic liposomes promise to be a viable alternative to the currently predominant viral vectors. Yet, despite a decade of investigation, little is known about the structure and properties of such complexes, or the relationship between system characteristics and transfection efficiency (see, for example, Behr, 1994; Bloomfield, 1996).

Cationic liposomes contain both a cationic and a nonionic (or zwiterionic) lipid. DNA mixtures with cationic liposomes can therefore be characterized by two compositional parameters: ρ, which defines the ratio of negative (DNA) charge to positive (cationic lipid) charge, and ν, which defines the molar ratio of nonionic to cationic lipid. (We assume, throughout this paper, that the cationic and nonionic lipids remain uniformly mixed. This assumption might fail, because DNA adsorption can reduce the mixing energy of the two lipids (in the same way added salt does). However, at low ν values, the results of Radler, et al. (1997) indicate good mixing.) Low ρ values indicate a low DNA concentration, high ρ values a high DNA concentration, and $\rho = 1$ is the isoelectric point, where the number of DNA anionic charges is equal to that of the cationic lipids.

Recent studies (Hirsch-Lerner and Barenholz, 1997; Lasic et al., 1997; Radler et al., 1997) have identified stable multilamellar aggregates of DNA with cationic liposomes (Fig. 1), which seem to exist over a wide composition range. The DNA in these complexes is ordered in smectic layers sandwiched between the bilayer lamellae and characterized by a regular interhelical spacing (Lasic et al., 1997; Radler et al., 1997). This multilamellar geometry is of special interest, because of its unique features and indications that it is an efficient DNA transfection agent (Lasic et al., 1997).

The relationship between system composition and the concentration of DNA in the complexes is of significant interest, because transfection efficiency can be related to the quantity of DNA entering a cell (Behr, 1994). It is known that flexible polyelectrolytes adsorb uniformly on an oppositely charged surface, and that the layer density increases monotonically with the polymer concentration in solution, until it reaches a limiting saturation value (see, for example, Van der Schee and Lyklema, 1984). Naively, one expects similar behavior for DNA adsorbing on cationic bilayers; the spacing, which is inversely proportional to the DNA concentration, should decrease monotonically when the DNA concentration in solution (ρ) is increased, until it reaches saturation point.

Yet Radler et al. (1997) find a very different behavior for DNA in cationic lamellar complexes. The interhelical spacing of DNA was shown to remain constant with increasing DNA concentration (ρ). Near the isoelectric point, a sharp transition occurred to a second, lower, spacing, which then remains unchanged as the DNA concentration is further increased (see Fig. 2). In the "positive regime," where $\rho < 1$, the lamellar aggregates were found to coexist with excess liposomes, and in the "negative regime," where $\rho > 1$, they were found to coexist with excess DNA. These trends seem to apply to various cationic/nonionic lipid ratios (ν).

In this paper we show that this unexpected relationship between the DNA density in lamellar complexes and the system composition can be explained by using a simple model for the adsorption of DNA molecules on fluid bilayers.

The overall free energy, F_{tot}, of molecules adsorbing from a finite, dilute solution onto a surface is given by three contributions, the entropy of the molecules in solution, the adsorption energy of the molecules, and the intermolecular interaction energy between the adsorbed molecules:

$$F_{tot} = V\phi \ln(\phi) + \frac{A}{\Sigma}(-\Delta + F_i) \qquad (1)$$

where ϕ is the number of molecules per unit volume in solution, and V is the solution volume. Σ is the area per molecule, and A is the overall surface area available for adsorption, so that $A/\Sigma = n_s$, the number of adsorbed molecules. F_i is the interaction energy, and Δ is the adsorp-

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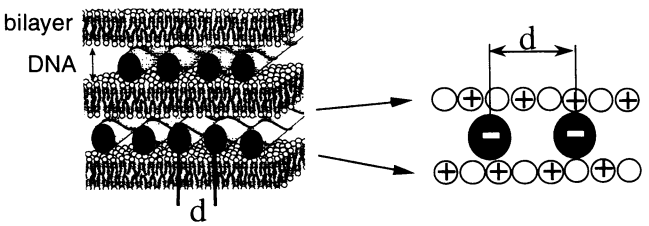

FIGURE 1 A multilamellar stack of DNA complexes with cationic bilayers. The DNA is ordered between the lipid bilayers in a nematic layer characterized by a uniform interhelical spacing, d. In most cases this spacing is larger than the DNA diameter (Strey, 1996; Radler, 1996; Fang, 1996). ν defines the ratio of nonionic to cationic lipids in the bilayer.

tion energy per molecule. All energies are given in units of kT (k is the Boltzmann coefficient, and T is the temperature). The overall number of molecules in the system, n_0, is equal to $V\phi + n_s$ and is taken to be constant. Because of the rigidity of DNA, we can define Σ as Ld, where L is the molecule length and d is the interhelical spacing between DNA molecules (Fig. 1). The number of charges per DNA molecule, z, is therefore proportional to L and is much larger than unity.

Minimizing F_{tot} with respect to d yields the relationship between the concentration of molecules in solution and their density on the surface. Taking into account that both Δ and F_i might vary as a function of the surface density,

$$\frac{\partial F_{tot}}{\partial d} = \frac{A}{Ld^2}\left(\ln(\phi) + 1 + \Delta - d\frac{\partial \Delta}{\partial \mathrm{d}} - F_i + d\frac{\partial F_i}{\partial d}\right) = 0 \tag{2a}$$

Contact between an anionic DNA charge and a cationic lipid charge releases two counterions, namely, $2kT$. The adsorption energy per molecule, Δ, is therefore of order $2z$. For simplicity, we assume that the effect of surface density on this adsorption energy is negligible, so that $\partial\Delta/\partial d \approx 0$. As will be shown presently, the intermolecular interaction

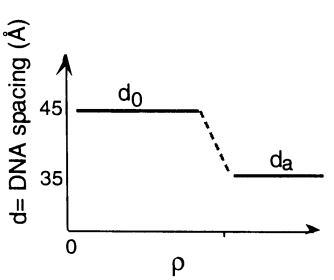

FIGURE 2 A schematic representation of the interhelical spacing, d, of DNA in multilamellar complexes as a function of the ratio between DNA and cationic charge, ρ, at constant bilayer composition ν (Radler et al., 1997). In this case the concentration of cationic lipids is equal to that of nonionic lipids, namely, $\nu = 1$. We see two regimes, above and below the isoelectric point ($\rho \approx 1$), where d is approximately constant. The experimental data show some scatter, with an average error in spacing measurements on the order of 3 Å. No measurements were taken in the transition region where, approximately, $0.8 \leq \rho \leq 1.1$. The theoretical value of ρ_c is 0.76.

energy (and its gradient) is much smaller than this adsorption energy. The number of adsorbed DNA molecules on the surface is therefore given by

$$\frac{n_0 - n_s}{V} \approx e^{-\Delta} \tag{2b}$$

which reduces to $n_s \approx n_0$ in this limit of $\Delta \gg 1$. This relationship defines the overall number of DNA molecules on the surface as a function of the overall number of molecules and adsorption energy. However, because of surface fluidity, the average spacing d is set by the intermolecular interactions, F_i. For example, regardless of n_s, DNA would aggregate on the surface if the intermolecular interactions were purely attractive. Repulsive interactions would lead to a dispersion where $d \approx A/(Ln_s)$.

Equation 2b is valid until the surface is saturated, which will occur at the point where the overall number of cationic adsorption sites, n_a, is equal to zn_s, the number of adsorbing negative charges. Above surface saturation the effective adsorption energy, Δ, becomes zero, and increasing the number of molecules in solution (n_0) can no longer increase the concentration of molecules on the surface. Therefore, the maximum value of n_s is set by n_a. (We implicitly assume that the spacing between adsorption sites is larger than the hard-core dimensions of the adsorbing molecules.) Note that using this notation, $\rho \equiv n_0 z/n_a$. The lateral spacing of the cationic charges, d_a, is equal then to A/Ln_a and is proportional to ν, the bilayer composition.

Now let us consider the interactions between DNA molecules adsorbed on cationic surfaces. These are composed of two contributions. The first is due to the sum of the various electrostatic forces on the surface (cationic-cationic, anionic-anionic, and cationic-anionic). We assume that, at relatively small spacing, the interactions between DNA molecules on a charged surface have a form similar to that of interactions between DNA in high-density liquid crystal phases (Podgornik et al., 1994; Strey et al., 1997). We will show presently that the model's qualitative predictions hold regardless of the exact form of these forces:

$$F_e = \theta e^{-d/\lambda} \tag{3}$$

where θ is a parameter related to the effective charge density per unit length of DNA, λ is an effective screening length, and d is the interhelical spacing.

The second contribution to the interactions between adsorbed DNA molecules is a membrane-induced one. It arises from the DNA-imposed perturbation of the equilibrium bilayer structure and is attractive because DNA aggregation minimizes the perturbed area (Dan, 1996). Although the full form of the energy is complicated, in the limit of small interhelical spacing it is linearly proportional to d (Dan, 1996):

$$F_m = \alpha d \tag{4}$$

where α is a membrane constant of order 10^{-5} $kT/\text{Å}^2$ for typical bilayers (where k is the Boltzmann constant and T is

the temperature). α is defined as $\Delta_0^2 B/\Sigma_0$, where Δ_0 is the degree of bilayer perturbation, B is the compressibility modulus, and Σ_0 is the lipid surface density (Dan, 1996). The overall interaction energy is, therefore, $F_i = (F_m + F_e)$. Minimizing the two contributions, we find the optimal spacing,

$$d_0 = \lambda \ln(\theta/\alpha\lambda) \quad (5)$$

The penalty for perturbation from d_0 is simply given by $\Delta F_i = F_i(d) - F_i(d_0) = \alpha(d - d_0)$. These interactions are extremely weak, but they are still orders of magnitude larger than the DNA entropy per unit length.

Interestingly, this simple model (exponential repulsion, linear attraction) yields a nonmonotonic dependence of the equilibrium spacing, d_0, on the electrostatic screening length λ, as shown in Fig. 3. Although the explicit form of λ is unknown, it is reasonable to assume that this effective screening length decreases when the average number of charges between adsorbed DNA increases. λ should therefore scale with the Debye screening length (Podgornik et al., 1994; Strey et al., 1997), or, in solutions with no added salt (such as in the Radler et al., 1997 experiments), λ should decrease with increasing cationic charge density, i.e., increase with ν.

Recently, Bruinsma and Mashl (1997) calculated the electrostatic interactions between charged rods confined between oppositely charged surfaces, using the Poisson-Boltzmann theory. They find that the force between the rods scales as $1/d$ in the limit where d is much larger than the rod diameter (which translates, in this case, to $d \gg$ DNA diameter). The interaction energy, therefore, has the form $F_e \approx -\beta \ln(d)$, where β is a function of the surface charge density and is purely repulsive. The combination of this form of F_e with the membrane-induced energy F_m (Eq. 4) again yields an optimal DNA spacing given by $d_0 = \alpha/\beta$. We will continue our discussion using the equilibrium spacing as defined by Eq. 5, because the analysis of Bruinsma and Mashl (1997) explicitly applies only to spacings much larger than those observed by Radler et al. (1997).

Let us now return to the experiments of Radler et al. (1997). Because of the large adsorption energy, all DNA molecules in solution are driven to the surface (Eq. 2b). This process stops when the system reaches the isoelectric point,

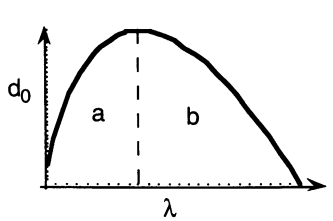

FIGURE 3 The DNA equilibrium spacing, d_0, as a function of the effective electrostatic screening length λ (Eq. 5). A large value of λ means long-range electrostatic repulsion between the DNA and little screening, i.e., low density of cationic charges (or added salt). Therefore we expect λ to increase with ν.

where $\rho = 1$, or $n_a = zn_s$. Increasing n_0, or ρ, above this value will not increase in the number of adsorbed DNA molecules. Thus, d_a defines the lowest spacing of DNA on the surface. This spacing remains constant with increasing ρ (Fig. 2) above the isoelectric point. The excess DNA ($n_0 - n_a/z$) will remain in solution, at equilibrium with the neutral complex.

When ρ is low, most or all DNA molecules adsorb on the surface ($n_s \approx n_0$). However, because n_s is small, there is no constraint on the arrangement of the DNA molecules on the surface. The interhelical spacing is determined in this limit solely by the equilibrium spacing, namely, d_0 (Eq. 5). Areas of bilayer devoid of DNA may be expelled from the lamellar aggregate to form cationic liposomes or micelles. Increasing the relative DNA concentration, ρ, increases n_s, the overall area of the domains, and decreases the amount of expelled lipids, but does not affect the spacing. This process continues until all of the bilayer surfaces are covered by domains of spacing d_0, which occurs at a critical value $\rho_c = d_a/d_0$, that is smaller than unity.

Above ρ_c DNA adsorption continues, because the gain in adsorption energy, Δ, is still much larger than the interaction energy penalty (using the Radler et al. (1997) numbers, $d_0 = 46$ Å and $d_a = 35$ Å, so the maximum value of $\Delta F_i \approx 10^{-4}$ kT/Å, much smaller than the adsorption energy). The interhelical spacing therefore decreases, above ρ_c, until the isoelectric point, or saturation, is reached. Fig. 4 illustrates this process. (Determining how d decreases with ρ in the transition region ($\rho_c \leq \rho \leq 1$) requires a detailed phase analysis, which is outside the scope of this note. We are now in the process of calculating the phase diagram, also taking into account possible lipid phase separation induced by the DNA adsorption (Dan, manuscript in preparation).

Our model therefore predicts that the interhelical spacing is independent of the relative DNA concentration below ρ_c and above the isoelectric point ($\rho = 1$). This is consistent with the observations of Radler et al. (1997). In the experiment, $\rho_c \approx 0.76$; the transition region $\rho_c \leq \rho \leq 1$ is then relatively narrow, which explains why Radler et al. (1997) observe a relatively sharp transition.

The analysis is based on several assumptions:

1. The form of the electrostatic repulsion (Eq. 1 and the resulting Eq. 5). Our qualitative predictions regarding the dependence of DNA spacing on ρ are independent of the exact form of these interactions. The analysis holds as long as the balance between membrane-induced attraction and the electrostatic forces yields an energetic minimum at some finite spacing, and the strength of the interaction is smaller than the adsorption energy.

2. The cationic and nonionic lipids do not demix.

3. The distance between cationic charges on the surface, d_a, is larger than the DNA diameter. Otherwise, the minimum interhelical spacing will be given by the DNA diameter (Fig. 5 *a*), where the isoelectric point could not be obtained.

4. $d_0 > d_a$. Otherwise, $d = d_a$ for all ρ.

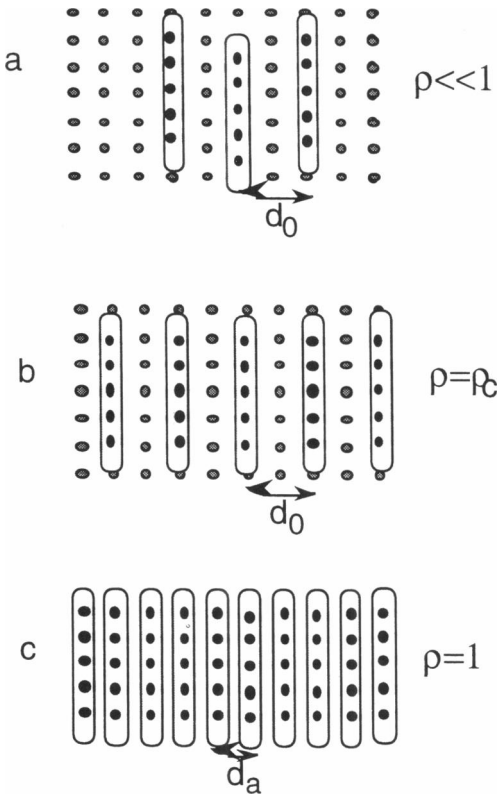

FIGURE 4 DNA adsorption on a cationic bilayer, top view. The gray circles denote the cationic charges, and the black ones the anionic DNA charges. (*a*) At low concentration, the area available for adsorption is large. The interhelical spacing is unconstrained and determined only by the DNA interactions, namely, it is equal to d_0. Excess area might be expelled from the multilamella complex. Increasing ρ increases the total area covered by DNA domains, but does not affect the spacing. (*b*) At the critical DNA concentration, ρ_c, DNA domains cover the overall area of the cationic lamella. Because d_0 is larger than the distance between cationic charges, not all of the cationic charges are neutralized and the overall complex charge is positive. (*c*) Because of the large adsorption energy, adsorption continues with increasing DNA concentration until all of the cationic charges are occupied. The overall complex charge is then neutral (ρ = 1), and the interhelical spacing is given by the distance between cationic charges on the surface, d_b. However, if d_b is smaller than the DNA diameter, adsorption will stop at a lower ρ value when the interhelical spacing is equal to the DNA diameter (close packing), and not all cationic charges are neutralized.

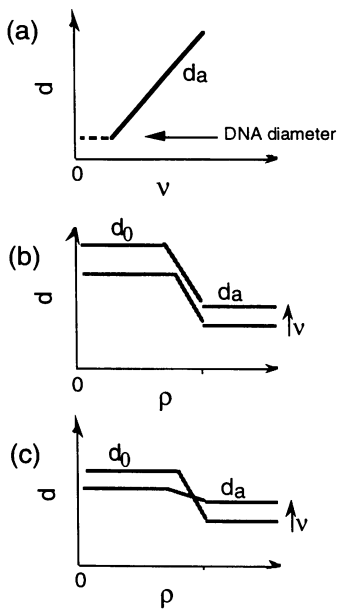

FIGURE 5 The dependence of the DNA interhelical spacing on ρ, the DNA concentration, and ν, the composition of the lipid mixture. (*a*) The interhelical spacing at and above the isoelectric point. d is equal to the spacing of the cationic charges on the surface, d_a, which increases linearly with ν. The dashed line describes systems where d_a is smaller than the DNA diameter; in this case the complex cannot reach the isoelectric point, because even close packing of the DNA cannot neutralize all of the cationic charges. (*b*) The interhelical spacing at high cationic lipid concentrations, namely, small ν. Both d_o and d_a increase with ν (regime *a* of Fig. 3). (*c*) The interhelical spacing at low cationic lipid concentrations, namely, large ν. d_o decreases and d_a increases with ν (regime *b* of Fig. 3).

The model allows us to make some specific predictions (Fig. 5). Examining the spacing of DNA as a function of ρ for different ν values, one should find a family of curves similar to that sketched in Fig. 2. We expect that the interhelical spacing at (and above) the isoelectric point, which is equal to d_a, will increase linearly with ν. Such a trend was indeed observed by Radler et al. (1997). In the limit of small ν, namely, at relatively low concentrations of nonionic lipids, the system is in regime *a* of Fig. 3. The higher spacing, d_0, then should also increase with ν. When the concentration of nonionic lipids in the bilayer is high (i.e., regime *b* of Fig. 3), d_0 will decrease with ν and the difference between the two limits of the interhelical spacing will decrease. The width of the transition region, which depends on $\rho_c = d_a/d_0$, will increase with the difference between the two spacings.

In conclusion, we have presented here a simple adsorption model for DNA complexes with mixed cationic and nonionic lipid bilayers. The interactions between adsorbed DNA molecules are characterized by a weak energetic minimum at a finite spacing. At low DNA concentrations, these interactions determine the interhelical spacing, which remains constant until a critical DNA concentration ρ_c. Above this point DNA continues to adsorb, because of the large adsorption energy. The average interhelical spacing thus decreases until the isoelectric point is reached, beyond which all adsorption sites are occupied and excess DNA is expelled. These predictions are in agreement with experimental observations (Radler et al., 1997).

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