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DNA–DNA electrostatic interactions within cationic lipid/DNA lamellar complexes

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Abstract

We present a simple theoretical analysis of the DNA–DNA electrostatic interactions within charge-neutral lamellar cationic lipid/DNA complexes (lipoplexes). Although always repulsive as a function of the DNA–DNA interaxial distance, the calculated electrostatic force shows a deep minimum for each value of lipid composition corresponding to an equilibrium distance of the system. The excellent agreement between the equilibrium distances predicted by the model and that experimentally observed in charge-neutral complexes as revealed by synchrotron X-ray diffraction, shows that the spatial dimensionality of both the lipids and the DNA may not be a crucial point to capture the essence of the DNA–DNA interactions within charge-neutral lipoplexes.

1. Introduction

The field of non-viral gene therapy has been receiving much attention since the pioneering studies by Felgner et al. [1] which showed that liposomal vectors can serve as gene delivery vehicles in the targeting of extracellular DNA into cell nuclei. Nowadays, cationic liposomes (CLs) based transfection is the most widespread non-viral method to deliver genes in clinical trials. CLs usually consist of a binary mixture of cationic and neutral lipids which spontaneously condense DNA in aqueous solutions [2,3].

A considerable breakthrought towards determining the supramolecular order of the self-assembled lipoplexes was provided by high resolution synchrotron X-ray diffraction (XRD) [4–9]. This technique has unequivocally revealed the existence of two different phases: a multilamellar structure (L_α^C) where a periodic one-dimensional lattice of parallel DNA chains is sandwiched between two-dimensional lipid bilayers (Fig. 1) and a columnar inverted hexagonal (H_{II}^C) liquid-crystalline structure where the DNA molecules are arranged on a two-dimensional hexagonal lattice. New formulations have been tested based on the exclusive use of neutral lipids which are not-cytotoxic. In these complexes, divalent electrolyte counterions common in biological cells (Mn^{2+}, Ca^{2+}, Co^{2+}, Mg^{2+}, Fe^{2+}) are used as DNA condensing agents [10–14]. Nowadays, it is strongly believed that a factual enhancement of transfection efficiencies requires a detailed understanding of the supramolecular structures of lipid/DNA complexes. A full knowledge of the structure/function relationship, could allow the researchers to rationally design the most efficient non-viral vectors [15]. With this purpose in mind, most work, both theoretical and experimental has been aimed to elucidate the principles and mechanisms governing the complex formation, internal structure and phase behavior [16–24]. It is now well recognized that the complex formation and its thermodynamic stability in solution can not be only explained in terms of simple Coloumb attraction but it is necessary to refer to the mechanism of counterion release. The interaction of a positively charged ligand with nucleic acids causes a perturbation

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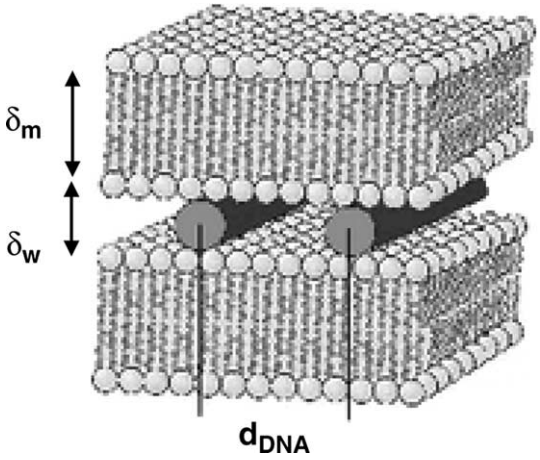

Fig. 1. Schematics of the ordered lamellar (L_α^C) structure of lipoplexes. It is a periodic multilayer structure with DNA adsorbed between cationic membranes. The repeat distance is $d = \delta_m + \delta_w$ whereas the interaxial distance between DNA molecules is d_{DNA}.

of the electrostatic potential of the charged surfaces with the result that some fraction of the Manning condensed counterions are released into the bulk solution with a remarkable entropy increase ($\sim 1\ K_B T$ per released counterion) [25].

From a physical point of view, electrostatic issues arise because of the interactions between the cationic lipids and the DNA. In addition, once condensed complexes are formed, also the interactions between charged DNA molecules are largely dominated by electrostatic effects and the membrane charge density is the constraint that sets the equilibrium spacing between the DNA chains [26].

From a theoretical point of view, the physical principles governing the lipoplex formation have been widely discussed and recently reviewed [27]. The most used approach to describe the complexes is the mean-field Poisson–Boltzmann (PB) equation. Solving numerically a modified PB equation with appropriate boundary conditions, Harries et al. [20] explained the structure and phase evolution of lamellar lipoplexes.

According to the accepted viewpoint that the dominant interaction is electrostatic, we focused on the DNA–DNA electrostatic interactions in the absence of electrolyte counterions. For a given cationic lipid surface charge, the absence of counterions within the lipoplex volume means we are in the presence of a particular cationic lipid/DNA molar ratio for which there is charge neutrality.

In this Letter, we present a simple electrostatic model aimed to shed more light on the role played by the spatial dimensionality on the DNA condensation between opposing lipid bilayers in the limit of zero salt concentration. The main goal of the forthcoming analysis is to derive an analytical expression of the interaction force between neighboring DNA chains by simplifying the molecular architecture of the unit cell of the complex. This analysis could indirectly allow us to gain insight into the real relevance of the molecular structure of both the DNA and the lipids in modulating the DNA–DNA electrostatic interactions within lamellar lipoplexes.

2. Theory

In this section, we give a description of the model used for calculating the electrostatic force between DNA molecules. As the system modeled in the present study is very complex, the theoretical analysis requires a few simplifying approximations. The L_α^C phase is an ordered smectic-like array with a periodic structure in the plane (x, y) perpendicular to the DNA axis that we label as the z-axis. Because the complex is translationally invariant along the z-axis, a unit cell is specified by the distance between apposed lipid surfaces δ_w, the thickness of lipid bilayers δ_m, and the DNA–DNA repeat distance d_{DNA} (Fig. 1). The complex is usually regarded as a periodic one-dimensional lattice of DNA molecules, modeled as infinite strands sandwiched between alternating lipid bilayers. Neglecting the effects associated with the flexibility and the curvature fluctuations of DNA is generally justified taking into account that the DNA persistence length ($\xi_p \sim 500$ Å) is much larger than the above mentioned length scales in the complex. Since numerical studies revealed that charge distribution at the DNA surface is approximately continuous, DNA molecules are frequently modeled as uniformly charged lines thereby ignoring the discrete distribution of the phosphate groups.

In our model, the DNA chains are modeled as one-dimensional parallel charged lines with uniform linear charge density $\lambda_- = e/l$, where e is the elementary charge and $l = 1.7$ Å is the distance between two phosphate groups projected on the DNA axis. In modeling the DNA strands as lines of charge we ignore the effects associated with the fluctuations, the DNA molecular structure and the charge distribution over the DNA surface.

In binary lipid mixtures the individual membrane components, although ideally mixed, are free to move within the plane of the membrane. Upon complexation, the Coloumb attraction between cationic lipids and DNA charged molecules induces the polarization of the positive charge carried by the lipid headgroups along the DNA helix axis. Cationic lipids are pushed out from between the DNA positions, migrate in the plane of the membrane to match the negative charge carried by the DNA and a charge segregation occurs (Fig. 2a and b). As a result of such 'lipid demixing', cationic lipids replace the DNA counterions and act like two-dimensionally condensed 'counter-lipids' [20].

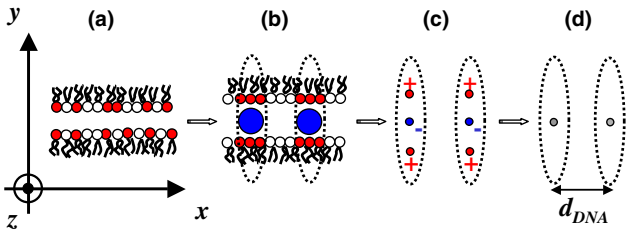

Fig. 2. Schematic illustration of the proposed model. Mixing DNA and cationic liposomes (a) results in the formation of locally ordered one-dimensional arrays of DNA chains (blue rods with helix axis parallel to the z-axis) intercalated between charged membrane bilayers (b). The electrostatic attraction between cationic lipids and DNA charged molecules induces polarization of the positive charge carried by the lipid headgroups along the DNA helix axis. These distributions of charge are schematized as parallel lines (c) and, finally, as a set of parallel lines of charge in the xz-plane (d). The distance between DNA ds chains is indicated as d_{DNA}.

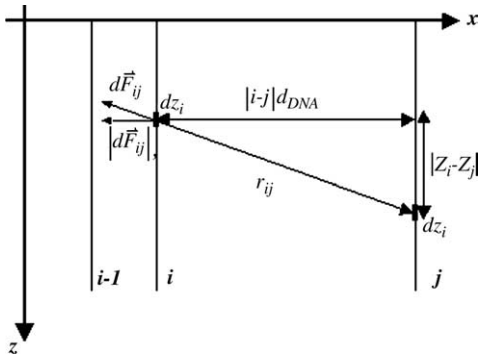

Fig. 3. Model for the calculation of the DNA–DNA electrostatic interaction. Each line represents the DNA distribution of charge screened by the polarized cationic lipids. The force on the infinitesimal charge dq_i due to the charge dq_j, positioned on two lines labeled as i and j respectively, is indicated as $\mathrm{d}\vec{F}_{ij}$.

In our simplified approach, the 'counter-lipids' merely modify the charge distribution along the length of the DNA chains. We assume that the condensation of demixed counterlipids results in a supplementary distribution of positive charge. We introduce the additional linear charge density $\lambda_+ = (Z\Phi d_{\mathrm{DNA}}/a)e$, where Z is the valence of the positive charge carried by the cationic lipid, Φ is the cationic/neutral lipid molar ratio and a is the lipid headgroup area ($a \sim 70$ Å^2) (Fig. 2c). In view of these approximations, the unit cell of the complex turns into a couple of lines of charge separated by a distance d_{DNA} (Fig. 2d), each line consisting of a charge distribution with a linear density $\lambda_{\mathrm{c}} = 2\lambda_+ + \lambda_-$ where the subscript 'c' means complexed. Under these assumptions, the interaxial distance between adjacent DNA chains corresponds to the average distance between adjacent charged lines d_{DNA}. The driving force for the formation of charge-neutral lipoplexes is the release of lipid and DNA counterions in the bulk solution. The counterion release mechanism implies one-to-one binding of opposite charges. Consequently, there exists a counterions vacuum inside isoelectric complexes [9]. Here, the absence of counterions within the complex means we refer to an isoelectric complex as above explained.

The complete dimensionless of the system in the xy-plane is the main result of the discussed approximations while the interaction between condensed DNA strands is reduced to the interaction between a set of parallel lines of charge along the z-axis (Fig. 3).

The resulting scenario is the starting point for the calculation of the interaction force between DNA molecules and appears to be very similar to that recently investigated by Arenzon et al. [28] who constructed a mean-field theory, based on Gibbs–Bogoliubov inequality, in order to study the interactions between two like-charged polyions. In the cited work, the effect of condensed counterions, was approximated by the renormalization of local charge and the horizontal component of the interaction force was calculated. Later, the same model has been successfully applied by Pastrè et al. [29] to describe the adsorption of the DNA to mica surfaces. Even if it is a rough approximation, it is particularly suitable to obtain quantitative information about the role of the spatial dimensionality on the DNA–DNA interaction within lamellar lipoplexes.

Consider the ith and jth lines of charge and two elements located at the point z_i and z_j, respectively, with infinitesimal thickness dz_i and dz_j (Fig. 3). The charge distributed on that elements are $\mathrm{d}q_j = \lambda_{\mathrm{c}} * \mathrm{d}z_{\mathrm{j}}$ and $\mathrm{d}q_i = \lambda_{\mathrm{c}} * \mathrm{d}z_{\mathrm{i}}$ so that the infinitesimal force exerted by the infinitesimal charge dq_j on the infinitesimal charged dq_i is given by

$$\mathrm{d}\vec{F}_{ij} = \frac{1}{4\pi\varepsilon}\frac{\lambda_{\mathrm{c}}\,\mathrm{d}z_i\lambda_{\mathrm{c}}\,\mathrm{d}z_j}{r_{ij}^2}\hat{r}_{ij}, \tag{1}$$

where r_{ij} is the distance between the elements, $\hat{r}_{ij}$ is the unit vector and ε is the dielectric constant of water ($\varepsilon \sim 80$). This equation can be broken into component form. To do so, we used the set of orthogonal axes reported in Fig. 2. Looking at the modulus of the component of the infinitesimal force along the x-axis, it is given by

$$\left|\mathrm{d}\vec{F}_{ij}\right|_x = \frac{1}{4\pi\varepsilon}\frac{\lambda_{\mathrm{c}}^2\,\mathrm{d}z_i\,\mathrm{d}z_j}{r_{ij}^3}\mid i-j\mid d_{\mathrm{DNA}}, \tag{2}$$

Moving along the jth line of charge, we sum all the elementary forces due to the infinitesimal charges distributed on the jth line and acting on the element dz_i. The force at the point z_i can be obtained by applying the superposition principle. Summing all these contributes, along the ith line of charge, the x-component of the net force exerted by the jth charged line on the ith one becomes

$$\left|\vec{F}_i\right|_x = \frac{\lambda_c^2}{4\pi\varepsilon}\int_0^{\xi_p} dz_i \int_0^{\xi_p} \frac{dz_j}{[(z_j - z_i)^2 + (\mid i-j \mid d_{DNA})^2]^{3/2}}. \quad (3)$$

Note that, we set the limits on the integrals according to where the DNA fluctuations can be neglected. In principle, the integration could be over an arbitrary length L along the line of charge ($\xi_p \geqslant L \geqslant 0$). Changing the upper limit of the integration does not absolutely modify the conclusions of the present analysis. The double integral that expresses the x-component of the force is simple to perform. Thus, the electrostatic force exerted by the jth line on the ith becomes

$$\left|\vec{F}_i\right|_x = \frac{Z^2}{2\pi\varepsilon}\frac{|e^-|^2}{l^2}\left(\frac{2ld_{DNA}\Phi}{a} - 1\right)^2 \left(\frac{\left((\mid i-j \mid d_{DNA})^2 + \xi_p^2\right)^{1/2}}{\mid i-j \mid d_{DNA}} - 1\right). \quad (4)$$

Finally, let us consider the interaction between adjacent lines of charge, i.e. neighboring DNA molecules, by taking $|i-j| = 1$.

$$\left|\vec{F}\right|_x = f(\Phi, d_{DNA}) = \frac{Z^2}{2\pi\varepsilon}\frac{e^2}{l^2}\left(\frac{2ld_{DNA}\Phi}{a} - 1\right)^2 \left(\frac{\left((d_{DNA})^2 + \xi_p^2\right)^{1/2}}{d_{DNA}} - 1\right). \quad (5)$$

As is evident, the x-component of the calculated electrostatic force only depends on the cationic/neutral lipid composition Φ and on the DNA–DNA spacings d_{DNA}. While Φ regulates the surface charge density, the latter parameter controls the DNA packing density within the complex [9]. In what it follows, we shall refer to monovalent cationic lipid ($Z = 1$) whereas the importance of this parameter will be discussed elsewhere.

3. Results and discussion

Fig. 4a shows the calculated electrostatic force as a function of Φ for several values of d_{DNA}. All curves exhibit a minimum which shifts to lower Φ values as d_{DNA} increases. These profiles closely resemble the electrostatic free energy curves of the unit cell of the L_α^C complexes reported by Harries et al. [20].

Similarly, Fig. 4b shows the d_{DNA}-dependence of the calculated electrostatic force for several values of Φ. On a logarithmic scale, deep minima related to equilibrium distances are clearly visible. Furthermore, the interaxial distances of stability move apart as a function of increasing membrane charge density. Moving around each minimum of ±1 Å we observe that the intensity

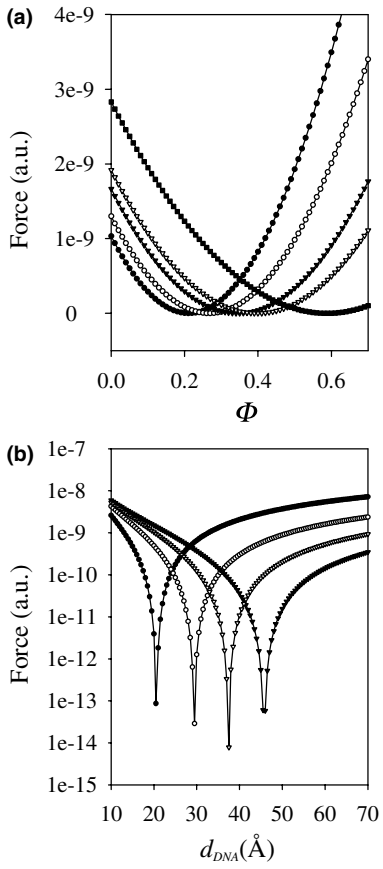

Fig. 4. DNA–DNA electrostatic force as a function of the cationic lipid mole fraction (a), for several values of the DNA–DNA interaxial distance: (black circles), d_{DNA} = 97.4 Å; (white circles), d_{DNA} = 77.0; (black triangles), d_{DNA} = 59.7; (white triangles), d_{DNA} = 51.8; (black squares), d_{DNA} = 35.0 Å; DNA–DNA electrostatic force, as a function of the interaxial d_{DNA} distance (b), for several values of the cationic lipid molar fraction: (black circles), Φ = 1 Å ; (white circles), Φ = 0.7; (white triangles), Φ = 0.55; (black triangles), Φ = 0.45.

of the repulsive force increases of about two orders of magnitude. This result enforces the idea that such minima in the calculated force profiles are points of high stability of the complex.

As is evident from Fig. 4b, the calculated DNA–DNA electrostatic interaction is a repulsive force as a function of d_{DNA}, in agreement with previous works [6,18,19]. Nevertheless, the magnitude of this force is not simply falling off with a power of the distance d_{DNA} as elsewhere reported [6]. Conversely, it becomes very weak and reaches a deep minimum at a single value of d_{DNA} we indicate as $(d_{DNA})_{eq}$ which only depends on the lipid composition, i.e. on the surface charge density. In the lipoplexes, the surface charge density has been already identified as the only constraint that sets not only the DNA packing density [26] but also significantly the transfection efficiency in lamellar lipoplexes [30]. Interestingly, the derived 'equilibrium spacings' are equal or very close to the interhelical distances, $(d_{DNA})_{iso}$, experimentally observed for isoelectric complexes.

In order to further validate this scenario, we report the values of $(d_{DNA})_{eq}$ superimposed to the experimental values obtained for different isoelectric complexes [5,8,9,24]. In these works, the cationic liposomes consisted of the cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and the neutral lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). One data set refers to a triple lipid system containing DOTAP, cholesterol (Chol) and DOPE [24]. The comparison is shown in Fig. 5.

For all investigated Φ values, the equilibrium distances predicted by the model (solid line) significantly match the distances revealed by XRD experiments (points) in the isoelectric complexes. Surprisingly, this suggests the existence of a master curve matching the experimental observations. Thus, the inner structure of the complex is well predicted by the proposed model, in particular when the extrapolated equilibrium distance $(d_{DNA})_{eq}$ is larger than diameter of a hydrated DNA molecule (~25 Å).

Our results show that the electrostatic screening acted by polarized cationic lipids is maximal at the isoelectric point resulting in a minimum of repulsion between neighbouring DNA chains and confirm that the charge neutrality is the most stable condition of the complexes. The fact that the isoelectric point is a point of stability is not surprising by itself, because it is predicted by the counterion release mechanism and represents one of the main conclusions of the self-consistent theoretical apparatus developed by the Ben-Shaul's group [20,21,27]. Indeed, the isoelectric stability of the complexes was attributed to the complete matching between the charges of cationic lipid and DNA and to the fact that the counterion release was maximal. The main result of the proposed model is the attainment of results in excellent agreement with experimental observations by using simple theoretical tools and making large approximations aimed to simplify the detailed molecular architecture of the unit cell of the complex. In principle, one-dimensional lines of charge may be a dramatic oversimplifications of the physical reality and the proposed modelization could appear too simple. More realistic models can not neglect the intrinsic charge pattern of DNA molecule. This seemingly contradicts the complexity of the inner structure of lamellar lipoplexes. Conversely, we believe that the physical meaning of our results is that the ignored molecular details do not remarkably affect the electrostatic DNA–DNA interactions inside the complex.

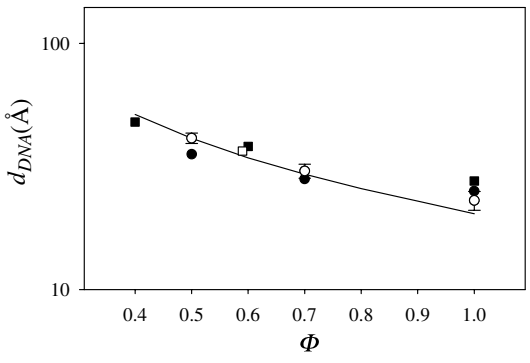

Fig. 5. Variation of DNA packing with Φ in isoelectric complexes and no salt added. Solid line is the prediction of the equilibrium distances corresponding to the minima of Fig. 4(b). The points are experimental interaxial distances in isoelectric complexes: DOTAP–DOPC complex (black squares) [6]; DOTAP–DOPC complex (black circles) [9]; DOTAP–DOPE complex (white squares) [8]; DOTAP–DC–Chol–DOPE complex (white circles) [24].

4. Conclusions

By treating the DNA and the polarized cationic lipids as lines of charge, we have provided a simple and analytical model describing the electrostatic interactions between DNA molecules inside lamellar lipoplexes. The DNA–DNA interaction force, depending on both the membrane charge density and the interaxial distance d_{DNA}, is always repulsive. Our simplified analytical model has confirmed that, for each value of membrane charge density, the interaxial distances $(d_{DNA})_{iso}$ are effectively related to the most stable DNA packing inside the complex. We have also shown that the disregarded microscopic details such as molecular size and shape of DNA and lipid molecules do not drastically affect large length-scales properties. The spatial dimensionality of both the lipids and the DNA, in the limit of zero salt concentration, may not be a crucial point to capture the essence of the DNA–DNA interaction within charge-neutral complexes. Once the CL–DNA complexes are formed, the DNA packing density could in principle be regulated by several contributions such as electrostatic forces, hydration force, attractive van der Waals forces. We emphasize that, since we focused on the electrostatic DNA–DNA interactions failing to consider any other contribution, the only electrostatic interactions can account for the DNA ordering in the isoelectric complexes. Other contributions should be introduced in the physical picture, to account for the discussed slight discrepancies.

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