Correlation Induce Association in Polyelectrolytes Solutions

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Abstract

We present a simple model for the formation of a DNA-surfactant complex and we show that correlational effects relevant for multivalent ions enhance the association. Our results suggest that divalent amphiphiles might be a good vehicle for gene therapy.

Key words:
PACS: core-softened potential, diffusion

1 Introduction

Gene therapy represents a promising way for the treatment of both genetic and acquired diseases. The basic idea is to replace the sick gene with a healthy one or in some cases to add a new gene to get a resulting synthesis of a therapeutic protein. The process of getting the new gene to its target involves the crossing of several barriers, such as the cell membrane and the nuclear membrane. Since both the DNA and the cell membranes are negatively charged, the naked polynucleotides are electrostatically prevented from entering into the cell. Viral vectors such as retroviruses and adenoviruses are very efficient

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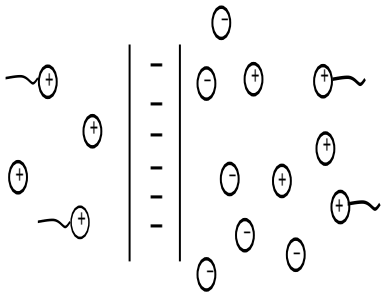

Fig. 1. Our Model. Here we represent the case in which monovalent surfactant and salt ions are present

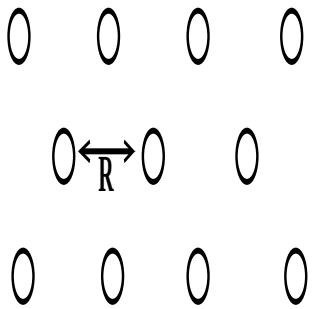

Fig. 2. Wigner Crystal representation. Correlation between associated ions will lead to the formation of a two dimensional crystal

and able to target a wide range of cells [1][2]. The gene transfection is accomplished by the use of a virus in which the native DNA has been replaced by the required DNA. Since the main purpose of the virus is to replicate itself, the new gene is successfully transfected to the cell nucleus by endocytosis or membrane fusion. This process suffers from the drawback that in cases where repeated treatment is needed, this might cause the immune system to react negatively due to the viral origin of the vector.

As a solution for this problem nonviral vectors have been developed. One of the approaches, pioneered by Felgner and Ringold [3][4] relies on the association between anionic nucleic acid and cationic lipid lipossomes. The process of association neutralizes the excess negative charge of the DNA and the DNA-lipossome penetrate into the cell by endocytosis. The efficiency of this method is low and they are toxic to the cell at the concentrated used.

Among the parameters needed for achieving efficient transfection is the requirement that the complex should have small size similar to a virus [5]. This factor indeed limits the efficiency of the DNA-lipossomes complexes. In contrast, cationic surfactant have been shown to condense to DNA into discrete particles containing a single nucleic acid molecule [6][7] that can decrease the charge of the DNA as we have showed recently [8]-[11]. Our previous calculations did not account for the correlation between the associated ions that are relevant for multivalent ions. In this work we will take into account correlations and show that for divalent ions the association is enhanced by correlational effects.

2 The Model

Our model, illustrated in Fig. 1, consists of a solution of *DNA* strands of length L and diameter a_p, surfactant and salt with the same valence. In this work we will consider the case of monovalent and divalent ions. In aqueous solution, the polyions become ionized resulting in a negative charge $-Zq$ distributed uniformly distributed with separation $b = L/Z$. The solvent, water, is modeled as a continuous medium of dielectric constant D. The ions of the salt are completely dissociated, forming an equal number of positive and negative ions. Similarly, the surfactants are assumed to be fully dissociated producing negative monovalent coions and polymeric chains with a divalent cationic head group. For simplicity, all the counterions and coions are treated as identical, independent of the molecules from which they were derived. The electrolytes are depicted as hard spheres of diameter a_c and charge $\pm vq$ and the surfactant is modeled as a polymer of s monomers each one considered as a rigid sphere of diameter a_c with the head monomer carrying a charge of $+vq$, where v is the valence.

The interaction between the hydrophobic tails is short ranged and characterized by the parameter χ. The density of DNA strands is $\rho_p = N_p/V$, the density of salt is $\rho_s = N_s/V$ and the density of divalent amphiphiles is $\rho_a = N_a/V$.

The strong electrostatic interactions between the polyions, the counterions, salt cations and surfactant heads leads to the formation of complexes, which in thermodynamic equilibrium will be made up of one polyion, n_c counterions and n_a surfactants. We do not consider the effects of of polydispersity in the size of the complexes, since it does not affect the final result. Due to the association and to the charge conservation, there are only two free quantities and so, $\rho_c = (Z/v - n_c)\rho_p + \rho_s \quad \rho_{a+} = \rho_a - n_a\rho_p \quad \rho_- = \rho_s + \rho_a \quad \rho_f = \rho_c + \rho_a + \rho_-$ where ρ_f is the total density of free ions made of ρ_c, the density of free counterions, ρ_{a+}, the density of free amphiphiles and ρ_-, the density of negative ion.

The objective of this theory is to determine the number of counterions n_c and surfactants n_a associated to each *DNA* strand. For this, we construct the Helmholtz free energy of the system and minimize it. The details about the model can be found elsewhere [8]-[11]. We give here the main steps. The relevant contributions for the Helmoltz free energy F are two, the electrostatic and the entropic namely, $f = F/V = f_{el} + f_{ent}$. f_{el} is the electrostatic interaction between the complex, free ions and free surfactants. In the framework of the Debye-Hückel-Bjerrum (DHBj) it is given by [8]-[11]:

$$\beta f^{el} = -\frac{\rho_p Z_c^2 (a/L)}{T^*(\kappa a)^2} \left\{ 2\ln\left[\kappa a K_1(\kappa a)\right] - I_0 + \frac{(\kappa a)^2}{2} \right\} \tag{1}$$

where $\beta = 1/k_BT$ and

$$I_0 \equiv \int_0^{\kappa a} \frac{xK_0^2(x)}{K_1^2(x)} dx \tag{2}$$

where $K_n(x)$ are the are the n-order modified Bessel functions, κ in $(\kappa a)^2 = 4\pi v^2 \rho_f^*/T^*$ is the inverse of the Debye screening length, $\rho_f^* = \rho_f a^3 = \rho_c a^3 + \rho_{a+} a^3 + \rho_- a^3$ is the reduced density and $T^* = Dk_BTa/q^2$ is the reduced temperature. Furthermore, $a = (a_c + a_p)/2$ is the effective radius and $Z_c = Z - vn_a - vn_c$ is the effective charge of the exclusion cylinder around each complex.

The calculation of the entropic contribution can be obtained with the aid of Flory [12] theory of mixing, namely

$$\begin{aligned}
\beta f^{ent} = & \rho_p \ln \left(\frac{\phi_p (1 + m_c + m_a)}{\zeta_{cl}(1 + m_c + m_a s_a)} \right) - \rho_p \\
& + \rho_c \ln \phi_c - \rho_c + \rho_{a_+} \ln \frac{\phi_{a_+}}{s_a} - \rho_{a_+} \\
& + \rho_- \ln \phi_- - \rho_- \ .
\end{aligned} \tag{3}$$

In the case of the particles without structure, the internal partition function $\zeta_- = \zeta_c = \zeta_{a+} = 1$. The volume fraction ϕ_s of the different species are:

$$\begin{aligned}
\phi_p &= \frac{\pi \rho_p^*}{4(a/L)} \left(\frac{a_p}{a} \right)^2 + \frac{Z\pi\rho_p^*}{6} (s_a m_a + m_c) \left(\frac{a_c}{a} \right)^3 \\
\phi_c &= \rho_c^* \frac{\pi}{6} \left(\frac{a_c}{a} \right)^3 \\
\phi_{a+} &= \frac{s_a \pi \rho_{a+}^*}{6} \left(\frac{a_c}{a} \right)^3 \\
\phi_- &= \frac{\pi \rho_-^*}{6} \left(\frac{a_c}{a} \right)^3 \ .
\end{aligned} \tag{4}$$

Here we introduce the fractions counterions, $m_c = n_c/Z$, and surfactant, $m_a = n_a/Z$, associated to each DNA strand. The internal partition function of the complex, ζ_{cl}, can be calculated by modeling the DNA by an one dimensional lattice with Z sites. If the number of associated ions to each site can be only zero or one, this problem becomes equivalent to finding the free energy of an one dimensional array with the three different states. The resulting partition function is given by

$$-\rho_p ln\zeta_c\left[m_c, m_a\right] = \rho_p[\xi K\left[\frac{Z_c}{Z^2}^2 - 1\right] + \beta\chi\left(Z-1\right)m_a^2 + Z m_c \ln m_c + Z m_a \ln m_a + Z\left(1-m_c-m_a\right)\ln\left(1-m_c-m_a\right)] + \beta f_{WC} \quad (5)$$

where $\xi \equiv \beta q^2/Da$ is the Manning parameter, $K = Z[\psi(Z) - \psi(1)] - Z + 1$, $\psi(n)$ is the digamma function and f_{WC} is the correlational free energy.

How can we obtain this correlational free energy? To begin with, let us assume that the n_a+n_c associated ions are in a planar surface and not in the cylindrical geometry. The dependence on the shape of the polyions for this contribution of the free energy is not dramatic, particularly if the polyion radius is bigger than the distance between the charged groups along the polymer. In this case, the two dimensional concentration of divalent ions is $(n_c+n_a)/(2\pi a_p L)$ and the surface area per ion can be characterized by R such that $\pi R^2 = 2\pi a_p L/(n_t)$ as illustrated in Fig. 2. Thus $R = \sqrt{2a_p L/(n_c+n_a)}$ and $\Gamma = 4q^2/(k_B T D R)$, where Γ is the plasma parameter, a Coulomb coupling constant that scales quadratically with the valence For $\Gamma >> 1$ the screening atmosphere is narrowly confined as a two-dimensional liquid of classical charged particles in an uniform neutralizing background located at the surface of the polyion. This liquid, also called one component plasma, at zero temperature acquires the minimal energy state of a Wigner Crystal showed in Fig. (4). The correlational energy associated to this plasma made of $n_c + n_a$ ions of valence v is given by $e = -3.92q^2/(DR)$ per ion.

Applying the charging process to this energy one gets the free energy associated with the correlational energy of surfactant and salt ions. The total correlational free energy associated with the polyelectrolyte solution is given by:

$$\beta f_{WC}(n_a + n_c) = -0.98\rho_p(\frac{(n_c+n_a)L/a_p}{2\pi})^{1/2}(n_a+n_c)\ . \quad (6)$$

The equilibrium configuration of the system is found by the minimization of the Helmoltz free energy, leading to two equations, namely

$$\frac{\partial F}{\partial m_c}\delta m_c = 0$$
$$\frac{\partial F}{\partial m_a}\delta m_a = 0\ . \quad (7)$$

Solving this system of two equations, it is possible to obtain the values of m_c and m_a.

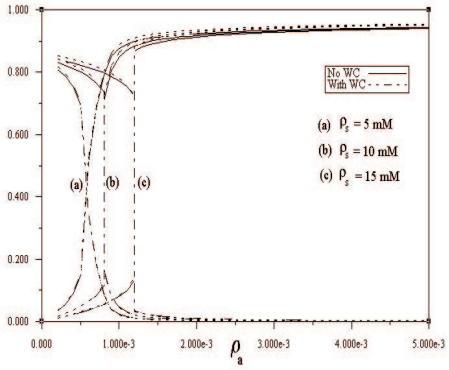

Fig. 3. Surfactant (left) and counterions (right) binding isotherms for salt concentrations (a) 5mM, (b)10mM and (c)15mM. The upper curve is the analysis with the correlational term and the lower curve without it. Both the electrolytes and the surfactant are monovalent

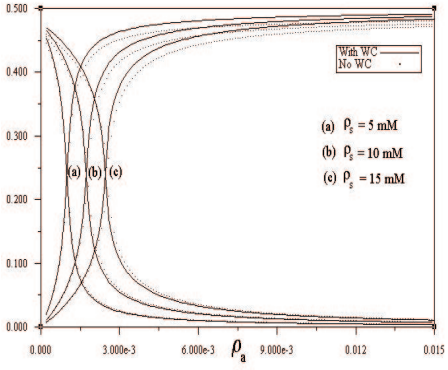

Fig. 4. Same as Fig.3 but for divalent surfactant and salt ions.

3 Results and Conclusions

We define a "surfoplex" to be a complex in which almost all of the DNA's phosphate groups are neutralized by the associated surfactant molecules. As mentioned earlier, we are interested in the minimum amount of cationic surfactant needed to transform naked DNA into surfoplexes. The effect of addition of high concentrations of salt to the system was analyzed by varying the amount of salt added to the system ρ_s.

Fig.3 and Fig.4 illustrate the surfactant and the counterion binding isotherm, m_a and m_c, as a function of total amphiphilic concentration for hydrophobicity parameter $\beta\chi = -4$ and salt concentrations $\rho_s = 5mM, 10mM, 15mM$ for monovalent and divalent electrolytes respectively. Comparing the case where the Wigner Crystal term f_{WC} were not taken into account with the case in which it was considered, one notice that the association of counterions and surfactant are enhanced by the correlational effects, particularly in the case of divalent ions. Consequently, the effective charge,$(Z - vn_a - vn_c)/Z$, illustrated in Fig. 5 and Fig 6, is smaller in the case in which the Wigner Crystal term is present. Due to the simplicity of our model that does not allow for the association of more than one surfactant molecule to each charged group along the DNA, there is no charge inversion.

In resume, we have presented a simple theory of DNA, for salt and surfactant solutions. Our results should be of direct interest to researchers working on the design of improved gene delivery systems. In particular, we find that addition of cationic divalent surfactants and divalent salt ions leads to a cooperative binding that due to correlational effects is much stronger than for

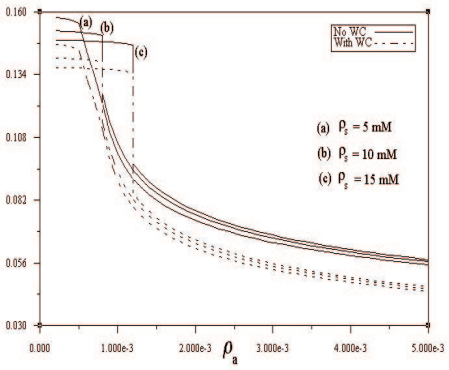

Fig. 5. Effective charge for the same parameters as in fig. 3

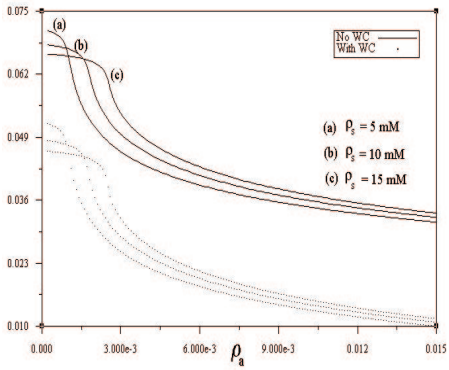

Fig. 6. Effective charge for the same parameters as in fig. 4

monovalent ions. This binding happens far below the critical micell concentration,suggesting that divalent surfactant would be appropriated for transfection.

Acknowledgments

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