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Effects of hydrophobicity in DNA surfactant complexation

Paulo S. Kuhn, M.C. Barbosa*, Yan Levin

Instituto de Física, Universidade Federal do Rio Grande do Sul Caixa Postal 15051, CEP 91501-970, Porto Alegre, RS, Brazil

Abstract

We study a simple model of DNA cationic surfactant complexation. It is found that a combination of electrostatic and hydrophobic effects leads to a cooperative binding transition in which a large fraction of the DNA's charge is neutralized by the condensed cationic surfactants. A further increase of surfactant density can result in charge inversion of the DNA–surfactant complexes. This regime should be of particular interest for application to gene therapy. In this paper we shall explore the dependence of the cooperative binding on hydrophobic interactions between the DNA and amphiphilic molecules.

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Electrostatic interactions play an essential role in many biological processes. The reason for this is that quite a few important macromolecules such as DNA, proteins, and phospholipids, in aqueous solutions are ionized. The problem of how to deal with strong Coulomb forces present in polyelectrolyte solutions has become particularly acute in view of recent attempts to construct effective gene delivery systems. The researchers working in this field are faced with a difficult problem of delivering a specific polynucleotide sequence inside cells of a living organism. This is particularly non-trivial since both the DNA and the phospholipid cell membranes are negatively charged [1–3]. A possible solution was suggested by Felgner and Ringold [2] and involves an attempt to reverse the DNA's charge by forming lipoplexes, complexes composed of the DNA and cationic liposomes. Liposomes are spherical bilayer vesicles made of cationic lipid molecules. By forming a lipoplex one can reverse the DNA's

* Corresponding author. Fax: +55-51-319-1762.
E-mail address: barbosa@if.ufrgs.br (M.C. Barbosa)

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negative charge, allowing a complex to approach the cell membrane. The mechanism of DNA's entry inside a cell is still not well understood and a certain specificity has been found, suggesting that some lipids are much better vehicles for gene delivery than others [4]. In this paper we shall not discuss the specifics of gene delivery but instead concentrate on the question of what is the minimum concentration of cationic surfactant or lipid necessary to reverse the DNA charge. This problem is of crucial importance since almost all cationic lipids and surfactants are toxic to an organism. One would, therefore, like to know the minimum dose of surfactant necessary to accomplish the charge inversion [5–7].

Since we will be interested in low surfactant concentrations, our attention will be restricted to densities below the critical micelle concentration (CMC). Recently, we have shown that when the concentration of amphiphilic molecules is small, the counterions preferentially adsorb to the DNA, forming DNA–counterion complexes. However, when a critical amount of surfactant is added to solution, a large number of surfactant molecules simultaneously condense onto the DNA strands, while the bound counterions are released back into solution. This cooperative phenomena is the result of hydrophobic interaction between the hydrocarbon tails of surfactant molecules [5–7]. Our theory was able to semi-quantitatively account for the cooperative binding observed in recent experiments on dodecyltrimethylamonium bromide and DNA [8,9]. The theory also predicted that for sufficiently hydrophobic surfactants or lipids, charge inversion could be achieved with very low concentration of amphiphile [7]. This calculations, however, did not take into account an additional gain in energy due to hydrophobic interactions between the amphiphile tails and the core of the DNA. In this paper, we shall attempt to account for this effect.

Our system consists of DNA segments of density ρ_{DNA}, surfactants of density ρ_{surf}, and monovalent salt of density ρ_{salt}. The solvent is idealized as a uniform medium of dielectric constant D. In solution, the Z phosphate groups of DNA become ionized. We shall model the DNA strands as rigid cylinders of length L and diameter a_p, with the negative phosphate groups spaced uniformly with separation $b \equiv L/Z$ along the major axis (see Fig. 1). $Z\rho_{\mathrm{DNA}}$ counterions are released into solution, preserving the overall charge neutrality. Similarly, the cationic surfactant molecule in aqueous solution becomes ionized, producing a free negative ion and a flexible chain consisting of one positively charged hydrophilic head group and a neutral hydrocarbon tail of $s-1$ monomers. The interaction between the tails is short ranged and characterized by the hydrophobicity parameter χ.

The ions of salt, the counterions, and the negative ions dissociated from the surfactant are modeled as hard spheres with point charge located at the center. We shall call the negative ions, "coions", and the positive ions, "counterions" – independent of the species from which they were derived.

The strong electrostatic attraction between the polyions and cations results in formation of complexes composed of one DNA molecule, n_{count} associated counterions, and n_{surf} associated surfactants (see Fig. 1). We shall assume that to each phosphate group can associate *at most* one counterion *or* $l = 1, \ldots, l_{\max}$ surfactants. The association of

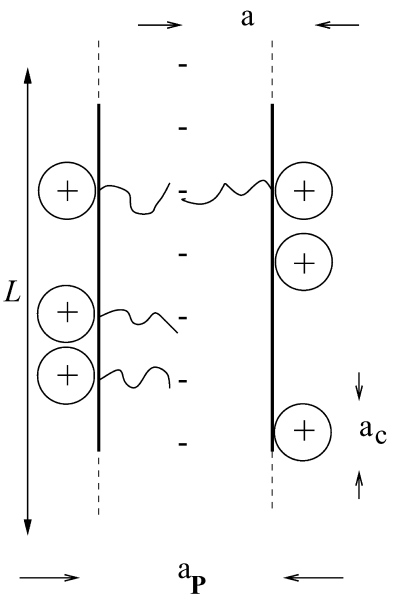

Fig. 1. A cylindrical polyion of diameter a_p and length L. Each monomer of a macroion is free or has *at most* one counterion, *or* l amphiphiles associated with it.

more than one counterion is prevented by the electrostatic repulsion. In the case of surfactant molecules, the electrostatic repulsion between the head groups can be compensated by a decrease in free energy due to favorable interaction between the hydrophobic tails. The amphiphiles can further lower their free energy by partially hiding the exposed hydrocarbon tails inside the groves of the DNA, as illustrated in Fig. 1.

The counterion and surfactant association has the effect of neutralizing $n_{\text{surf}} + n_{\text{count}}$ charges of DNA molecule, decreasing the net charge of a complex to $q_{\text{complex}} = -(Z - n_{\text{surf}} - n_{\text{count}})q$ (Fig. 1). All the physical properties of this system depend on the values of n_{count} and n_{surf} which are thermodynamically favored, i.e. which minimize the overall Helmholtz free energy of solution, $F = F_{\text{complex}} + F_{\text{solvation}} + F_{\text{mixing}}$.

The first term is the energy needed to form an isolated complex, the second is the solvation energy, while the last term represents the entropy of mixing of different species.

To obtain F_{complex}, the cluster composed of one DNA molecule, n_{count} counterions, and n_{surf} surfactants is modeled as a one-dimensional lattice with phosphate groups represented by Z charged sites. To each site i, we associate an occupation variables $\sigma_c(i)$ and $\sigma_l(i)$, which are nonzero if the site is occupied by a counterion or by a ring composed of l amphiphiles. The partition function is obtained as the Boltzmann sum over all configurations v. The energy of each configuration E^v is due to electrostatic and hydrophobic interactions between the monomers of the DNA and the condensed counterions and amphiphiles, $E^v = E^v_{\text{el}} + E^v_{\text{hyd}}$. The first term E^v_{el} [5–7], accounts for the electrostatic interactions between all the charges within a complex. The second term

$E^v_{\rm hyd}$, is the result of interactions among the condensed amphiphiles, and between the condensed amphiphiles and the DNA. In order to account for hydrophobic interactions between the hydrocarbon tails of the amphiphilic molecules and the core of the DNA one of the contributions to $E^v_{\rm hyd}$ is,

$$E^v_{\rm DNA-AMPH} = \chi_{\rm DNA} \sum_{i,l}^{Z,l_{\max}} l\sigma_l(i)\,. \tag{1}$$

This term was not included in previous calculations [5–7]. The parameter $\chi_{\rm DNA} < 0$ measures the attraction between the hydrocarbon tails and the DNA core.

To obtain the exact partition function of even this simplified model is a very difficult task. To simplify the calculations we shall adopt a mean-field approximation. The actual free energy of the system will be replaced by an upper bound, calculated using the Gibbs–Bogoliubov–Feynman inequality. This approximation is particularly accurate for long-ranged potentials [10].

Next, we must account for the free energy gained when the complex constructed in isolation is introduced into the bulk solution. This "solvation energy" can be split into two parts, $F_{\rm solvation} = F_{\rm complex/\mathit{complex}} + F_{\rm complex/\mathit{ions}}$. The term $F_{\rm complex/\mathit{complex}}$ represents the electrostatic interaction between the complexes, while $F_{\rm complex/\mathit{ions}}$ is the result of interactions between the complexes and the free counterions and surfactants [5,11–14].

The solvation energy is calculated in the framework of Debye–Hückel [15,18] theory. Let us fix the position of one cluster and ask what is the electrostatic potential Φ that this cluster feels as a result of the presence of all the other clusters, surfactants, counterions, and coions. To answer this question, it is necessary to solve the Poisson equation, $\nabla^2\Phi = -4\pi\rho_q/D$. Following the pioneering ideas of Debye and Hückel we shall assume that the free (unassociated) surfactants, counterions, and coions are distributed around the complex in accordance with the Boltzmann distribution, with the other clusters providing a neutralizing background, $\rho_q = q_{\rm complex}\rho_{\rm DNA} + q\rho_{\rm count}\exp(-\beta q\Phi) - q\rho_{\rm coion}\exp(+\beta q\Phi) + q\rho_{\rm surf}\exp(-\beta q\Phi)$ where $\beta = 1/(k_BT)$.

Substituting this charge density into Poisson equation, we obtain the non-linear Poisson–Boltzmann equation (PB). Since the non-linear interactions between the surfactants, counterions, and the DNA have already been accounted for by renormalization of the effective polyion charge, the PB equation can be linearized. The new equation is analytically soluble, allowing the calculation of the potential Φ. The electrostatic free energy can be obtained from the usual Debye charging process [10,15–18].

The free energy due to mixing of various species i is the sum of individual entropic contributions, $F_{\rm mixing} = F_{\rm counterion} + F_{\rm coion} + F_{\rm surfactant} + F_{\rm complex}$. approximated by the ideal gas form $\beta F_i/V = \rho_i \ln(\phi_i/\zeta) - \rho_i$. Here ρ_i represents the density of specie i and ϕ_i its volume fraction. Since the coions and counterions contain no internal structure, $\zeta = 1$. The surfactant is modeled as a flexible chain with s monomers for which $\zeta = s$ [19]. For complexes, we find $\zeta = (Z + n_{\rm count} + n_{\rm surf}s)/(Z + n_{\rm count} + n_{\rm surf})$ [5].

The number of condensed counterions and surfactants is found from minimization of the total free energy, $\partial F/\partial n_{\rm count} = \partial F/\partial n_{\rm surf} = 0$. Fig. 2 illustrates the surfactant

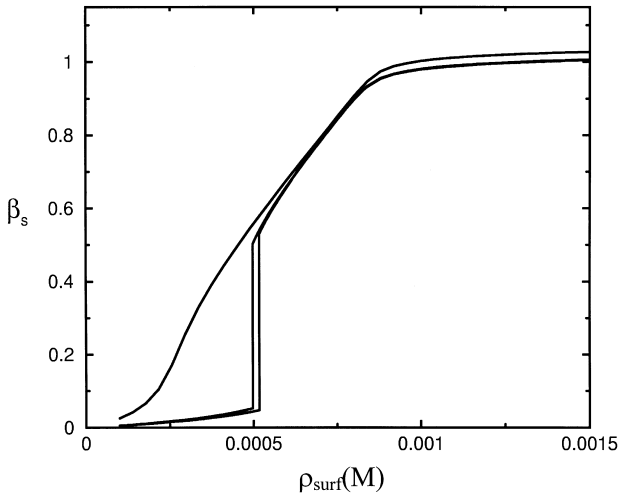

Fig. 2. Effective binding fraction of amphiphiles $\beta_s \equiv n_s/Z$, as a function of concentration ρ_{surf}. The concentrations of DNA and of added salt is 2×10^{-6} M and 18 mM, respectively. The length of the DNA segments is 220 base pairs. The hydrophobicity parameter is $\chi = -6\ k_BT$. The diameter of the DNA is 27 Å. The diameter of surfactant monomers and the ions is $a_c = 3.52$ Å as the other free ions. The solvent is water at room temperature. From right to left, $\chi_{DNA} = 0, -0.1, -1.5\ k_BT$.

binding isotherm, $\beta_s = n_{surf}/Z$, as a function of total amphiphilic concentration. In order to evaluate the relevance of hydrophobic interactions between the amphiphile and the DNA, the parameter χ_{DNA} was varied from 0 to $-1.5k_BT$. For all the values, the same qualitative behavior is observed. For small concentrations of surfactant, the number of associated surfactant molecules was very small. Above certain threshold, ρ^*_{surf}, a cooperative binding is observed. The sharpness of transition strongly depends on χ_{DNA}. When ρ_{surf} is increased further, on average, more than one surfactant molecule associates to each phosphate group and the charge inversion of the surfoplex appears. The hydrophobic interactions between the DNA and the amphiphilic tails, lower the critical density of amphiphile needed for cooperative binding transition.

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References

[1] P.L. Felgner, T.R. Gadek, M. Holm, M. Roman, M. Wentz, J.P. Northrop, M. Ringold, H. Danielsen, Proc. Natl. Acad. Sci. USA, 84 (1987) 7413.
[2] P.L. Felgner, G.M. Ringold, Nature 337 (1989) 387.
[3] P.L. Felgner, Sci. Am. 276 (1997) 86.
[4] M.J. Hope, B. Mui, S. Ansell, Q.F. Ahkong, Mol. Membr. Biol. 15 (1998) 1.
[5] P.S. Kuhn, Y. Levin, M.C. Barbosa, Chem. Phys. Lett. 298 (1998) 51.
[6] P.S. Kuhn, M.C. Barbosa, Y. Levin, Physica A 269 (1999) 278.
[7] P.S. Kuhn, Y. Levin, M.C. Barbosa, Physica A 274 (1999) 433.

[8] A.V. Gorelov, E.D. Kudryashov, J.-C. Jacquier, D. McLoughlin, K.E. Dawson, Physica A 249 (1998) 216.
[9] K. Shirahama, K. Takashima, N. Takisawa, Bull. Chem. Soc. Jpn. 60 (1987) 43.
[10] P. Kuhn, Y. Levin, M.C. Barbosa, Macromolecules 31 (1998) 8347.
[11] B.V. Derjaguin, L. Landau, Acta Phys. (USSR), 14 (1941) 633.
[12] E.J.W. Verwey, J.Th.G. Overbeek, Theory of the Stability of Lyophobic Colloids, Elsevier, Amsterdam, 1948.
[13] X.-J. Li, Y. Levin, M.E. Fisher, Europhys. Lett. 26 (1994) 683.
[14] M.E. Fisher, Y. Levin, X.-J. LI, J. Chem. Phys. 101 (1994) 2273.
[15] P.W. Debye, E. Hückel, Z. Phys. 24 (1923) 185.
[16] N. Bjerrum, Kgl. Dan. Vidensk. Selsk. Mat.-Fys. Medd. 7 (1926) 1.
[17] D.A. McQuarrie, Statistical Mechanics, Harper and Row, New York, 1976.
[18] M.E. Fisher, Y. Levin, Phys. Rev. Lett. 71 (1993) 3826.
[19] P. Flory, Principles of Polymer Chemistry, Cornell University Press, Ithaca, NY, 1971.