Electrostatic Zipper Motif for DNA Aggregation

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Counterion specificity of DNA condensation is rationalized from a theory of electrostatic interaction between helical molecules that accounts for different charge distribution patterns. An axial charge separation due to ion binding in helical grooves allows close approach of opposite charges along the DNA-DNA contact and forms an electrostatic "zipper" that "fastens" the molecules together. Predictions of the theory are in agreement with experimental data. [S0031-9007(99)09128-0]

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Meters of genetic material are packed by nature into compact structures by a variety of methods optimized to specific requirements. In chromosomes, this is done by specialized proteins. In phage heads and sperm, DNA is packaged by relatively simple counterions. This counterion-induced DNA condensation was studied extensively, but its mechanism remains puzzling [1].

DNA has two charged phosphate strands spiraling around a rodlike core formed by nucleotide base pairs (Fig. 1); i.e., electrostatics is likely to be involved [2,3]. Models for attraction between rodlike polyelectrolytes were proposed [4]. However, when applied to DNA condensation, these models offer no explanation for the observed cation specificity. Namely, none of the alkali-earth metal ions (Mg^{2+}, Ca^{2+}, etc.) induce aggregation of double-stranded DNA, despite their high affinity to phosphates [1,5]. In contrast, transition metal ions (Mn^{2+} and Cd^{2+}), which have lower affinity to phosphates, precipitate guanine-cytosine (GC) rich DNA [6,7]. Both groups of ions precipitate nonhelical, single-stranded DNA [5]. *In vivo*, complexes of DNA are stabilized by polycations, e.g., spermidine, spermine, protamine, and some polypeptides. These and cobalt hexammine are also widely used for condensing DNA in laboratory [1]. They form distinct surface charge patterns by binding in DNA grooves via hydrogen bonds [8].

Here we extend the theory of interaction between helical macromolecules [9–12]. We find that details of surface charge pattern may determine the specificity and energetics of DNA aggregation. We explicitly describe fixed, adsorbed, and condensed [13] charges while using the Debye-Hückel model for the diffuse cloud of free ions. Similar Debye-Hückel-Bjerrum approximation has been successful in the theory of concentrated electrolyte solutions [14].

Consider interaction between two molecules ($\nu = 1, 2$) that have parallel, cylindrical, water-impermeable cores of the radius b. We previously derived that the potential created by a charge density $\sigma_\nu(z, \phi)$ on cylindrical surfaces of the radius $r_\nu \geq b$ around each core is [9]

$$\tilde{\varphi}_\nu(r, q, n) = \sum_{\nu,\mu=1}^{2} \sum_{m=-\infty}^{\infty} \tau_{n,m}^{\nu,\mu}(q, R)\tilde{\sigma}_\mu(q, m), \quad (1)$$

where

$$\tilde{\varphi}_\nu(r, q, n) = \frac{1}{2\pi} \int_0^{2\pi} d\phi \int_{-\infty}^{\infty} dz\, \varphi_\nu(r, z, \phi) e^{in\phi} e^{iqz} \quad (2)$$

is the Fourier transform of the potential near the core of molecule ν in cylindrical coordinates (r, z, ϕ) associated with the molecular axis, $\tilde{\sigma}_\nu(q, m)$ is a similar transform

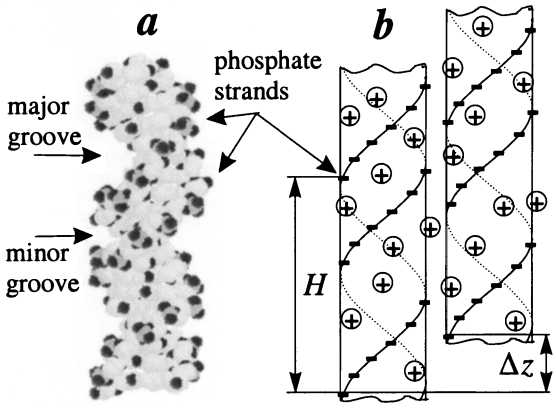

FIG. 1. (a) *B*-DNA structure based on crystallographic coordinates. (b) Schematic illustration of DNA surface charge pattern. Negatively charged helical lines of phosphates and positively charged counterions adsorbed in the grooves form stripes of positive and negative charges. The molecules may align so that closely opposing stripes have complementary charges along the length of DNA-DNA contact (as shown). This creates a "zipper" which pulls the molecules together via electrostatic attraction. The strength of the attraction depends on the distribution of counterions between the two grooves and on the ratio of the axial shift Δz to the helical pitch H.

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of $\sigma_\nu(z,\phi)$, and R is the interaxial separation. At $R \geq 2b + \lambda_D$, where λ_D is the Debye length ($\sim$7 Å *in vivo*),

$$\tau^{\nu,\mu}_{n,m}(q,R) - \tau^{\nu,\mu}_{n,m}(q,\infty) \approx \frac{r_\mu}{b^2}\, Q^{\nu,\mu}_{n,m}(q,R) \times \zeta^\nu_n(q,r_\nu)\zeta^\mu_m(q,r_\mu) \quad (3)$$

(see [9] for more details). Here

$$Q^{\nu,\mu}_{n,m}(q,R) = \frac{4\pi(-1)^{\mu n-\nu m}K_{n-m}(\tilde\kappa R)}{\varepsilon_w\tilde\kappa^2 K'_n(\tilde\kappa b)K'_m(\tilde\kappa b)} \qquad (\nu \neq \mu), \quad (4)$$

$$Q^{\nu,\nu}_{n,m}(q,R) = -\frac{4\pi(-1)^{\mu(n-m)}\Omega_{n,m}(\tilde\kappa R,\tilde\kappa b)}{\varepsilon_w\tilde\kappa^2 K'_n(\tilde\kappa b)K'_m(\tilde\kappa b)}, \quad (5)$$

$$\Omega_{n,m}(x,y) = \sum_{j=-\infty}^{\infty}\left[K_{n-j}(x)K_{j-m}(x)\,\frac{I'_j(y)}{K'_j(y)}\right], \quad (6)$$

$$\zeta^\nu_n(q,r_\nu) = \left[\frac{K'_n(\tilde\kappa b)I_n(\tilde\kappa r_\nu) - K_n(\tilde\kappa r_\nu)I'_n(\tilde\kappa b) + \frac{\varepsilon_c q I'_n(qb)}{\varepsilon_w\tilde\kappa I_n(qb)}\left[I_n(\tilde\kappa b)K_n(\tilde\kappa r_\nu) - K_n(\tilde\kappa b)I_n(\tilde\kappa r_\nu)\right]}{\left[K'_n(\tilde\kappa b)I_n(\tilde\kappa b) - K_n(\tilde\kappa b)I'_n(\tilde\kappa b)\right]\left(1 - \frac{\varepsilon_c q K_n(\tilde\kappa b)I'_n(qb)}{\varepsilon_w\tilde\kappa K'_n(\tilde\kappa b)I_n(qb)}\right)}\right], \quad (7)$$

ε_w and ε_c are the dielectric constants of water and dielectric cores; $\tilde\kappa = \sqrt{\kappa^2 + q^2}$, $\kappa = \lambda_D^{-1}$; K_n and I_n are the modified Bessel functions of nth order, $K'_n(x) = dK_n(x)/dx$, $I'_n(x) = dI_n(x)/dx$. Since $\varepsilon_c/\varepsilon_w \ll 1$, $\zeta^\nu_n(q,r_\nu) \approx 1$ may be used at $(r_\nu - b)/b \ll 1$.

Assume that adsorbed and condensed counterions lie within nonoverlapping layers around DNA cores [15] ($b \leq r \leq B$, $R > 2B$). Calculating $\frac{1}{2}\int\varphi(\mathbf{r})\rho(\mathbf{r})\,d^3\mathbf{r}$ from Eqs. (1)–(7), we find the interaction energy per unit length

$$u_{\rm int}(R) = \tfrac{1}{2}\sum_{\nu,\mu=1}^{2}\sum_{n,m=-\infty}^{\infty}\int_{-\infty}^{\infty} dq\, Q^{\nu,\mu}_{n,m}(q,R)s^{\nu,\mu}_{n,m}(q). \quad (8)$$

Here

$$s^{\nu,\mu}_{n,m}(q) = \lim_{L\to\infty}\left\{\frac{\tilde\sigma^{\rm eff}_\nu(q,n)\tilde\sigma^{\rm eff}_\mu(-q,-m) + \tilde\sigma^{\rm eff}_\nu(-q,-n)\tilde\sigma^{\rm eff}_\mu(q,m)}{2L}\right\}, \quad (9)$$

$$\tilde\sigma^{\rm eff}_\nu(q,n) = \int_b^B \tilde\rho_\nu(r,q,n)\zeta^\nu_n(q,r_\nu)\,\frac{r\,dr}{b}, \quad (10)$$

$\tilde\rho_\nu(r,q,n)$ is the Fourier transform of the charge density, and L is the length of the molecules [16]. Equations (4)–(10) define the interaction Hamiltonian for given $s^{\nu,\mu}_{n,m}(q)$. To account for fluctuations in $\tilde\rho_\nu(r,q,n)$, one can add the energy of isolated DNA and chemical interaction of ions with DNA and calculate the partition function.

The theory of counterion condensation [13] and most models of attraction between polyelectrolytes [4] presume that all counterions are freely mobile. Such an assumption may hold for alkali metal ions. It is doubtful already for divalent alkali-earth ions [17]. It breaks down for DNA-condensing counterions since they possess strong chemical affinity to specific sites on the DNA surface [8]. Therefore, while heuristically useful, theories of this type may not apply to DNA condensation. Based on experimental evidence [8], we assume [17] that DNA-condensing counterions are chemisorbed and form a rigid, R–independent $s^{\nu,\mu}_{n,m}(q)$ pattern. Then Eq. (8) gives the *free energy* of interaction between the molecules.

We explicitly describe phosphate strands as two helical lines of charges and approximate various patterns of chemisorbed counterions by a three-state model, so that [16]

$$s^{\nu,\mu}_{n,m}(q) = 2\pi\overline{\sigma}^2\delta_{n,m}\delta(q + ng)\cos[q\Delta z(1 - \delta_{\nu,\mu})] \times [f_1\theta + (-1)^n f_2\theta - (1 - f_3\theta)\cos(n\tilde\phi_s)]^2. \quad (11)$$

Here $\overline{\sigma} \approx 16.8\ \mu{\rm C/cm}^2$ and $-\theta\overline{\sigma}$ are the average effective surface charge densities of phosphates and adsorbed counterions, respectively [18]; $\delta_{x,y}$ and $\delta(x)$ are the Kronecker's and Dirac's deltas; $\tilde\phi_s \approx 0.4\pi$ is the azimuthal half-width of the minor groove; $g = 2\pi/H$, $H \approx 34$ Å is the DNA pitch; f_i are the fractions of ions in the middle of the minor (f_1) and major (f_2) grooves and on the strands (f_3); and $f_1 + f_2 + f_3 = 1$. DNA alignment is described by the axial shift $0 \leq \Delta z \leq H$ (Fig. 1) [19].

After substitution of Eq. (11) into Eqs. (8) and (9), we find the free energy of interaction,

$$\frac{u_{\rm int}(R)}{u_0} = \sum_{n=-\infty}^{\infty}[f_1\theta + (-1)^n f_2\theta - (1 - f_3\theta)\cos(n\tilde\phi_s)]^2\frac{(-1)^n\cos(ng\Delta z)K_0(\kappa_n R) - \Omega_{n,n}(\kappa_n R,\kappa_n b)}{(\kappa_n/\kappa)^2[K'_n(\kappa_n b)]^2}. \quad (12)$$

Here $u_0 = 8\pi^2\overline{\sigma}^2/\varepsilon\kappa^2$ ($\approx 2.9\kappa_B T/$Å at physiological ionic strength) and $\kappa_n = \sqrt{\kappa^2 + n^2 g^2}$. The sum rapidly converges, and it can be truncated after $|n| = 2$. Since $\kappa_n R > 3$ and $g \sim \kappa$, each of the terms in the sum decreases exponentially at increasing R with the decay length $\kappa_n^{-1} \propto 1/n$.

The homogeneously charged rod model is the $n = 0$ case of Eq. (12). The $n = 0$ term has the slowest decay, but it is proportional to $(1 - \theta)^2$, unlike the $n = \pm 1, \pm 2$ terms. Even for pure Manning condensation of counterions [13], $\theta \approx 3/4$. Chemisorption increases θ so that $(1 - \theta)^2 < 1/16$, unless DNA overcharging ($\theta > 1$)

occurs [18]. Thus, the structure-sensitive $n \neq 0$ terms can never be neglected *a priori*. Not only is it essential that DNA is a double helix rather than just a rod, but its specific surface charge pattern is also important.

From Eq. (12) we find that DNA helices may attract each other even at $\theta < 1$ as a result of counterion binding in DNA grooves that produces axial separation of positive and negative charges (Figs. 1 and 2). Negatively charged strands may come close to positively charged grooves of the opposing molecule so that the attraction between them keeps the molecules together (Fig. 1). This works as an *electrostatic zipper* running along the whole length of DNA-DNA contact. Counterion adsorption onto phosphate strands reduces the attraction [Fig. 2(a)] due to weaker charge separation, consistent with the observation [1] that Ca^{2+} and Mg^{2+}, which have high affinity to phosphates, do not induce DNA condensation.

The energetically optimal alignment of opposing DNA helices depends on the interaxial separation and on the pattern of counterions [Figs. 2(a) and 2(b)]. When 70% of ions are in the minor groove, $\Delta z \approx H/4$ is optimal at all R [Figs. 2(a), inset, and 2(b)]. When 70% of ions are in the major groove, $\Delta z = 0$ at $R \geq 29$ Å and $\Delta z \neq 0$ at $R < 29$ Å. Optimization of $\Delta z \neq 0$ for all neighbor pairs in a multimolecular aggregate requires special symmetry of lateral packing. Accurate predictions for such aggregates need a many-body theory, which is beyond the scope of this Letter. Instead, here we estimate the lower bound for the aggregation energy by calculating it at $\Delta z = 0$. Although $\Delta z = 0$ may not be optimal, if the energy gain at $\Delta z = 0$ is larger than $k_B T$, the aggregation will occur.

Figure 2(c) suggests that DNA aggregation will take place at 70%–30%, but not at 50%–50% or 30%–70% partitioning of ions between the major and minor grooves. This may explain the observed counterion specificity of DNA condensation. Indeed, most DNA-condensing ions, e.g., spermine, protamine, cobalt hexamine, and Mn^{2+}, are known to bind preferentially in the major groove [8,20]. The aggregation becomes possible when $0.9 < \theta < 1.1$ (Fig. 3), as observed [1]. At smaller or larger θ, uncompensated charge on the opposing molecules prevents aggregation. Effective DNA overcharging [18] ($\theta > 1$) may explain the observed [2] dissociation of DNA aggregates at high bulk concentration of counterions.

The depth of the energy minimum (Fig. 3) at optimal conditions is $\sim 10 k_B T$/persistence length ($\sim 0.1 k_B T$/base pair), close to the estimate based on osmotic stress measurements [3,7]. The distance between DNA helices at the energy minimum is $R \sim 25$–27 Å, close to what is seen by x rays [3,7]. Further approach is prevented by the image-charge repulsion of one helix from the core of the other helix. The relatively small value of the energy is a feature of the symmetry of *B*-DNA; it is much larger for *A*-DNA. This may have an important biological function of allowing stable packing and quick unpacking of *B*-DNA.

Ion adsorption specificity may also explain base pair sequence effects. For example, Mn^{2+} preferentially binds in the major groove to the N7 atom of GC base pairs

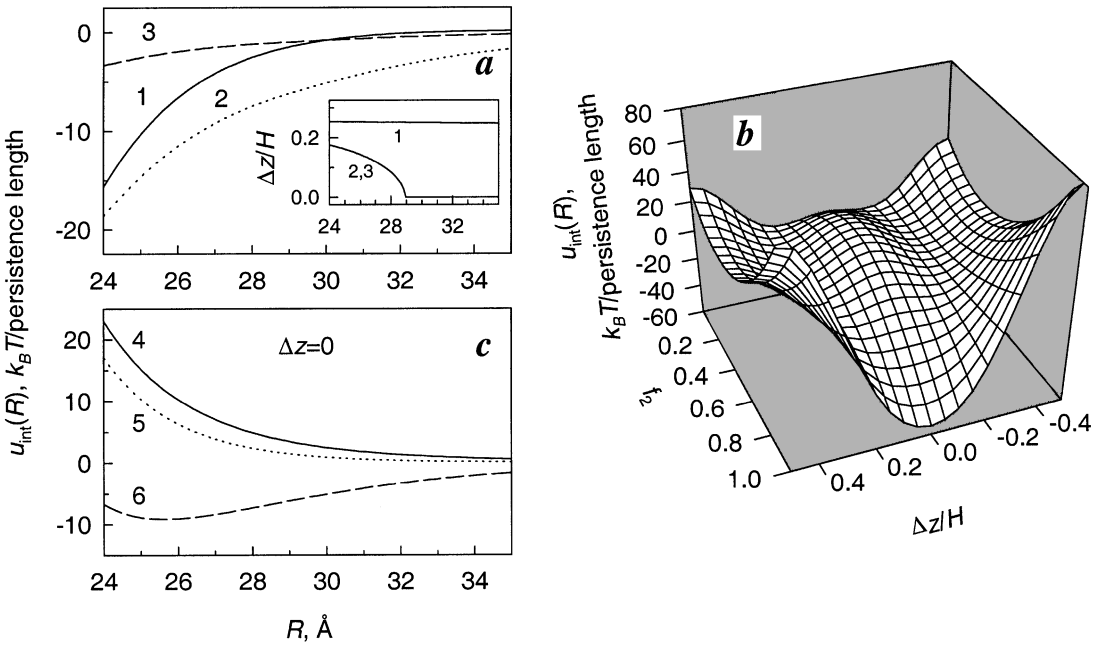

FIG. 2. Effect of counterion adsorption pattern on interaction between DNA helices at physiological ionic strength ($\lambda_D = 7$ Å). Pair interaction potential is shown at $\theta = 0.9$ (as typical for DNA condensation [1]) and (a) at "optimal" mutual alignment, Δz, which minimizes the interaction energy; (b) at $R = 26$ Å and all counterions distributed between the minor and major groove, i.e., $f_3 = 0$, $f_1 = 1 - f_2$; and (c) at $\Delta z = 0$. The curves in (a) and (c) correspond to the following: (1) and (4) $f_1 = 0.7$, $f_2 = 0.3$, $f_3 = 0$; (2) and (6) $f_1 = 0.3$, $f_2 = 0.7$, $f_3 = 0$; (3) $f_1 = 0.15$, $f_2 = 0.35$, $f_3 = 0.5$; and (5) $f_1 = 0.5$, $f_2 = 0.5$, $f_3 = 0$. The inset shows the optimal Δz for the curves (1)–(3) in (a).

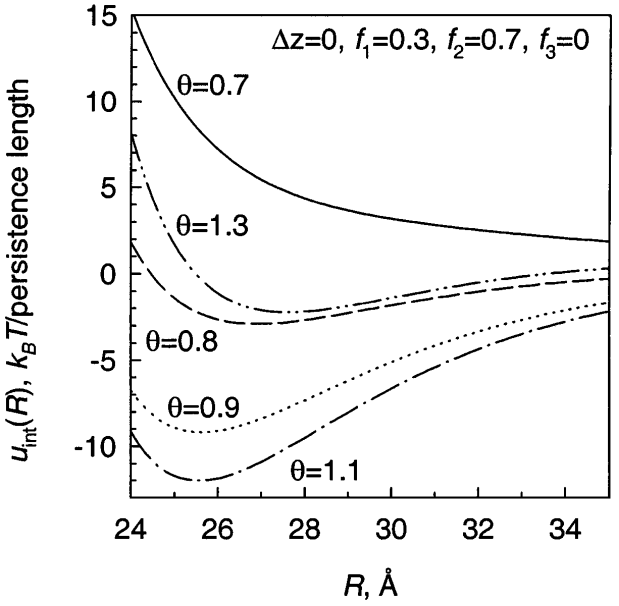

FIG. 3. Effect of the counterion coverage, θ, on DNA condensation at $\Delta z = 0$ and at 30% counterion partitioning in the minor groove and 70% in the major groove ($\lambda_D = 7$ Å). Note that $\theta > 1$ may be caused either by net DNA charge overcompensation or by finite size effects [18].

[8,20]. High GC content increases Mn^{2+} fraction in the major groove and enhances DNA condensation [6,7].

More elaborate theories that account for imperfect helical structure of DNA, for torsional fluctuations, for many-body effects in aggregates, etc., may reveal new phenomena. However, the agreement with experimental data suggests that the present model may have already captured crucial factors for DNA aggregation, i.e., the specific helical structure of charged phosphate strands, the nature of counterion-DNA interaction, and the resulting pattern of ion binding sites.

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[16] For a helical line that crosses the (x, y) plane at $\phi = \phi_0$ and has the average surface charge density $\overline{\sigma}$, $\tilde{\sigma}(q, n) = (L\overline{\sigma}e^{-in\phi_0}\delta_{q,-ng})_{L\to\infty} = 2\pi\overline{\sigma}e^{-in\phi_0}\delta(q + ng)$. Equation (11) directly follows from this expression.
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[18] The effective surface charge density depends on the radial distance of charges from the DNA center [Eq. (10)]. Since some bound ions may lie at a larger radial distance than phosphates, $\theta > 1$ is possible even when phosphates have the number density larger than counterions.
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