Polymer- and Salt-Induced Toroids of Hexagonal DNA

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ABSTRACT A model is proposed for polymer- and salt-induced toroidal condensates of DNA, based on a recent theory of the undulation enhancement of the electrostatic interaction in the bulk hexagonal phase of semiflexible polyions. In a continuum approximation, the thermodynamic potential of a monomolecular toroid may be split up in bulk, surface, and curvature contributions. With the help of an approximate analytical minimization procedure, the optimal torus dimensions are calculated as a function of the concentrations of inert polymer and added salt. The stability of the torus is analyzed in terms of its surface tension and a bulk melting criterion. The theory should be applicable to ψ-toroids that are not too thick.

INTRODUCTION

Under a wide variety of conditions, randomly coiled DNA in dilute aqueous solution may be forced to condense into globules of varying degrees of order. This collapse may be induced by neutral polymers like polyethylene oxide with added monovalent salt (Lerman, 1971), or by a number of polyvalent cations like polylysine (Laemmli, 1975), the polyamines spermidine (3+) (Gosule and Schellman, 1976) and spermine (4+) (Chattoraj et al., 1978; Gosule and Schellman, 1978), cobalt hexammine (3+) (Widom and Baldwin, 1980), or magnesium (2+) in water/ethanol mixtures (Post and Zimm, 1982).

The polymer-and-salt-induced or ψ-condensation of DNA was first studied by Lerman (1971) who discovered that the rate of sedimentation of DNA in highly dilute solutions increased strongly when the concentrations of polyethylene oxide and NaCl were above certain critical levels. He was able to estimate an average hydrodynamic radius of the sedimenting particles, which indicated that within the globules the DNA strands were tightly packed, the volume of a globule being of the order of the self-volume of one DNA molecule.

In additional investigations, the spatial structure of the condensates was resolved to some extent. From x-ray studies it was concluded that the DNA strands were stacked in a lattice, the hexagonal order of which increased with increasing polymer concentration (Maniatis et al., 1974; Evdokimov et al., 1972). Spectroscopic studies indicated that the DNA double helix did on the whole retain its normal B form (Jordan et al., 1972). In electron micrographs a large fraction of the globules adopted more or less simple geometries, like loosely wound toroidal globules (Evdokimov et al., 1972; Evdokimov et al., 1976). The packing of the DNA strands in the globules tended to be tighter the greater the polymer concentration. ψ-Condensation appears to be fully reversible; on diluting the solution to concentrations of polymer or salt lower than the critical concentrations needed for condensation to occur, the solution becomes isotropic again (Lerman, 1973).

Several years after the discovery of ψ-condensation, it was observed that low concentrations of spermidine or spermine, polyamines that are found in vivo, could also induce a collapse of DNA. Again, regular morphologies of the globules were observed. Bloomfield and coworkers reported rather well defined toroids making up the larger fraction of the globules, which are often also rodlike structures (Arscott et al., 1990; Plum et al., 1990). In electron microscopy studies it was shown that the DNA strands were circumferentially wrapped around the centers of the toroids (Marx and Ruben, 1983, 1986), thereby allowing a close packing of the DNA strands without the need for sharp kinks or bends.

In both types of condensation, small globules of well defined geometry are observed only at low concentrations of DNA. At higher DNA concentrations, aggregation sets in, giving rise to much larger and less compact structures. For example, in the case of the condensation induced by magnesium it was shown that sometimes the monomolecular condensates are in coexistence with the random DNA coils (Post and Zimm, 1982).

Quantitatively, the condensation phenomena are not well understood, although a wide variety of models has been proposed. For instance, ψ-condensation has been interpreted in terms of the coil-to-globule transition (Frisch and Fesciyan, 1979; Post and Zimm, 1979) that is observed in the case of *flexible* polymers in a poor solvent (Lifshitz et al., 1978). A persistence segment of DNA has a large aspect ratio implying orientational order within a tightly packed globule as has been stressed by Grosberg and colleagues (Grosberg, 1979; Grosberg and Khokhlov, 1981; Grosberg et al., 1982; Grosberg and Zhestkov, 1986). The free energy of toroidal globules has been estimated assuming the DNA phase is nematic (Grosberg and Khokhlov, 1981; Grosberg and Zhestkov, 1986). In a recent paper by Bloomfield (1991) an overview is given of the free energy of hexagonally packed condensates.

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The general statistical problem of DNA condensation is greatly complicated by the stiffness of DNA causing the orientational and translational degrees of freedom to be strongly coupled. Here, we focus on the relatively modest problem of ψ-condensation of toroidal DNA based on a recent, apparently successful theory of the hexagonal phase (Odijk, 1993a, b, 1994). The collapse of the DNA coil is caused by the osmotic pressure of the surrounding semidilute polymer solution (Lifshitz et al., 1978). We do not describe the initial stages of the compression of the coil but rather approach the problem from the opposite point of view, i.e., the hexagonally ordered globule and its melting behavior. In particular, we do not justify why the globule should adopt a toroidal shape; this is merely a supposition, although we note that some justification for *nematically* ordered toroids was presented by Grosberg and Zhestkov (1986). We do give a novel treatment of the effect of undulations and surface terms, the onset of deviations from a toroidal shape and the possible import of the interaction energy of curvature.

The outline of the paper is as follows. We first neglect undulations as it turns out that their effect is easily included at the end of the analysis. We have to evaluate the electric potential of curved DNA so as to derive the electrostatic free energy of DNA hexagonally packed within a toroid, which is split up in volume, surface, and curvature terms. The bulk polymer solution is supposed semidilute; otherwise the osmotic pressure is too weak to induce condensation. The free energy is again separated in volume and surface (depletion) terms. The optimal dimensions of a torus are calculated by minimizing the total potential of the whole system.

ELECTRIC POTENTIAL EXERTED BY CIRCULAR DNA

The DNA double helix may be approximated by a uniformly charged cylinder of diameter D, bent into a circle of radius R much larger than the Debye length κ^{-1}, so that the polyion may be considered to be slightly curved on the scale of a Debye length. For a 1–1 electrolyte the Debye length is given by $\kappa^2 \equiv 8\pi Q n_s$, with $Q \equiv q^2/\epsilon k_B T$ the Bjerrum length, q the elementary charge, ϵ the dielectric constant of the solvent, and n_s the excess salt concentration. To a good approximation, the electrostatic potential around the polyion may be obtained by solving the nonlinear Poisson-Boltzmann equation (Fixman, 1979). However, as under excess salt conditions the distance between adjacent DNA winds in the hexagonal lattice is larger than $D + 2\kappa^{-1}$, we are interested only in the outer double layer of the potential, which decays essentially as a superposition of Debye-Hückel potentials.

If the charged cylinder is straight, various workers have argued that it may be replaced by a line charge of effective linear charge density adjusted so as to let the tails of the respective potentials match (Brenner and Parsegian, 1974). A convenient means of calculating ν_{eff} is via the Philip-Wooding solution to the nonlinear Poisson-Boltzmann equation (Philip and Wooding, 1970; Stroobants et al., 1986). Upon bending the cylinder and the concomitant line charge, ν_{eff} will decrease a bit because more counterions are attracted to the bent cylinder in view of the enhancement of the bare potential. Nevertheless, we neglect this renormalization as it has the following form:

$$\nu_{\text{eff}}(R) = \nu_{\text{eff}}\,[1 - \text{constant}\,(\kappa R)^{-2}] \tag{1}$$

for it will turn out that only the thin double layer approximation ($D\kappa > 2$) is of practical interest. (Note that Eq. 1 must be an expansion in the square of the curvature.) Furthermore, effects of image charges may be disregarded at high ionic strengths. Ultimately, our analysis will retain solely the leading terms in an expansion in terms of the small parameter $(a\kappa)^{-1}$, where a is the distance between the centerlines of adjacent DNA winds in the torus (in the case of interest here, $a\kappa > 5$). A second small parameter will be a/R; therefore, in addition, we expand all results systematically in terms of the curvature.

Next, we choose a Cartesian coordinate system with the origin fixed at a point on the line charge, with the z axis aligned tangentially along the line charge, and the x axis pointing along its outward normal (see Fig. 1). The distance r between a point P in the xy plane and a point S on the line charge is given by:

$$\begin{aligned} r^2 &= r_0^2 \sin^2\gamma + (r_0 \cos\gamma - R(\cos\theta - 1))^2 + R^2 \sin^2\theta \\ &= r_0^2 + \frac{r_0 s^2}{R}\cos\gamma - \frac{s^4}{12R^2} + s^2 + \mathcal{O}\left(\frac{r_0 s^4}{R^3}\right) \end{aligned} \tag{2}$$

where γ is the angle between the line OP and the x axis, and $\theta \equiv (s/R)$, with s the length of the arc OS. Equation 2 represents the deviation from the straight line in

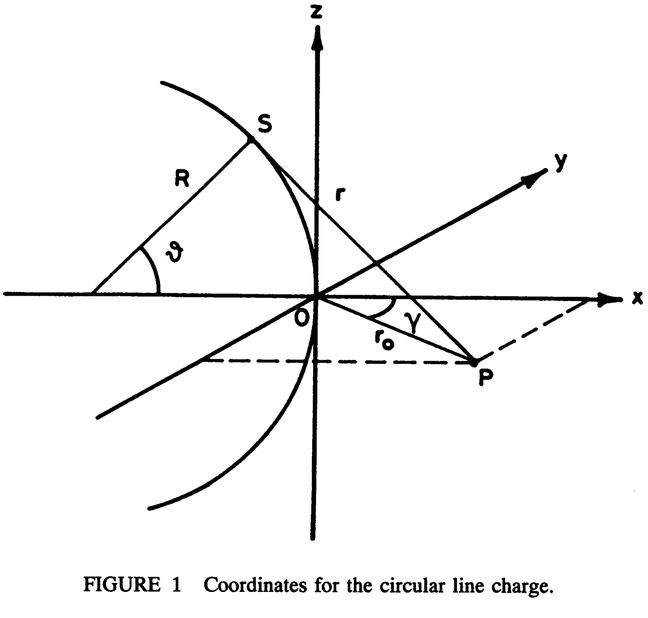

FIGURE 1 Coordinates for the circular line charge.

terms of the curvature. As argued above, the electrostatic potential, scaled by $-q/k_BT$, at point P is a sum of Debye-Hückel potentials:

$$\psi = \psi(r_0, \gamma, R) \approx 2\Gamma \int_0^{\pi R} \mathrm{d}s\, \frac{\exp[-\kappa r]}{r} \tag{3}$$

where $\Gamma \equiv Q\,\nu_{\mathrm{eff}}$.

The best way of expanding this integral asymptotically is by introducing the variable $t = r - r_0$:

$$r\,\mathrm{d}t = \left(1 + \frac{r_0}{R}\cos\gamma\right)s\,\mathrm{d}s - \frac{s^3}{6R^2}\,\mathrm{d}s \tag{4}$$

The integral is transformed to:

$$\psi \sim 2\Gamma\exp[-\kappa r_0] \int_0^{\infty} \mathrm{d}t\, \frac{\exp[-\kappa t]}{\left(1 + \frac{r_0}{R}\cos\gamma\right)s - \frac{s^3}{6R^2}} \tag{5}$$

Because $\kappa R \gg 1$, the errors introduced as we let the upper limit of integration go to infinity are exponentially small.

Expanding the denominator to $O(R^{-2})$ gives:

$$\psi \sim 2\Gamma\exp[-\kappa r_0] \int_0^{\infty} \mathrm{d}t\, \frac{\exp[-\kappa t]}{s} \times \left[1 - \frac{r_0}{R}\cos\gamma + \frac{r_0^2}{R^2}\cos^2\gamma + \frac{s^2}{6R^2}\right] \tag{6}$$

To dispose of s in the integrand, it is convenient to introduce:

$$W \equiv 2r_0\,t + t^2 \approx \left[1 + \frac{r_0}{R}\cos\gamma\right]s^2 - \frac{s^4}{12R^2} \tag{7}$$

Equation 6 may then be written as:

$$\psi \sim 2\Gamma\exp[-\kappa r_0] \int_0^{\infty} \mathrm{d}t\, \frac{\exp[-\kappa t]}{W^{1/2}} \times \left[1 - \frac{r_0}{2R}\cos\gamma + \frac{3r_0^2}{8R^2}\cos^2\gamma + \frac{W}{8R^2}\right] \tag{8}$$

With the help of the leading terms:

$$\int_0^{\infty} \mathrm{d}t\, \frac{\exp[-\kappa t]}{W^{1/2}} \sim (2r_0)^{-1/2} \int_0^{\infty} \mathrm{d}t\, t^{-1/2}\exp[-\kappa t] = \left(\frac{\pi}{2\kappa r_0}\right)^{1/2} \tag{9}$$

$$\int_0^{\infty} \mathrm{d}t\, \exp[-\kappa t]W^{1/2} \sim (2r_0)^{1/2} \int_0^{\infty} dt\, t^{1/2}\exp[-\kappa t] = \left(\frac{\pi r_0}{2\kappa^3}\right)^{1/2} \tag{10}$$

we obtain the asymptotic form of the potential of the circular DNA or its equivalent circular line charge:

$$\psi \sim \psi_{\mathrm{rod}}(r_0) + \psi_{\mathrm{c}}(r_0, \gamma, R) \tag{11}$$

$$\psi_{\mathrm{rod}}(r_0) = \Gamma\left(\frac{2\pi}{\kappa r_0}\right)^{1/2} \exp[-\kappa r_0] \tag{12}$$

where ψ_{c} is the correction to the electrostatic potential for slight curvature:

$$\psi_{\mathrm{c}} = \psi_{\mathrm{rod}}(r_0)\left[\frac{r_0}{2R}\cos\gamma - \frac{3r_0^2}{8R^2}\cos^2\gamma - \frac{r_0}{8\kappa R^2}\right] \tag{13}$$

The coefficient of the last term is not very meaningful as terms of $\mathcal{O}(1/(\kappa r_0))$ have been deleted in Eqs. 9 and 10.

FREE ENERGY OF A HEXAGONALLY PACKED TORUS

We consider a DNA globule, which is assumed to be toroidal in shape and the size of which is determined by the two radii R_{c} and R_{t} (Fig. 2). One DNA molecule is wrapped circumferentially around the center of the torus. In its bulk, the DNA exhibits perfect hexagonal order with a lattice spacing a. We momentarily suppose the DNA undulations are negligible. Hence, the hexagonal packing must continue all the way to the surface. Provided the DNA is long enough, we adopt a continuum approximation so that the DNA strands are aligned with an average spacing a on the toroidal surface, the cross section of which is circular (Fig. 2).

The total free energy of the DNA torus may be written as:

$$\Omega_{\mathrm{t}} = F + A = F_{\mathrm{el,0}} + F_{\mathrm{el,c}} + F_{\mathrm{b}} + A_{\mathrm{el,0}} + A_{\mathrm{el,c}} \tag{14}$$

where F is the total bulk free energy of the torus, A is its surface free energy, $F_{\mathrm{el,0}}$ and $A_{\mathrm{el,0}}$ are, respectively, the bulk and surface electrostatic free energies of the hexagonal lattice as if it were straight, $F_{\mathrm{el,c}}$ and $A_{\mathrm{el,c}}$ are the

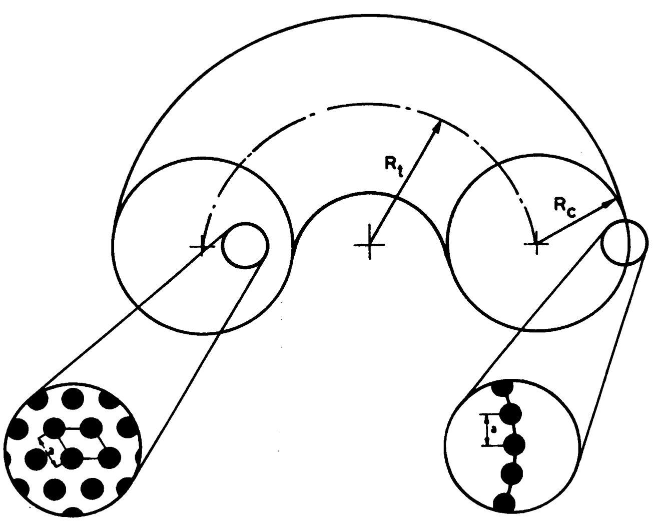

FIGURE 2 The hexagonally packed DNA toroid.

respective correction terms to the electrostatic free energy of interaction due to the slight toroidal curvature, and F_b is the bending free energy.

To obtain the electrostatic free energy per unit length of strand in the bulk of the toroid we simply multiply the effective charge density ν_{eff} on the test strand by the electrostatic potential at its axis. As only the far field of the Poisson-Boltzmann potential is relevant and the superposition approximation holds, we may obtain the total potential at the test strand by just summing over the hexagonal lattice. Only the nearest neighbors in the lattice (i.e., three-pair interactions) contribute significantly because the electrostatic potential falls off exponentially and $\kappa a \gg 1$. The total bulk electrostatic free energy is then obtained by integrating the free energy density, i.e., the free energy per unit length of strand divided by the surface area of a hexagonal unit cell ($3^{1/2}a^2/2$), over the torus volume $V = 2\pi^2 R_c^2 R_t$.

$$\frac{F_{\text{el,0}}}{k_B T} = 4\,3^{1/2}\,\pi^2 \nu_{\text{eff}} \psi_{\text{rod}}(a)\,\frac{R_c^2 R_t}{a^2} \tag{15}$$

The total electrostatic free energy of the torus is lower than its total bulk electrostatic free energy, because on the surface two of the six nearest neighbors assumed to be present in the bulk calculation are missing. We thus have a negative electrostatic surface free energy. This is consistent with our neglect of entropy or undulations. A DNA strand is repelled by the toroidal bulk. The surface free energy as if the lattice were straight is obtained by multiplying the free energy density by the surface area $S = 4\pi^2 R_t R_c$ of the torus:

$$\frac{A_{\text{el,0}}}{k_B T} = -4\pi^2\,\nu_{\text{eff}}\,\psi_{\text{rod}}(a)\,\frac{R_t R_c}{a} \tag{16}$$

Next, the free energy density of interaction and curvature is given by (see Eq. 13):

$$\begin{aligned}\frac{\mathcal{F}_{\text{el,c}}}{k_B T} &= \frac{2\nu_{\text{eff}}}{3^{1/2}a^2}\left[\psi_c\!\left(a, \frac{\pi}{6}, R + \frac{3^{1/2}a}{2}\right) + \psi_c\!\left(a, \frac{\pi}{2}, R\right)\right.\\ &\qquad\left. + \psi_c\!\left(a, \frac{5\pi}{6}, R - \frac{3^{1/2}a}{2}\right)\right] \\ &= \frac{7\,3^{1/2}\,\nu_{\text{eff}}\,\psi_{\text{rod}}(a)}{8R^2}\left[1 + \frac{2}{7\kappa a}\right]\end{aligned} \tag{17}$$

where R is the local radius of curvature. By symmetry, the term of order R^{-1} disappears as it should. The elastic free energy density is given by:

$$\frac{\mathcal{F}_b}{k_B T} = \frac{P}{3^{1/2}a^2R^2} \tag{18}$$

where P is the persistence length of DNA.

We again adopt a continuum approximation: $a \ll R_c$, $a \ll R_t$. A summation over the strands amounts to an integration over the volume even though R may be small, as we shall see. The free energies of curvature may be written as:

$$\frac{F_{\text{el,c}}}{k_B T} = \frac{7}{2}\,3^{1/2}\,\pi\,\nu_{\text{eff}}\,\psi_{\text{rod}}(a)\left[1 + \frac{2}{7\kappa a}\right] I \tag{19}$$

$$\frac{F_b}{k_B T} = \frac{4\pi P}{3^{1/2}a^2} I \tag{20}$$

where the integral I is given by:

$$\begin{aligned} I &= \int_{R_t - R_c}^{R_t + R_c} \frac{[R_c^2 - (R - R_t)^2]^{1/2}}{R}\,dR \\ &= \pi R_t - \pi(R_t^2 - R_c^2)^{1/2} \approx \frac{\pi R_c^2}{2R_t} \qquad \text{for} \quad R_c \ll R_t \end{aligned} \tag{21}$$

Note that the potential divergence has disappeared, which justifies our continuum approximation. The calculation of I is effected by the substitution $R = R_t + R_c \cos\gamma$.

Similar considerations allow us to evaluate the analogous surface terms. The density of the electrostatic surface free energy of curvature is:

$$\begin{aligned}\frac{\mathcal{A}_{\text{el,c}}}{k_B T} & \\ \approx \frac{\nu_{\text{eff}}\psi_{\text{rod}}}{a}&\left[\frac{3^{1/2}\cos\theta}{2R} + \frac{7a\cos^2\theta}{16R^2} + \left[\frac{7a}{32} + \frac{1}{8\kappa}\right]\frac{1}{R^2}\right]\end{aligned} \tag{22}$$

where $R = R_t + R_c\cos\theta$ is the local radius of curvature. The surface free energy of curvature is obtained by an integration over the surface:

$$\frac{A_{\text{el,c}}}{k_B T} = \int_0^{2\pi} R_c c(\theta)\,\frac{\mathcal{A}_{\text{el,c}}}{k_B T}\,d\theta \approx \frac{16}{7}\,\pi\nu_{\text{eff}}\psi_{\text{rod}}(a)\,\frac{aR_c}{R_t} \tag{23}$$

where only the first term has been retained. $c(\theta) = 2\pi R(\theta)$ is the local circumference of the torus. Eq. 23 is simply the surface equivalent of Eq. 17.

LOCAL EQUILIBRIUM IN A SEMIDILUTE POLYMER SOLUTION

We may assume that the DNA and polymer phases are completely immiscible so that the polymer is not present in the DNA phase, or vice versa. The interface between the DNA globule and the polymer solution is considered to be well defined, in the sense that it is stable, relatively smooth, and permeable only to water and salt.

The polymer is neutral, flexible, highly soluble, and sufficiently high in concentration to ensure that we are in the semidilute regime. According to scaling theory, the properties of the solution are governed by the correlation length ξ, which is a measure of the average mesh size in the polymer network. The correlation length decreases quite strongly with increasing polymer volume fraction v (de Gennes, 1979):

$$\xi = Av^{-3/4} \tag{24}$$

where A is the Kuhn length. Eq. 24 is valid if the polymer chain is long enough.

In the stabilization of the DNA globule by the polymer solution, two effects have to be discerned. First, we have the osmotic pressure that scales as (de Gennes, 1979):

$$\frac{\Pi_p}{k_B T} = C_3 \xi^{-3} \tag{25}$$

where C_3 is a constant. The second effect that is of importance here is the depletion of the polymer at the surface of the globule (Joanny et al., 1979). If we suppose the polymer is inert with respect to both DNA and salt, the polymer concentration in a layer of characteristic thickness ξ surrounding the toroidal surface is lower than in the bulk. The surface tension that results from this depletion layer scales as:

$$\frac{\gamma_p}{k_B T} = C_2 \xi^{-2} \tag{26}$$

A renormalization theory of γ_p does not exist at present; the numerical coefficients C_2 and C_3 are close to unity in Eqs. 25 and 26.

The thermodynamic potential of the DNA torus in equilibrium with the polymer solution may thus be written as the sum of the total free energy of the torus and the work of expanding it against the polymer stress (Kirkwood and Oppenheim, 1961):

$$\Omega = \Omega_t + \Pi_p V + \gamma_p S \tag{27}$$

Here, the polymer solution is a very large reservoir so that the polymer concentration remains virtually constant under variations in volume and surface area of the torus.

The numerical minimization of Ω does not yield a great deal of insight. Hence, we apply an approximate analytical procedure. In the continuum limit ($R_c/a = \mathcal{O}(10)$ or more), the two volume terms in Eqs. 15 and 27 overwhelm the other terms in view of the estimates $R_c = \mathcal{O}(R_t) = \mathcal{O}(P)$ and $\nu_{\text{eff}}\psi_{\text{rod}} = \mathcal{O}(Q^{-1})$. In addition, we note that the potential ψ decays exponentially so that the equilibrium lattice spacing must be very close to the hypothetically straightened hexagonal phase in equilibrium with the same polymer solution. As the total amount of DNA in a globule is constrained, knowledge of the lattice spacing also fixes the volume of the globule, $V = 3^{1/2}La^2/2$, where L is the length of the DNA molecule.

Therefore, we have:

$$\frac{\partial \Omega}{\partial V} \approx \frac{\partial F_{\text{el},0}}{\partial V} + \Pi_p = 0 \tag{28}$$

which, together with Eq. 16, leads to:

$$C_3 \xi^{-3} = \frac{3^{1/2} E \kappa}{Qa} \tag{29}$$

where E is defined by $E \equiv Q\nu_{\text{eff}}\psi_{\text{rod}}(a) = \Gamma^2(2\pi/\kappa a)^{1/2} \exp[-\kappa a]$. In Eq. 29 terms of $\mathcal{O}((1/\kappa a))$ have been deleted.

At a fixed volume of the torus, we have one remaining degree of freedom, R_c for instance. The balance of surface and curvature free energy terms determines the aspect ratio R_t/R_c of the globule. Positive surface free energy contributions will tend to keep the torus short and thick; the free energy of curvature in general favors a more extended configuration.

At this stage it is convenient to consider the ratio of the surface terms:

$$g_1 \equiv \frac{-A_{\text{el},0}}{\gamma_p \cdot S} = \frac{\nu_{\text{eff}}\psi_{\text{rod}}(a)\xi^2}{C_2 a} = \frac{E\xi^2}{C_2 Qa} = (3^{1/2} C_2 \kappa \xi)^{-1} \tag{30}$$

where $A_{\text{el,c}}$ may be neglected. If $g_1 > 1$, the total surface free energy is negative and the torus is unstable, for it tends to increase its surface area without bound. We therefore have to adopt the condition $g_1 \leq 1$ as a stringent criterion for stability. The ratio of the curvature terms becomes:

$$g_2 \equiv \frac{F_{\text{el,c}}}{F_b} = \frac{21Ea^2}{8QP} = \frac{7\,3^{1/2} C_2 a^3}{8\kappa P \xi^3} \tag{31}$$

The thermodynamic potential may be written as (see Eqs. 14–16, 19–21, 23, and 25–26):

$$\Omega(R_c, V) \approx \frac{2k_1 V}{R_c} + \frac{k_2 V}{2\pi^2 R_c^2}\left[1 - \left(1 - \frac{4\pi^4 R_c^6}{V^2}\right)^{1/2}\right] + \text{constant} \times V \tag{32}$$

with:

$$k_1 = \frac{C_2}{\xi^2} - \frac{E}{Qa} \tag{33}$$

$$k_2 = \frac{7\,3^{1/2}\pi^2 E}{2Q} + \frac{4\pi^2 P}{3^{1/2} a^2} \tag{34}$$

Accordingly, $\partial\Omega/\partial R_c = 0$ yields:

$$\frac{k_1 V^2}{3\pi^2 k_2 R_c^5} = -\frac{2R_t^2}{3R_c^2} + \left[\frac{1}{3} + \frac{2R_t^2}{3R_c^2}\right]\left(1 - \frac{R_c^2}{R_t^2}\right)^{-1/2} \tag{35}$$

One useful limit is at high salt and fairly low polymer concentrations ($k_1 \approx C_2\xi^{-2}$ and $k_2 \approx 4\pi^2 P/3^{1/2}a^2$) for aspect ratios $R_t/R_c > 2$.

$$\frac{3^{1/2} C_2 a^2 R_t^2}{2PR_c \xi^2} \approx 1 \tag{36}$$

$$R_t = \left(\frac{L\,P^2 \xi^4}{3^{1/2}\pi^2 C_2^2 a^2}\right)^{1/5} \tag{37}$$

$$R_c = \left(\frac{3^{3/2} C_2 L^2 a^6}{32\pi^4 P \xi^2}\right)^{1/5} \tag{38}$$

DISCUSSION

In our calculation of the free energy of the torus, undulations of the DNA chain were neglected. Potentially, however, even chain undulations much smaller than the lattice spacing may strongly enhance the electrostatic interaction between the winds, leading to a significant increase in the equilibrium lattice spacing. Moreover, if the undulations become larger than a certain fraction of the lattice spacing, the lattice may undergo a melting transition (Odijk, 1993b).

We now show that the incorporation of the polyion undulations into the previous analysis is straightforward. The total free energy of a straight lattice of weakly undulating semiflexible polyions (undulatory amplitude $d \ll a$), balancing the electrostatic interaction against entropic confinement, may be calculated by assuming a Gaussian distribution of the undulations. For details of the undulation theory, see Odijk 1993a (in Table I the entry for R = 4 nm should read 0.19 (0.011); the theory is compared with osmotic experiments on DNA (Podgornik et al., 1989), muscle filament (Millman, 1986), and tobacco mosaic virus (Millman et al., 1984)). The viewpoint that the decay length ought to be renormalized in a straightforward way (Podgornik and Parsegian, 1990) has been criticized in the review by Odijk (1994). The status of these theories has also been discussed in the context of field theory by de Vries (1994).

Minimization of the total free energy leads to an asymptotic relation between a and d (Odijk, 1993a):

$$d^{8/3}\,\tilde{E} = \frac{2cQ}{9P^{1/3}\kappa^2} \tag{39}$$

where the coefficient c is estimated to be $2^{-2/3}$. $\tilde{E}$ is defined by $\tilde{E} \equiv UE$, where U is an undulatory factor:

$$U \equiv \frac{\exp[\tfrac{1}{2}\kappa^2 d^2]}{1 + \kappa d^2/2a} \tag{40}$$

The osmotic pressure is given by:

$$\frac{\Pi_{\mathrm{el}}}{k_B T} = \frac{2c}{3^{3/2}\kappa a d^{8/3} P^{1/3}} = \frac{3^{1/2}\tilde{E}\kappa}{Qa} \tag{41}$$

The equilibrium lattice spacing a and the undulation amplitude d may be obtained numerically from Eqs. 39–41. We see that the effect is most pronounced at high concentrations of added salt, because the undulation enhancement of the electrostatic potential becomes significant when the undulatory amplitude d is $\mathcal{O}(\kappa^{-1})$. In Eq. 41, the bare interaction E (see Eq. 29) is replaced with the undulation-enhanced interaction $\tilde{E}$. In a new analysis of the electrostatics of a DNA torus incorporating undulations, we would have to substitute $\tilde{E}$ for E wherever the latter occurs, at least to within a factor of unity. Hence, our main result, Eq. 36, remains legitimate even when there are undulations. In the continuum limit, the undulations and spacing are given by Eqs. 28, 39, and 41.

Recently, a melting criterion for the hexagonal phase of DNA was formulated by combining the theory of undulation enhancement with the Lindemann melting rule (Odijk, 1993b). The latter states that on the melting curve of a positionally ordered system, the ratio l of the undulation amplitude and the lattice spacing is a constant of order 0.1. For macroscopic hexagonal phases of DNA, l turns out to be ~0.13 (Odijk, 1993b). For a hexagonal toroid, we expect a melting transition at about the same value of the Lindemann ratio $l = d/a \approx 0.13$, at least in the continuum limit ($a \ll R_c$ and $\mathcal{O}(R_c) = \mathcal{O}(R_t) = \mathcal{O}(P)$), where the bulk free energy terms are much larger than the surface and curvature free energies.

As the critical deflection length of the toroidal lattice is much larger than the lattice deflection length $\lambda = P^{1/3}d^{2/3}$, the undulations will not be suppressed by curvature (Odijk, 1993c). The higher order term discussed in Odijk's paper is much smaller than those discussed here.

Our theory for DNA toroids is applicable for aspect ratios $R_t/R_c > 2$, i.e., for toroids that are not too thick. Hence, we impose an upper limit on the size of the globule because the aspect ratio decreases with increasing length of the DNA molecule (as $L^{-1/5}$ for $R_t/R_c > 2$). Of course, this limit depends on the salt and polymer concentrations. The dimensions of the torus are thus confined within rather narrow bounds in view of our insistence on the continuum limit (which restricts the minimum size of the DNA).

If the aspect ratio becomes larger than 2, the simple model presented here does not suffice quantitatively. As R_c tends to R_t, the local density of curvature free energy will increase very strongly, leading to a deformation of the toroidal shape. The surface will tend to flatten near the hole in the torus, thereby increasing the surface free energy, but reducing the otherwise strong increase in curvature free energy. We hope to return to this issue.

For the globule to be stable the surface tension should be positive; otherwise, an unlimited increase in surface area would be favorable and the torus would cease to exist. Therefore, $g_1 \geq 1$, so that the salt and polymer concentrations requiring stability are related by the following inequality:

$$\nu \leq 4\,3^{1/3}\pi^{2/3}C_2^{2/3}\Lambda^{4/3}Q^{2/3}n_s^{2/3} \tag{42}$$

This condition neglects entropic contributions (first postulated for crystals of hard particles by Kirkwood (1950) long ago) that might enforce absolute stability of the hexagonal phase.

Finally, although we would like to compare Eq. 36 with experiments, they seem to lie outside the limits of validity of the present analysis. Laemmli's nontoroidal globules studied at very high salt (Laemmli, 1975) appear to be in the regime where R_c is very close to R_t and where the toroid model must fail as we have already pointed out. Toroids have been witnessed in the ψ-condensation of DNA by Evdokimov et al. (1976). The dimensions of the torus photographed in their Fig. 3 agree well with Eq. 36: $R_c \approx 25$ nm, $R_t \approx 50$ nm, $P \approx 50$ nm, $a \approx 3.7$ nm, $\xi = \mathcal{O}(a)$. However, the globule consists of many DNA molecules so the agreement with our single molecule theory may be fortuitous. We hope the simple rule Eq. 36 may incite experimentalists to reinvestigate ψ-toroids.

CONCLUSION

We again stress the limited scope of this work. The DNA macromolecule is viewed as a uniformly charged cylinder the undulations of which are strictly elastic or wormlike. The surrounding ionic distribution is evaluated within the

TABLE 1 Approximations used to derive Eq. 36

Polymer scaling theory	$\nu \leq 0.1$
Double layer superposition	$a \geq D + \kappa^{-1}$
Continuum approximation	$R_c \geq 5\,a$
Ideal torus	$R_t \geq 2\,R_c$
Leading order approximations	$\kappa a \gg 1$

Poisson-Boltzmann approximation (Fixman, 1979). The approximations leading to our main result are listed in Table 1.

A referee has remarked on the difficulty of the charged worm model for DNA providing an explanation for the isotropic-precholesteric-cholesteric transition (Livolant, 1987; Strzelecka and Rill, 1991; Merchant and Rill, 1994). We may add to that the peculiarities found recently in the behavior of semidilute isotropic solutions of DNA (Strzelecka and Rill, 1992; Wissenburg et al., 1994). On the other hand, neutron scattering work on cholesteric DNA (Groot et al., 1994), extended to study the ionic strength dependence of the orientational order, is well represented by electrostatic theory (K. Kassapidou and J. van der Maarel, University of Leiden, manuscript in preparation). It is important to note that the isotropic-cholesteric transition is very sensitive to perturbative influences (e.g., weak attractive forces). In isotropic and cholesteric solutions long-range positional order is absent (disregarding the helicoidal organization of the cholesteric) and the DNA molecules sample the entire configurational space beyond the effective diameter of each polyion (Stroobants et al., 1986). By contrast, in the hexagonal phase the molecules are pinned within a sharply defined potential trough, which is considerably less sensitive to perturbations. Hence, difficulties in explaining the isotropic-cholesteric transition need not falsify the usefulness of the electrostatic model for the hexagonal phase.

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