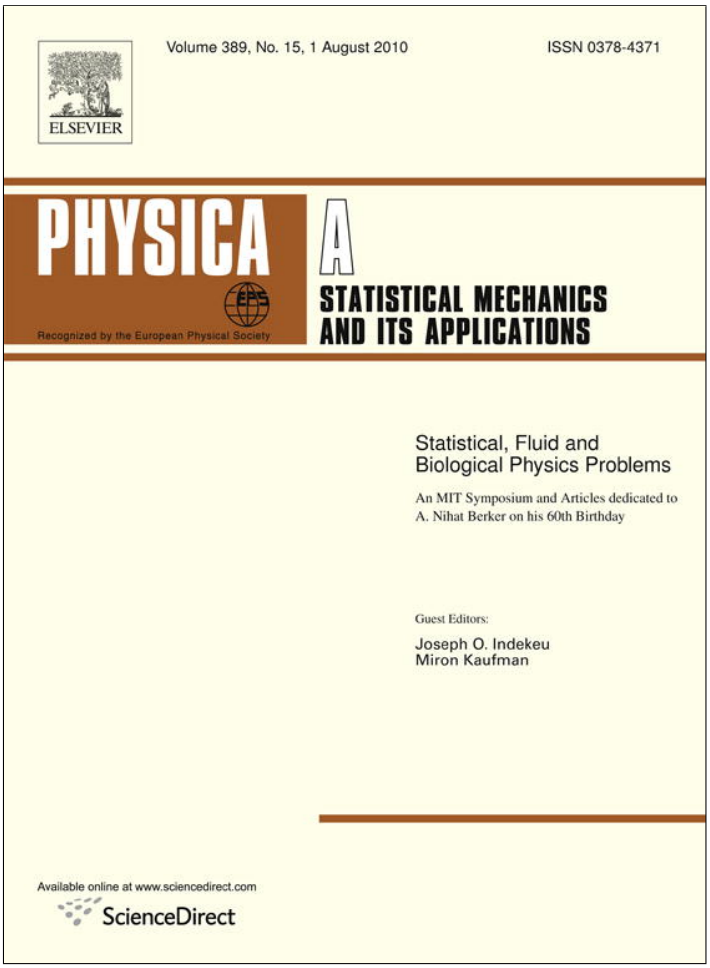

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New results on the melting thermodynamics of a circular DNA chain

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ABSTRACT

We investigate the impact of supercoil period and nonzero supercoil formation energy on the thermal denaturation of a circular DNA. Our analysis is based on a recently proposed generalization of the Poland–Scheraga model that allows the DNA melting to be studied for plasmids with circular topology, where denaturation is accompanied by formation of supercoils. We find that the previously obtained first-order melting transition persists under the generalization discussed. The dependence of the size of the order-parameter jump at the transition point and the associated melting temperature are obtained analytically.

1. Introduction

There is a renewed interest in DNA denaturation [1] fueled by recent experiments that perform elastic measurements at the level of a single molecule [2], perform force-induced denaturation [3] and monitor denaturation dynamics in real-time [4]. Although some of these experimental observations can be addressed by standard models [5,6], some require a representation of the DNA chain at a higher level of sophistication.

A signature of the DNA molecule is its intrinsic twist, quantified at equilibrium by a stacking angle of approximately 36° between successive base pairs. In a circular B-DNA (plasmid) of L base pairs, it amounts to a topological invariant called the linking number, i.e., the number of turns along the chain which is roughly L/Ω_0 where $\Omega_0 \simeq 10.5$ bps [7] is the helical pitch of the relaxed B-DNA. The denaturation process in a plasmid is inevitably accompanied by bending and/or overtwisting on the rest of the chain, as the entropic expulsion of twist from the denaturation loops induces a torsional stress on the bordering bound portions. Recent theoretical models which assume that the torsional stress is absorbed by an increase in the stacking angles (either uniformly distributed [8] or otherwise [9]) suggest that the first-order melting transition observed by the experiments [1] and predicted by theory [5,10–12] for a DNA chain with free ends should disappear in a circular topology. Recent experiments suggest that a circular DNA also melts through a sharp transition [13,14] and at a temperature lower than that of an identical sequence with free ends. The reduction in the melting temperature upon the imposed constraint is associated with the fact that only a fraction of the bases are unbound at the high temperature phase.

We recently developed an extension of the Poland–Scheraga model [5] that incorporates an alternative mechanism for the allocation of the turns entropically expelled from the denaturation loops [15]: a backbone deformation that amounts to a finite "writhe". As is well known, the linking number of two closed curves (such as the two strands of the DNA chain) can be decomposed as

$$\text{Linking number} = \text{Twist} + \text{Writhe} \tag{1}$$

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in the limit where the separation between the two curves is small [16]. Let the two curves be parametrized by the variable s, while their centerline and the unit tangent and normal vectors on it be given by $\vec{r}(s)$, $\hat{t}(s)$, $\hat{u}(s)$, respectively. Then one can show that (see, e.g., Ref. [17])

$$\text{Writhe} = \frac{1}{4\pi}\oint \mathrm{d}s \oint \mathrm{d}s'\, \vec{t}(s)\times\vec{t}(s')\cdot\frac{\vec{r}(s)-\vec{r}(s')}{|\vec{r}(s)-\vec{r}(s')|^3}$$
$$\text{Twist} = \frac{1}{2\pi}\oint \mathrm{d}s\, \vec{t}(s)\cdot\left[\vec{u}(s)\times\frac{\mathrm{d}\vec{u}(s)}{\mathrm{d}s}\right]. \tag{2}$$

We here assume that the twist density of the dsDNA is fixed at its native value, i.e., the twist rigidity is infinite (while Refs. [8,9] considered the other extreme where the persistence length of the dsDNA is assumed infinite). While the latter extreme is relevant for small torsional stress $\sigma = \Delta\mathrm{Lk}/L \ll 1$ in short DNA rings where bending is costly, numerical simulations [18] suggest that the former is a better approximation for DNA circles of about ten persistence lengths (∼kbps) or longer where most of the extra linking number is stored in the writhe. A full treatment of the problem involves a free energy minimization that fixes both the amount of twist and writhe, as well as their sum at a given temperature (work in progress).

Even with the above simplification, the evaluation of the partition function as a sum over all closed curves with a fixed linking number is a formidable task. Therefore, we further assume that the dominant configurations are those where straight segments of dsDNA are separated either by denaturation loops or by regions of uniformly interwound dsDNA chains forming supercoils of arbitrary length that end in a sharp turn. The writhe integral in Eq. (2) is then approximately equal to the number of crossings between the two interwound halves of the supercoil (more precisely, its average value over all projections). Then, the condition in Eq. (1) reduces to a simple constraint involving the total length of the loops L_{loop} and that of supercoiled regions L_{sc} given by

$$L_{\mathrm{loop}}/\Omega_0 = L_{\mathrm{sc}}/\Omega_1 \tag{3}$$

where Ω_1 is the superhelical period varying between $(1.7\text{–}5)\times\Omega_0$ [19] depending on the salt concentration as well as the torsional stress. Since we are interested in a uniform solution and a narrow temperature interval, it is reasonable to consider a constant value for Ω_1. Also note that, we ignored above the correction to L_{sc} due to the end-loop of the supercoil, although the energetic cost of supercoil initiation will be taken into account below.

The limit described above can be studied analytically by a generalization of the Poland–Scheraga model [20]. The partition function is a sum over configurations including denatured regions, supercoiled segments and relaxed dsDNA with arbitrary order, number and sizes consistent with the total length of the chain and Eq. (1).

Let us express the number of base pairs in a loop by l_i, in a relaxed dsDNA by λ_i and in a supercoil by Λ_i, so that $L_{\mathrm{loop}} = \sum_i l_i$, $L_{\mathrm{sc}} = \sum_i \Lambda_i$ and $L_{\mathrm{bound}} = L - L_{\mathrm{loop}} - L_{\mathrm{sc}} = \sum_i \lambda_i$. Then, a DNA segment given by the sequence $\lambda_1 - l_2 - \lambda_3 - \Lambda_4 - \lambda_5$ will be associated with the Boltzmann weight of a configuration can be expressed as

$$\omega^{\lambda_1}\Omega(2l_2)\omega^{\lambda_3}\gamma\omega^{\Lambda_4}\omega^{\lambda_5}$$

where ω, γ and $\Omega(l)$ are Boltzmann factors determined the energy parameters described below. We assume a fixed binding energy E_b between the base pairs in double stranded regions, in the spirit of the Poland–Scheraga model. The possibility of a different effective binding energy for supercoils associated with the winding of the two strands around each other is considered in Ref. [20] and will also be ignored here. On the other hand, the initiation of a supercoil requires formation of an end-loop which will be assigned a fixed energy cost of $E_{\mathrm{sc}} > 0$ per supercoil. Corresponding Boltzmann factors are $\omega = \exp(-E_b/k_BT)$ and $\gamma = \exp(-E_{\mathrm{sc}}/k_BT)$, respectively, where we have $\omega > 1 > \gamma > 0$ at any finite temperature. For the actual DNA molecule, $E_b \simeq 10\text{–}25$ kcal/mol depending on the base-pair type and the nearby bases. E_{sc} depends on the size of the end-loop and is not more than a few kcal/mol [19].

The melting transition is driven by the interplay between the energetic preference for base pair formation and the entropic advantage of more flexible denaturation bubbles. It is well established [21] that the entropy of a denaturation bubble composed of l subsequent unpaired complementary bases bounded by dsDNA on both sides is given by $k_BT\log\Omega(l)$, where $\Omega(l) \equiv As^l/l^c$. c is a universal exponent >2, s and A are nonuniversal constants. The widely used DNA melting simulation tool MELTSIM adopts $s = e^{12.5}$ and $A \simeq 10^{-4}$ [22], although these values are optimized for the Poland–Scheraga model and probably need the modified for the present case.

2. Thermodynamics of the melting transition under fixed linking number

The "tight supercoil" limit of the model with $\Omega_1 = \Omega_0$ was analyzed earlier in Ref. [20], and a first-order transition was obtained for $c > 2$. The transition in the model takes place at the critical temperature given by

$$\omega_c \sim \frac{s}{A\zeta_{c-1}} \tag{4}$$

where ζ_c is the Riemann-zeta function. We here extend this result to the case of an arbitrary superhelical period quantified by the dimensionless parameter $p = \Omega_1/\Omega_0 > 1$ and obtain a closed-form expression for the order parameter at or near the

melting temperature. For a supercoil diameter of 3.5 nm (limiting geometric value valid for high electrolyte concentrations) and a winding angle of 55° [19], one finds $p \simeq 1.7$.

The canonical partition function must be evaluated under two constraints: a fixed DNA length and a fixed linking number. Since the writhe of a supercoil per period (Ω_1) is the same as the twist of a dsDNA per pitch (Ω_0), the condition for the linking number conservation in Eq. (1) reads

$$\sum_i \Lambda_i = p \sum_i l_i \tag{5}$$

where we assumed that the supercoils are not over- or undertwisted. Both constraints can be relaxed by defining associated fugacities z and μ, respectively, and substituting

for dsDNA coils: $\omega \to (\omega z)$,
for supercoils: $\omega \to (\omega z/\mu)$,
for loops: $s \to (sz\mu^p)$.

After these substitutions, the grand sum $Q(z, \mu)$ for the system can be evaluated following the methodology in Ref. [20], yielding in closed form

$$Q(z, \mu) = \frac{\tilde{V}(z, \mu)}{1 - U(z, \mu)\tilde{V}(z, \mu)}$$

where

$$\tilde{V}(z, \mu) = \frac{\omega z}{1 - \omega z - \gamma \omega^2 z^2/(\mu - \omega z)}$$

$$U(z, \mu) = A\Phi_c(sz\mu^p).$$

$\Phi_c(x)$ is the polylog function which at $x = 1$ reduces to the Riemann-zeta function ζ_c. The total DNA length is set by

$$L = \partial \log Q / \partial \log z$$

and the thermodynamic limit $L \to \infty$ is attained at the smallest value of z that satisfies

$$1 - U(z, \mu)\tilde{V}(z, \mu) = 0$$

or

$$\left(\frac{1}{\omega z} - 1\right) - \gamma \frac{\omega z}{\mu - \omega z} = A\Phi_c(sz\mu^p). \tag{6}$$

μ above is fixed such that the linking number is conserved (on average), i.e., $\partial \log Q / \partial \log \mu = 0$, which yields

$$\gamma \frac{\omega z}{(\mu - \omega z)^2} = \frac{Ap}{\mu}\Phi_{c-1}(sz\mu^p). \tag{7}$$

Eqs. (6) and (7) generalize those in Ref. [20] to arbitrary superhelical period given by p and nonzero supercoil initiation energy associated with $\gamma > 1$. Even though they are transcendental equations, an analytical investigation of the transition is still possible.

Let us note that, in absence of the topological constraint in Eq. (5), one sets $\mu = 1$ and recovers the earlier result for the standard Poland–Scheraga model [11,20], i.e., a first-order phase transition for $c > 2$, a second-order transition for $1 < c \leq 2$ and a smooth crossover for $c \leq 1$. With the constraint in Eq. (5), the model exhibits a phase transition (in the limit $L \to \infty$) only if Eqs. (6) and (7) can be solved simultaneously at the point where the polylog function is nonanalytic, i.e., at the phase transition point we have $sz_c\mu_c^p = 1$. Substituting in Eq. (7), we get a quadratic equation for $\omega_c z_c/\mu_c$. Picking the solution with smaller $|z_c|$ and considering the limit $A/\gamma \ll 1$. one finds

$$z_c^{1+p} = \left(\frac{1}{s\omega_c^p}\right)\left(\frac{Ap\zeta_{c-1}}{\gamma}\right)^{2p},$$

$$\mu_c^{1+p} = \left(\frac{\omega_c}{s}\right)\left(\frac{\gamma}{Ap\zeta_{c-1}}\right)^2 \tag{8}$$

where both z_c and μ_c are real and positive. The melting temperature follows from substituting the critical fugacities in Eq. (6) to get

$$\omega_c \sim \frac{s\gamma}{Ap\zeta_{c-1}} \Rightarrow \begin{cases} sz_c = (s/\omega_c)^{3p/(1+p)} \\ \mu_c = (s/\omega_c)^{1/(1+p)} \end{cases} \tag{9}$$

and recalling that $\omega = \exp[-E_b/k_BT]$:

$$k_BT_c \sim \frac{E_b}{\ln(Ap\zeta_{c-1}/\gamma s)}. \tag{10}$$

Note that $E_b < 0 < A \ll 1 < \gamma s/p\zeta_{c-1}$, so that $T_c > 0$. As expected, when the phenomenological parameters s and A increase, the loop entropy gets more prominent and the melting temperature is reduced. By the same reasoning, melting takes place at a higher temperature if the supercoil formation is penalized by an increase in E_{sc}.

With a higher superhelical period and a fixed value of the order parameter (the fraction of bound pairs), a larger portion of the dsDNA is found in supercoils in order to preserve the linking number. At first sight, it is not clear why this should result in a decrease in the melting temperature. In order to address this question, one should look more carefully at the order parameter near the transition.

3. The order parameter at and near the melting point

The order parameter is the average bound pair density given by

$$\theta = \frac{L_{\text{bound}}}{L} = -\frac{\partial \log z}{\partial \log \omega} = -\frac{\omega}{z}\frac{\partial z}{\partial \omega}. \tag{11}$$

Eqs. (8) cannot be used to evaluate the derivative above, since they are only valid at the phase transition point and not in its vicinity. Therefore we have to work with Eqs. (6) and (7) without imposing the condition $sz\mu^p = 1$. By evaluating $\partial/\partial\omega$ [Eq. (7)] and using the identity $\mathrm{d}\Phi_c(x)/\mathrm{d}x = \Phi_{c-1}(x)/x$, one can derive θ in closed form as a function of the fugacities and the phenomenological parameters as:

$$\theta = \frac{(\mu - \omega z)^2 + \gamma\mu\omega^2 z^2}{(\mu - \omega z)^2 + (1 + 1/p)\gamma\mu\omega^2 z^2}. \tag{12}$$

Note that $0 \le \theta \le 1$ as expected. Using Eq. (6), the order parameter at the transition temperature is found as

$$\theta_c^< = \frac{1 + pA\omega_c z_c \zeta_{c-1}}{1 + (1+p)A\omega_c z_c \zeta_{c-1}}. \tag{13}$$

At the high temperature regime one expects maximal denaturation, which in this model corresponds to a fraction $p/(1+p)$ of the DNA in supercoils while the rest is denaturated. Eq. (13) confirms that the order parameter is discontinuous at the transition and becomes continuous only in the limit $c \to 2$, consistent with Ref. [20]. On the other hand, as the superhelical period increases, the transition point shifts to a regime with a higher percentage of bound bases. This observation is in accord with Eq. (10) where increasing p was shown to shift the transition to lower temperatures. A self-consistent determination of the value of p requires a generalization of the present model which includes an energy penalty for the supercoils as a function of the superhelical period. As a final note, strictly speaking, these results are valid only in the thermodynamic limit $L \to \infty$. However, as is the case with the open-ended DNA chains and the Poland–Scheraga model, the theory should be applicable to circular chains with tens of kbps or longer with a smoothening of the transition due to finite size effects and sequence heterogeneity. The topological shift in the melting temperature has been indirectly measured through gel electrophoresis [13,14], although it should also be directly observable by comparing the melting curves of identical DNAs in circular and nicked forms.

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