

Effects of torsional strain on thermal denaturation of DNA

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A class of simple statistical mechanical models for DNA melting, first proposed by Poland and Scheraga, has been demonstrated to exhibit a first or second order thermodynamic singularity, notwithstanding the intrinsic one-dimensional nature of the problem. In the present paper we consider the case of circular DNA and show that the inclusion of twist elastic energy in the Poland-Scheraga models leads either to suppression of the thermodynamic singularity or to a weak, third order singularity. Such behavior may also be present in linear DNA under mechanical influences that preclude the release of torsional strain.

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The thermodynamic properties of DNA near the melting, or denaturation, point have become the focus of intense interest in the theoretical physics community [1–5]. A class of simple models, proposed in their original form by Poland and Scheraga (PS) [1], exhibits a thermodynamic singularity, notwithstanding the essentially one-dimensional nature of the DNA molecule. In these PS models the single-stranded DNA sections inside denaturation bubbles are treated as excluded-volume polymers performing a random walk. This produces an effective long-range interaction that leads to a thermodynamic singularity in this one-dimensional system. Depending on whether excluded-volume effects between single and duplexed strands are or are not taken into account, the thermodynamic singularity can have the character of either a first [2] or a second order [3] phase transition. We denote those two alternatives as case I and case II, respectively.

Experimental studies of DNA melting have not yet provided any clear support for the presence of a thermodynamic singularity. In particular, optical absorption studies of the transition usually yield smoothly sigmoidal behavior [6], although this might be attributed to finite size effects or to the sequence heterogeneity of DNA. However, the PS models do not incorporate an additional important effect, namely, *elastic strain*. It is well known that stretching DNA can cause denaturation [7]. We will focus on the case of denaturation-induced *torsional strain*. As shown in Fig. 1, because of the interwinding of the two strands of duplexed DNA, a denaturation bubble induces extra elastic twist in the undenatured portions of the strand. For circular DNA, this strain energy cannot be relieved in the absence of special cutting enzymes, and progressive supercoiling can be expected to arrest the denaturing transition. The thermal denaturation of circular DNA is well known to be sensitive to the degree of over- or underwinding of the loop before it is closed [8]. In the biophysics literature, sigmoidal thermodynamic behavior is also connected with the development of supercoiling [9].

It is the aim of this Rapid Communication to demonstrate that, when supercoiling is included in the PS model for the case of circular DNA, the thermodynamic singularity is, indeed, smeared out or seriously weakened by induced torsional strain. The starting point is the inclusion of distributed twist energy [9] in the PS Hamiltonian:

$$\mathcal{H} = K \frac{[N_2 - \sigma(N_2 + N_1)]^2}{N_1} - \epsilon_0 N_1 + U_{EV}. \quad (1)$$

The first term in Eq. (1) is the distributed twist elastic energy. The untwisting imposed by the unbinding of N_2 base pairs is assumed to produce a twist proportional to N_2 on the remaining N_1 intact bases. Here, σ quantifies the extent to which the circular chain is underwound or overwound. The limit $\sigma = 1$ corresponds to complete unwinding of the DNA. When $\sigma < 0$, the DNA duplex is overwound. The constant K is proportional to the torsional rigidity of double helical DNA ($K \sim 10^{-12}$ erg). The second and third terms describe, respectively, the base-pairing energy ($\epsilon_0 \sim 2$ kcal/mole) and the excluded-volume interaction mentioned above.

We will examine the phase behavior of this generalized Poland-Scheraga model in the grand canonical ensemble, in which the partition function $Z^K(z_1, z_2)$ depends on the fugacities z_1 and z_2 of, respectively, intact and broken base pairs as

$$Z^K(z_1, z_2) = \sum_{N_1, N_2} z_1^{N_1} z_2^{N_2} Z_{N_1, N_2}^K. \quad (2)$$

The partition function Z_{N_1, N_2}^K of a chain with prescribed numbers $N_{1,2}$ of, respectively, intact and broken base pairs is related to the corresponding partition function $Z_{N_1, N_2}^{K=0}$ of the stress-free PS model by

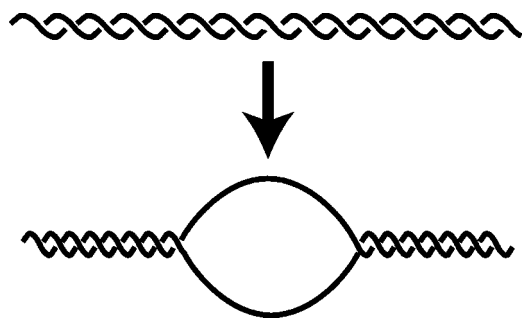


FIG. 1. The creation of a denaturation bubble induces extra twist in the undenatured portions of DNA.

$$Z_{N_1, N_2}^K = Z_{N_1, N_2}^{K=0} \exp \left[-\beta K \frac{[N_2 - \sigma(N_1 + N_2)]^2}{N_1} \right]. \quad (3)$$

Applying Cauchy's theorem to Eq. (2), we obtain

$$Z_{N_1, N_2}^K = \left(\frac{1}{2\pi i} \right)^2 \oint dz_1 \oint dz_2 \frac{e^{-\beta K N_2^2 / N_1} Z^{K=0}(z_1, z_2)}{z_1^{N_1+1} z_2^{N_2+1}}. \quad (4)$$

The functional form of $Z^{K=0}(z_1, z_2)$ near the thermodynamic singularity of the PS models adopts one of two different forms:

$$Z^{K=0}(z_1, z_2) \propto \{ [z_e(\text{I}) - z_1] + c(\text{I})t + b(\text{I})[z_e(\text{I}) - z_2] - a(\text{I})[z_e(\text{I}) - z_2]^{p_{\text{I}}} \}^{-1}, \quad (5a)$$

$$Z^{K=0}(z_1, z_2) \propto \{ [z_e(\text{II}) - z_1] + c(\text{II})t + a(\text{II})[z_e(\text{II}) - z_2]^{p_{\text{II}}} \}^{-1}, \quad (5b)$$

where $z_e(\text{I,II})$, $c(\text{I,II})$, $a(\text{I,II})$, and $b(\text{I,II})$ are positive numbers that depend on $\beta\epsilon_0$. The parameter t is the reduced temperature, which vanishes at the melting point. The exponents $p_{\text{I}} > 1$ and $0 < p_{\text{II}} < 1$ play a key role in the thermodynamic properties of the melting transition.

Case I [Eq. (5a)] corresponds to PS models in which excluded-volume interactions are included, both for the more flexible single strands and for double strands. These interactions lead to a first order melting transition. However, in case II [Eq. (5b)] excluded-volume interactions are included only between single strands. In case II, at the critical temperature $t=0$ a pole in the complex plane merges with the branch cut starting at $z_e(\text{II})$, corresponding to a continuous phase transition. The mean length of a denaturation bubble diverges at $t=0$, while the correlation length $\xi(t)$ diverges as $t^{1/p_{\text{II}}}$, and the specific heat exponent $\alpha = 2 - 1/p_{\text{II}}$. The power p_{II} thus plays the role of a critical exponent, and hyperscaling is obeyed with the dimensionality d equal to 1. In case I, the mean size of a bubble remains finite at the critical temperature. The melting transition is first order.

Performing the integration over z_1 in Eq. (4) we obtain

$$Z_{N_1, N_2}^K \propto \oint dz \exp[-N f_{\text{I,II}}(z, M)] \quad (6)$$

with $M \equiv N_2/N$ the fraction of all pairs that are broken, and $z = z_2$. The function $f_{\text{I,II}}$ is the sum of entropic and enthalpic terms:

$$f_{\text{I,II}}(z, M) = M \ln z + (1-M) \ln [Z_{\text{I,II}}^{K=0}(0, z)] + \beta K (M - \sigma)^2 / (1-M). \quad (7)$$

In the thermodynamic limit $N \rightarrow \infty$, but with M finite the partition function is dominated by the minimum of $f_{\text{I,II}}$ with respect to both z and M . Effectively $f_{\text{I,II}}(z, M)$ plays the role of a Landau variational free energy per base pair. This leads to the following coupled equations of state for $M(t)$:

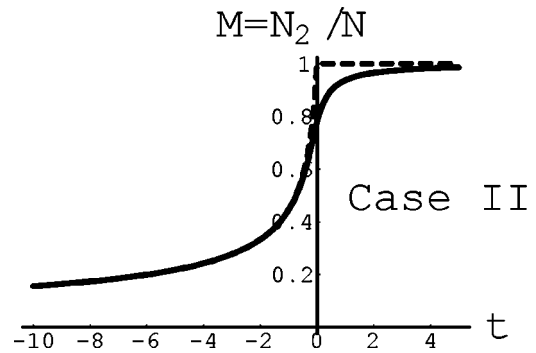


FIG. 2. Dependence of the fraction of broken pairs $M = N_2/N$ on the reduced temperature t for case II with $p_{\text{II}} = 0.5$ and $\sigma = 0$. The dashed curve shows $M(t)$ for the $K=0$ case, i.e., with denaturation-induced twist energy fully relaxed. The solid curve shows $M(t)$ for $\beta K = 1$. In the second case there is no thermodynamic singularity. The melting transition in the strain-unaffected case is at $t=0$.

$$\ln \left[\frac{Z^{K=0}(0, z)}{z} \right] = \beta K \frac{(M - \sigma)(2 - M - \sigma)}{(1 - M)^2}, \quad (8a)$$

$$\frac{M}{1 - M} = - \frac{z}{Z^{K=0}(0, z)} \frac{\partial}{\partial z} Z^{K=0}(0, z). \quad (8b)$$

We first focus on the case $\sigma = 0$. Using Eq. (5b) for $Z^{K=0}(0, z)$, we obtain the results for $M(t)$ shown in Fig. 2. For case II *there is no thermodynamic singularity at $t=0$* . The function $M(t)$ has a smooth, sigmoidal behavior, even for very small values of K , and there is no specific heat anomaly. Physically, this is due to the continuous arrest of the denaturation by the buildup of strain energy as more and more bubbles open. The transition is never completed, and the “denatured” case consists of large bubbles connected by tightly twisted double-stranded sections. For case I, the thermodynamic singularity is not completely removed, but the first order phase transition is transformed into a weak, third order transition [10] (see Fig. 3).

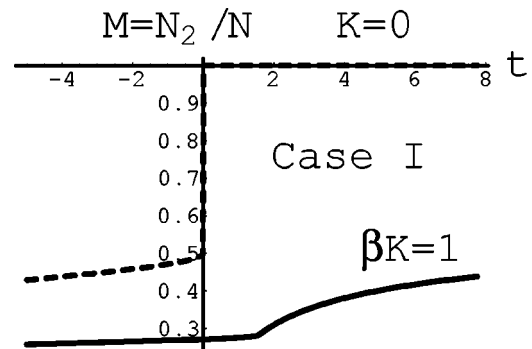


FIG. 3. Dependence of the fraction of broken pairs $M = N_2/N$ on the reduced temperature t for case I with $p_{\text{I}} = 1.5$. The dashed curve shows $M(t)$ for $K=0$, and the solid curve shows $M(t)$ for $\beta K = 1$. In the second case, there is a third order thermodynamic instability.

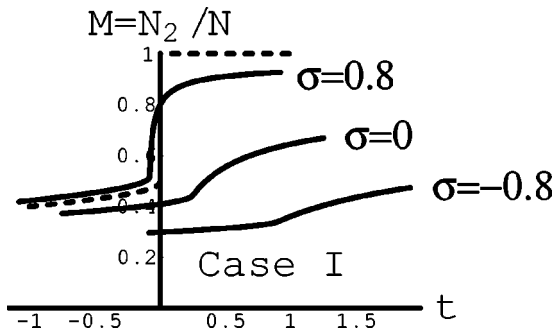


FIG. 4. Dependence of the fraction of broken pairs $M=N_2/N$ on reduced temperature t for different values of the degree of over- and underwinding for case I with $p_1=1.5$ and $\beta K=1$. For $\sigma \neq 1$ there is a third order thermodynamic singularity. For $\sigma=1$ the first order singularity of standard case I PS models is recovered. The dashed curve is the fraction of broken pairs when $K=0$.

We now turn to the effects of nonzero values for the underwinding parameter σ on the melting transition. In the case of a transition that is first order in the absence of strain-related effects (case I), we find that, with one exception to be outlined below, underwinding or overwinding does not change the qualitative effect of strain energy on the transition. The “renormalization” of the order of the transition is the same, although the transition temperature will change with σ . Figure 4 displays the dependence of the denatured ratio N_2/N on the reduced temperature for different values of σ . Of note is the fact that underwinding can lower the transition temperature *below* its value for $K=0$. Underwinding also enhances denaturation at low temperatures, even though the full transition is frustrated. This qualitative result is in general accord with recent experimental observations [11].

Figure 5 displays the transition temperature of under- and overwound DNA as a function of σ . Note the minimum in the transition temperature at $\sigma=1$. At the special value $\sigma=1$, which corresponds to a strand of duplexed DNA that has been underwound to the point that the two individual strands are completely unwound, the transition temperature is lowered, and the first order transition to full denaturing is recovered.

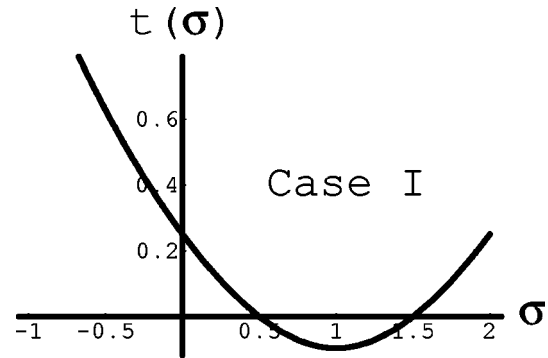


FIG. 5. A plot of the transition temperature $t(\sigma)$ of under- and overwound DNA as a function of the degree σ of over- or underwinding, for case I with $p_1=1.5$ and $\beta K=1$. When $\sigma=1$, the interwinding of the two strands of duplexed DNA has been removed.

It thus seems clear that inclusion of denaturing-induced stresses in the PS model for circular DNA either removes the thermodynamic singularity (case II) or weakens it to such an extent that one would encounter great difficulty in observing it experimentally (case I). The one exception is the introduction of “complete” underwinding, corresponding to the underwinding parameter $\sigma=1$. However, an important limitation on the present model is that we have assumed that the strains created as the result of the appearance of denaturation bubbles are taken up entirely in the form of torsion of non-denatured sections. On the other hand, the denatured section may be able to absorb some part of the torsional stress. It appears very unlikely that either effect will “restore” the critical behavior encountered in the absence of coupling to strain. It is well known that torsional stress on undenatured portions of DNA also produces *writhing*, e.g., formation of heterogeneous three-dimensional interwound plectonemic structures. It is likely that the effect of writhing will be to suppress the reappearance of the first order transition for $\sigma=1$, because plectonemic structures are inherently inimical to strand separation. Such structures might account for the nonmonotonicity of melting temperature as a function of σ observed in [11].

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