Theoretical analysis of the helix-coil transition in positively superhelical DNA at high temperatures

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This paper presents a statistical mechanical analysis of strand separation in a topologically constrained (i.e., superhelical) DNA homopolymer. Our calculations show that positive superhelicity can efficiently stabilize the *B*-form DNA duplex at temperatures substantially above the transition temperature T_m for denaturation of the linear molecule. Moderate superhelix densities ($\sigma < 0.08$) suffice to keep a DNA molecule virtually entirely in the *B*-form conformation at temperatures where, if it were relaxed, it would be substantially denatured. This behavior persists up to a predicted critical temperature $T_c > T_m$. When $T > T_c$ the molecule remains entirely denatured regardless of the amount of superhelicity imposed. The value of T_c is shown to depend on the torsional stiffness C associated with interstrand twisting of the unpaired strands within denatured regions. Thus, experiments that measure T_c would provide another method to evaluate C.

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INTRODUCTION

DNA within living organisms is partitioned into topological domains, either by closure of the molecule into a ring or by the formation of loops through periodic attachments to a matrix. This fixes the linking number $\mathcal{L}$ of each domain, the number of times either strand of its DNA duplex links through the closed loop formed by the other strand. Because $\mathcal{L}$ is a topological invariant, it can only be altered by processes that transiently cut one or both strands of the DNA duplex. Suppose a domain containing N base pairs having helicity characteristic of the unstressed *B*-form duplex (10.4 base pairs per turn) is closed into a planar loop. This defines a reference state, called the relaxed state of the domain, having linking number $\mathcal{L}_0 = N/10.4$ turns. If the linking number of the domain differs from this relaxed value, the linking difference $\alpha = \mathcal{L} - \mathcal{L}_0$ must be accommodated by deformations of curvature or of helicity, both of which require free energy. A domain on which a linking difference has been imposed is said to be either negatively or positively superhelical, depending on the sign of α. All the essential physiological activities of DNA, including gene expression and replication, are stringently regulated by the extent of superhelicity imposed on the domain involved.

Superhelicity can strongly affect the molecular structure of the DNA within a domain. Negative superhelicity can destabilize the *B*-form duplex, disrupting base pairing within local regions to form denatured bubbles [1,2]. This change of secondary structure decreases the unstressed helicity of the regions involved. Although free energy is required for this local denaturation, the resulting decrease of helicity accommodates a portion of the negative linking difference, thereby allowing the balance of the domain to relax by a corresponding amount. Negative superhelicity will drive local denaturation when the deformation strain free energy relieved by this partial relaxation exceeds the cost of the transition [3–6].

Most bacteria and higher organisms produce topoisomerase enzymes that regulate the negative superhelicity of their DNA through processes that involve transient strand breaks. Type I topoisomerases relax the DNA, while type II enzymes, also called gyrases, introduce negative superhelicity into domains. However, thermophilic organisms produce a reverse gyrase, whose biological function is to introduce positive supercoils into DNA [7–9]. Because these organisms live at elevated temperatures, often above the transition temperature T_m for DNA denaturation, it has been suggested that positive supercoiling may serve to stabilize the DNA of these organisms in the *B*-form duplex state. Although the disruption of the duplex by negative superhelicity below T_m has been analyzed previously [3–6], the symmetric question of whether positive superhelicity stabilizes the *B*-form DNA structure above T_m has not been treated before. The paper presents a theoretical analysis of denaturation in positively superhelical DNA at elevated temperatures.

STATISTICAL MECHANICAL ANALYSIS

Consider a circular DNA containing N base pairs on which a linking difference of α turns is imposed. This superhelicity is accommodated by three types of deformations. First, n base pairs may denature in r runs. This changes the regions involved from the *B*-form structure, whose unstressed conformation has 10.4 base pairs per turn, to an unpaired configuration that has no interstrand twist when unstressed. Thus, the change of unstressed helicity consequent on transforming one base pair from the *B*-form to the denatured state is $1/A = -(1/10.4)$ turns. Nucleation of each region of denaturation requires free energy a, while in a homopolymeric transition the opening of each base pair requires the same free energy b. The total free energy required for this transition is $ar + bn$. Second, the unpaired strands within the dena-

tured regions may twist around each other at a rate of τ rad per base pair (i.e., per pair of bases, which in the B-form state comprised one base pair). The free energy required for this deformation is modeled here as being quadratic, having the form $Cn\tau^2/2$. Finally, the residual portion α_r of the imposed linking difference, the part not absorbed by these deformations, is expressed through twisting of the remaining B-form duplex and writhing (i.e., bending) of the entire molecule. Our statistical mechanical analysis does not require that α_r be explicitly decomposed in this way, however, because the free energy associated with residual superhelicity is known from experiments to be quadratic, $K\alpha_r^2/2$, and its coefficient K has been measured [10–13]. The invariance of the linking number results in the following conservation law among these deformations:

$$\alpha=\frac{n}{A}+\frac{n\tau}{2\pi}+\alpha_r \ . \tag{1}$$

A conformational state of this system is determined by first specifying the secondary structure of each base pair, B-form or denatured, and then specifying τ. The residual linking difference α_r then is determined from this information and from α, using Eq. (1). There are 2^N distinct states of secondary structure of the base pairs, while τ may vary continuously. The partition function associated with this system is

$$Z=e^{(-\beta K\alpha^2/2)}+\sum_{n>0}\left[e^{[-\beta(ar+bn)]}\int_{-\infty}^{\infty}\exp\left\{-\beta\left[\frac{nC\tau^2}{2}+\frac{K}{2}\left[\alpha-\frac{n}{A}-\frac{n\tau}{2\pi}\right]^2\right]\right\}d\tau\right] ,$$

where $\beta=1/kT$. The first term in this expression arises from the entirely B-form state, while the summation is over all states of secondary structure in which denatured base pairs occur (i.e., $n>0$). In this homopolymeric transition the separation energy of every state having n separated base pairs in r runs is the same, and there are

$$M(n,r)=\frac{N}{r}\begin{bmatrix}n-1\\r-1\end{bmatrix}\begin{bmatrix}N-n-1\\r-1\end{bmatrix}$$

such states [5]. The integral appearing in the partition function may be evaluated as

$$Q(n)=\left[\frac{8\pi^3}{n\beta(4\pi^2C+Kn)}\right]^{1/2}\exp\left[\frac{-2\pi^2CK\beta}{4\pi^2C+Kn}\times\left[\alpha-\frac{n}{A}\right]^2\right] ,$$

so the partition function simplifies to

$$Z=\exp\left[-\frac{\beta K\alpha^2}{2}\right]+\sum_{n=1}^{N}\left[Q(n)\sum_{r=1}^{r_{\max}}M(n,r)\exp[-\beta(ar+bn)]\right] , \tag{2}$$

where $r_{\max}=\min(n,N-n)$. The ensemble average number of denatured base pairs at equilibrium is given by

$$\bar{n}=\frac{\sum_{n=1}^{N}nQ(n)\sum_{r=1}^{r_{\max}}M(n,r)\exp[-\beta(ar+bn)]}{Z} .$$

This equation is used to calculate the extent of denaturation experienced by a positively superhelical, homopolymeric DNA molecule comprising $N=5000$ base pairs. The results are shown in Fig. 1, which plots $\bar{n}$ against α for several temperatures above T_m. The parameter values used in this analysis are characteristic of DNA having high AT content in low salt solution [13]. The transition temperature is $T_m=321$ K and the transition enthalpy is $\Delta H=7.25$ kcal/mol base pairs [14]. These results show that the onset of transition in relaxed molecules (i.e., molecules having linking difference $\alpha=0$) is delayed by closure into a domain, only commencing near the transition temperature T_m, which is the mid-

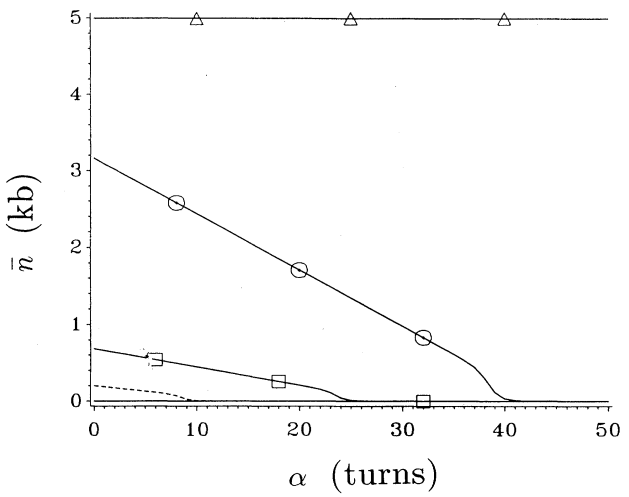

FIG. 1. The ensemble average number $\bar{n}$ of denatured base pairs is plotted as a function of the imposed linking difference α at temperatures $T=325$ K (solid line), $T=335$ K (dashed lines), $T=345$ K (squares), and $T=350$ K (circles). The curve marked by triangles is found at any temperature exceeding T_c. Calculations at $T=352$ K and $T=355$ K both produced this same curve. These results were calculated by the procedure described in the text, assuming a circular DNA molecule containing $N=5000$ base pairs. The free energy parameters used are $K=1945RT/N$, $a=10.8$ kcal/mol, and $C=3.6$ kcal/mol base pairs, values pertaining in low salt solution ($[Na^+]=0.01$ M). Relaxed molecules experience rapid denaturation above the transition temperature that is complete when $T=T_m+30$ K. Below the critical temperature $T_c=351$ K, this denaturation can be reversed by moderate amounts of positive superhelicity. Above T_c the molecule remains entirely denatured, regardless of the amount of superhelicity imposed.

point of transition for the identical linear molecule. As the temperature increases beyond T_m, relaxed molecules experience rapid and substantial denaturation, unpairing being virtually complete when $T=T_m+30$ K. The influence of positive superhelicity on the extent of denaturation depends strongly on temperature. A critical temperature T_c is found, which separates two distinct regimes of behavior. When the temperature T is in the range $T_m<T<T_c$, the imposition of positive superhelicity induces a rapid reversion to the B-form duplex configuration, even under conditions where the relaxed molecule is predominantly denatured. In all cases the number of turns of B-form duplex that are stabilized greatly exceeds the number of superhelical turns introduced. The superhelix density $\sigma=\alpha/\mathcal{L}_0$ required for complete reversion to B-form increases with temperature but is always moderate, $\sigma\leq 0.08$. When $T>T_c$, however, the molecule remains entirely denatured regardless of the amount of positive superhelicity imposed. In this sample calculation the critical temperature was found to be $T_c=351$ K.

The results for a GC-rich homopolymer have the same qualitative characteristics. However, the greater thermal stability of this homopolymer results in higher transition and critical temperatures.

EVALUATION OF THE CRITICAL TEMPERATURE

The critical temperature T_c at which this qualitative change of behavior occurs may be evaluated as follows. Denote by $Z(n,r)$ the contribution to the partition function from all states having n denatured base pairs in r runs. From Eq. (2) one finds that

$$Z(n,r)=Q(n)M(n,r)\exp[-\beta(ar+bn)] .$$

So the ratio of the fractional occupancies of $(n+1,r)$-states to (n,r)-states is given by

$$\frac{Z(n+1,r)}{Z(n,r)}=\mathcal{S}_0\exp\left\{-\beta\left[b+\frac{2\pi^2CK}{4\pi^2C+K(n+1)}\left[\alpha-\frac{n+1}{A}\right]^2-\frac{2\pi^2CK}{4\pi^2C+Kn}\left[\alpha-\frac{n}{A}\right]^2\right]\right\},$$

where $\mathcal{S}_0$ is a term of order zero in n whose value is close to unity. Because the exponential term dominates this ratio, states having $n+1$ denatured base pairs will be more populated than states with n separated pairs when the exponent is positive. Algebraic manipulation shows that this condition is equivalent to the satisfaction of a quadratic inequality, $a_2n^2+a_1n+a_0<0$, whose second order coefficient is

$$a_2=K^2\left[b+\frac{2\pi^2C}{A^2}\right] .$$

When $a_2>0$, the range of values n for which $Z(n+1,r)\geq Z(n,r)$ is limited to the interval between the roots of this quadratic. One can show that, under the present conditions, one root is negative and the other, denoted by n_p, is positive. The most populated states in this case will have $[1+n_p]$ denatured base pairs, where the square brackets denote the greatest integer function. However, when $a_2<0$, one finds that $Z(n+1,r)/Z(n,r)$ increases for all $n\geq n_p$. As $n_p<N$, this implies that the most populated states will be virtually entirely denatured. The transition between these two types of behaviors occurs when $b=-2\pi^2C/A^2$. The separation energy b appearing in this expression depends on temperature as

$$b=\Delta H\left[1-\frac{T}{T_m}\right] ,$$

where ΔH is the enthalpy of denaturation of a base pair of the homopolymer, which has been experimentally measured [14]. It follows that the critical temperature T_c, beyond which no level of superhelicity can maintain the DNA in a B-form configuration, is given by

$$T_c=T_m\left[1+\frac{2\pi^2C}{(\Delta H)A^2}\right] . \tag{3}$$

The critical temperatures under the low salt conditions used by Kowalski ($[Na^+]=0.01$ M) are $T_c=351$ K for an $A+T$-rich homopolymer and $T_c=389$ K for a $G+C$-rich one. These are approximately 30 K above the corresponding transition temperatures. Increasing the ionic strength delays the onset of transition. A molecule with 50% $G+C$ content in $[Na^+]=0.2$ M salt solution will have $T_c=393$ K, assuming the value of C found under the environmental conditions of Kowalski [2,13] remains correct under these conditions.

The dependence of T_c on C displayed by Eq. (3) can be understood by considering the changes in internal stresses that occur within a circular superhelical DNA as the molecule is transformed from being entirely B-form to entirely denatured. (Recall that the unstressed helicities of these two forms differ by N/A turns.) Because the linking number of a closed circular DNA does not change, the complete denaturation of a slightly superhelical B-form molecule produces an unpaired conformation that is torsionally highly deformed away from the unstressed denatured state. The free energy required for this interstrand twisting depends linearly on C. The smaller the value of C, the less the free energy needed to form this configuration and the better it competes with partially denatured states. Conversely, increasing the value of C makes the totally denatured state less competitive, thereby delaying criticality until a temperature where base pairing is energetically more unfavorable. We note that, as all other parameters in Eq. (3) are accurately known, the experimental determination of T_c would al-

low the evaluation of C, a quantity that has been difficult to measure in other ways.

DISCUSSION

These calculations predict that positive superhelicity by itself can maintain DNA in a substantially or entirely B-form state at temperatures significantly above T_m. Our results provide strong theoretical support for the claim that the positive DNA superhelicity induced by reverse gyrases in thermophilic organisms can stabilize the B-form duplex at elevated temperatures. If thermophiles use positive DNA superhelicity for this purpose, then the critical temperature T_c should place an upper limit on the temperature range over which these organisms can perform vital DNA metabolic functions. Moreover, if stringent regulation of the extents and locations of denaturation is required for correct DNA function in thermophiles, as it is in other organisms, then the degree of positive superhelicity imposed would have to depend sensitively on temperature. The theoretical analysis of this phenomenon will require a rigorous, heteropolymeric treatment. Monte Carlo techniques have been developed that can be used for this purpose [15]. This approach will be considered elsewhere.

Recently, an experiment has been reported that indicates that positive superhelicity does indeed stabilize the duplex at temperatures substantially above T_m [16]. Unfortunately, this experiment did not measure either the extent of denaturation of the DNA or the positive superhelicity imposed, so direct comparisons with the present predictions are not possible.

This analysis should be regarded as predicting the qualitative properties of superhelical DNA duplex stabilization at high temperatures. Quantitatively precise predictions would require accurate knowledge of energy parameters that have not been evaluated experimentally at the high temperatures considered here. However, the qualitative features seen—efficient duplex stabilization by positive superhelicity below a critical temperature T_c and a complete loss of stabilization above it —are entirely robust. Their occurrence does not depend on the precise parameter values used or their local variations with base sequence.

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