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Onset of writhing in circular elastic polymers

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A perturbation analysis is presented of an exchange of stability between two mechanical equilibrium conformations of a torsionally deformed, linearly elastic, cylindrical polymer whose ends are held together to form a topologically circular structure. The imposed constraint is the twist angle ζ through which one end of the polymer is rotated relative to the other. The value $\zeta=0$ corresponds to the relaxed state, in which no torsional deformation is imposed. Close to the relaxed state, the stable equilibrium conformation is a torsionally deformed circle in which the imposed constraint is expressed entirely as twist. A threshold value of ζ is shown to exist, whose location depends on the ratio of the polymer bending stiffness A to its torsional stiffness C. Beyond this threshold the stable equilibrium conformation involves nonplanar bending, also called writhing. As this threshold is surpassed, a smooth onset of writhing occurs as a form of torsional buckling. These results are applied to the analysis of torsional fluctuations in nicked circular DNA. A critical molecular length is found, at which the root-mean-square twist induced by thermal fluctuations just suffices to initiate writhing. Comparison with the analogous critical length for linear polymers shows that the constraint imposed by circularity greatly increases the stability of the unwrithed conformation. Although twisting and bending are not coupled either energetically or topologically in nicked circular molecules, the exchange of stability between unwrithed and writhed conformations provides a mechanical coupling between them. Because sufficiently large torsional deformations drive writhing, fluctuations in the parameters $\mathcal{T}$ and $\mathcal{W}$ are not statistically independent in nicked molecules, but instead are positively correlated.

INTRODUCTION

DNA has the mechanical properties of a symmetric, linearly elastic rod.[1,2] Deformation of the molecule away from its unstressed conformation (which is the B form under physiological conditions) induces a system of internal restoring stresses which are resolvable at each cross-section into a force $\mathbf{N}(s)$ and a torque $\mathbf{M}(s)$.[3–6] The magnitudes of these forces and torques are linearly related to the deformations which they induce. It follows that the free-energy densities associated to torsional and flexural deformations are quadratic, with coefficients the torsional stiffness C and the bending stiffness A, respectively.

Although the values of $\mathbf{M}$ and $\mathbf{N}$ may vary continuously with position s along the molecule, at mechanical equilibrium this system of internal stresses must be balanced.[7] This condition implies that the mechanical equilibria of a constrained molecule are precisely those conformations in which the extremals of the analogous action

$$S=\int_0^L \frac{A\kappa^2(s)}{2}+\frac{C\tau^2(s)}{2}ds-\int_0^L N(s)\cos\theta(s)ds \qquad (1)$$

are achieved, consistent with whatever constraints may be imposed.[4] Here s is contour length along the DNA, $\kappa(s)$ is the curvature, $\tau(s)$ is the change of local right-handed helicity, and $\theta(s)$ is the angle between the local tangent vector and the force $\mathbf{N}$. Stable equilibria occur in conformations of least analogous action. Metastable equilibria occur at local minima of S that are not absolute minima, while unstable equilibria occur at local maxima and saddle points.

DNA within living systems is maintained in a superhelically stressed condition. The prototype example of this superhelical constraint occurs in closed circular molecules. Here the two ends of each strand of the DNA duplex are covalently joined together, so the molecule has the topological structure of two interlinked circles. This fixes a molecular linking number $\mathcal{L}$, which is the essential superhelical constraint. The linking number is partitioned between twisting and bending according to White's formula,[8]

$$\mathcal{L}=\mathcal{T}+\mathcal{W} . \qquad (2)$$

Here $\mathcal{T}$ is the total molecular twist, the number of times either strand of the duplex twists around the molecular central axis. $\mathcal{W}$ is the writhing number, a measure of the large-scale shape of the molecular central axis in space. The constraint imposed by the constancy of the linking number is a topological coupling between $\mathcal{T}$ and $\mathcal{W}$. The relaxed state is phenomenologically defined to have planar central axis, for which $\mathcal{W}=0$, and linking number equal to the total unstressed twist of the B-form helix. Experiments have demonstrated that important physiological functions are modulated by the enzymatic regulation of DNA superhelicity, including the initiation of transcription, replication and repair.[9,10]

When $\mathcal{L}$ does not equal its relaxed value $\mathcal{L}_0$, the molecule must respond to this linking difference $\alpha=\mathcal{L}-\mathcal{L}_0$ by either twisting or bending away from its unstressed shape, or by undergoing local transitions to alternate secondary structures having different unstressed helicities than the B form. Extensive analyses have been performed of the distribution of deformations among these alternatives in superhelical molecules.[11] It has been shown that local transitions occur only when the imposed linking difference surpasses a substantial threshold, the precise value depending upon the nature of the secondary structures involved and environmental factors. Under physiologically reasonable *in vitro* conditions, small linking differences are partitioned entirely between smooth elastic twisting and bending of the B-form secondary structure. This paper examines the competition between these two modes of equilibrium deformation in a superhelically constrained elastic polymer.

The large-scale spatial conformation of the polymer is described using the local Euler angles $\theta(s)$, $\phi(s)$, and $\psi(s)$. The writhing number may be expressed in terms of Euler angles as[5]

$$\mathcal{W}=\frac{1}{2\pi}\int_0^L \frac{\dot\phi\sin\theta\cos\theta\cos(ks-\phi)-k\cos\theta-\dot\theta\sin(ks-\phi)}{1-\sin\theta\cos(ks-\phi)}ds\ , \tag{3}$$

where $k=2\pi/L$ and L is the contour length of the polymer.

Mechanical equilibria occur as extremals of the analogous action S associated with the conformation of the molecule, appropriately constrained by its linking number. In the equilibrium conformations the parameter $u=\cos\theta$ oscillates between limits u_1 and u_2,[4,5]

$$u=u_1+(u_2-u_1)\mathrm{sn}^2(\omega s)\ , \tag{4}$$

where sn is a Jacobi elliptic function and ω is a constant. In a conformation having a smoothly closed tangent (such as circular DNA) there must be an integral number j of periods of these oscillations along the length of the molecule. The differential equation satisfied by the Euler angle ϕ at equilibrium is

$$\dot\phi=\frac{\beta_2-\beta_1 u}{1-u^2}\ , \tag{5}$$

where β_1 and β_2 are constants of integration. In all equilibrium conformations of circular polymers, the second integral in the expression for the action [Eq. (1)] vanishes.[5] Thus the action coincides with the elastic deformation strain energy in these conformations.

ONSET OF WRITHING

Consider a nicked circular DNA molecule in which the covalent continuity of one of the strands is disrupted. Although the central axis of the molecule remains topologically circular, at the site of this lesion the two ends of the discontinuous strand can rotate around the central axis relative to each other. The angle through which one end of the discontinuous strand rotates relative to the other is denoted by ζ. Closed circular conformations correspond to values of ζ where these two ends are held in correspondence. The zero point of ζ is chosen to correspond to the relaxed conformation. Thus the linking difference is $\alpha=\zeta/2\pi$, which may vary as a continuous parameter in this case.

When $\zeta=0$ the molecule is relaxed and the equilibrium conformation is an undeformed circle.[5] As ζ is varied away from zero, two important classes of equilibrium conformations occur. Either the deformation can be entirely absorbed as twist so that the tertiary structure is unaltered, or the molecule can also writhe.

The torsionally deformed, unwrithed equilibrium conformation is a planar circle, which has $u_1=u_2=0$, $\theta(s)=\pi/2$, and $\zeta=\tau L$. Here τ is the torsional deformation density measured in radians per unit length, with positive values associated to increases in the right-handed helicity. The Euler angle ϕ is given by $\phi(s)=2\pi s/L$. The action S_c associated with this conformation is

$$\frac{2S_c}{A}=\frac{4\pi^2}{L}+\frac{C\zeta^2}{AL}\ , \tag{6}$$

where A is the bending stiffness of duplex DNA. These values satisfy the equations of equilibrium with $\mathcal{W}=0$ for all values of ζ.[5] This paper investigates the limits of the range of values of ζ over which this equilibrium is stable.

Now consider the alternative, slightly writhed conformation. To quantitate its deviation from the planar configuration, let $u_1=-\epsilon<0$, where ϵ is a small perturbation. Expressions for all relevant quantities need only be expanded to low order in ϵ. For many quantities having appropriate symmetry properties this order is two. The equilibrium solution for $u=\cos\theta$ given by Eq. (4) may be found to low order in ϵ using the series expressions at small values of the elliptic parameter m for the complete elliptic integrals K and E and for the Jacobi elliptic function sn.[12] The result is

$$u=-\epsilon\{\cos(2qs)+(m/4)[\sin^2(qs)-\sin^2(2qs)]\}\ , \tag{7}$$

where $q=\pi j/L$. Using this result, Eq. (5), the differential equation satisfied by the Euler angle ϕ at equilibrium, may be integrated and the low-order terms in ϵ retained

$$\phi(s)=\frac{2\pi s}{L}+\frac{C\tau\epsilon}{A}\left[\frac{\sin(2qs)}{2q}+\frac{m}{16q}[\sin(2qs)+\tfrac{1}{2}\sin(4qs)]-\frac{ms}{4}\cos(2qs)\right]+\frac{\pi\epsilon^2}{4Lq}\sin(4qs)\ . \tag{8}$$

These expressions for $\phi(s)$ and $u=\cos\theta(s)$ together are used to evaluate the writhing number of the infinitesimally deformed equilibrium conformation from the integral in Eq. (3). After considerable calculation, the results to lowest order in ϵ are found to be

$$\mathcal{W}=\frac{C\tau L\epsilon^2}{4\pi A}\ . \tag{9}$$

Note that the writhing number has the same sign as the imposed torsional deformation τ. Positive linking differences produce torsional strains which act to increase right-handed helicity. According to the above Eq. (9), in this case the infinitesimally deformed equilibrium conformation will have $\mathcal{W} > 0$, hence be a right-handed toroidal superhelix.[13] Negative linking differences will yield left-handed toroidal superhelices. The writhing number of the deformed equilibrium conformation is seen in this equation to depend upon the values of the elastic parameters A and C because the conformation itself depends upon them. This equation corrects an expression of LeBret.[6]

As ζ parametrizes the linking difference, we have

$$\zeta = \tau L + 2\pi \mathcal{W} . \tag{10}$$

Substituting Eq. (9) yields, to second order in ϵ

$$\tau = \frac{\zeta}{L}\left[1 - \frac{C\epsilon^2}{2A}\right] . \tag{11}$$

The curvature κ in the action integral [Eq. (1)] may be expressed in terms of the Euler angles θ and ϕ. The above expression for τ may also be inserted there, and the integration performed. In this way the action S_d associated to this infinitesimally deformed conformation is found to second order in ϵ to be

$$\frac{2S_d}{A} = \frac{4\pi^2}{L} + \frac{C\zeta^2}{LA} + \epsilon^2\left[\frac{2\pi^2}{L}(j^2-1) - \frac{C^2\zeta^2}{2A^2L}\right] . \tag{12}$$

The difference in action between the twisted circular and the infinitesimally writhed conformations is given by

$$\frac{2(S_d - S_c)}{A} = \epsilon^2\left[\frac{2\pi^2}{L}(j^2-1) - \frac{C^2\zeta^2}{2A^2L}\right] . \tag{13}$$

When $S_d - S_c < 0$, the infinitesimally deformed conformation is favored. This occurs precisely when

$$\alpha^2 = \frac{\zeta^2}{4\pi^2} > A^2(j^2-1)/C^2 . \tag{14}$$

For the infinitesimally deformed structure the apsidal angle Φ is related to j, the integer number of oscillations of θ, as $\Phi = \pi/j$.[5] Applying Kirchhoff's kinetic analogy[4] to the results of Diaz and Metcalf,[14] one finds that the permissible values of Φ are restricted to satisfy $\Phi < \pi$. From this it follows that $j > 1$. Equation (14) above finds a sequence of threshold values, one for each of value of j in excess of unity. The first transition has $j = 2$ and occurs when

$$|\alpha| = \frac{A\sqrt{3}}{C} . \tag{15}$$

This is the critical value of the linking difference at which an exchange of stability occurs between the torsionally deformed but unwrithed circle and the nonplanar, writhed equilibrium conformation having $j = 2$.

This result has several intriguing properties. First, it is symmetric with respect to the sign of the imposed linking difference. The magnitude of α at which writhing commences is the same for positively supercoiled molecules as it is for negatively supercoiled ones. More surprisingly, the threshold linking difference does not depend upon the length of the polymer. Thus, the transition will occur at smaller specific linking differences (i.e., $\alpha/\mathcal{L}_0$) in longer molecules.

Using reasonably average published values of the elastic parameters at moderate salt concentration of $A = 2.63 \times 10^{-19}$ erg cm,[1] and $C = 2.75 \times 10^{-19}$ erg cm,[15–17] one finds this threshold to be $\alpha = \pm 1.66$ turns. Although there is considerable variation in the published values of A and C,[17] all reasonable choices yield a threshold between 1.5 and 2 turns at physiologically reasonable salt concentrations of $[\mathrm{Na}^+] = 0.2M$. We note that the transition to writhed structures calculated here involves conformations that differ infinitesimally from circles. The onset of substantial writhing occurs somewhat beyond this threshold, probably near two turns.

This provides another example of the concept of regimes of superhelicity.[18] The transition to writhed structures defines the boundary between two such regimes. Within each regime the equilibrium conformations have qualitatively similar structures and the governing energetics obeys a simple law. However, the energetics and equilibria experience a qualitative change as the boundary between regimes is passed. A similar boundary between distinct regimes of superhelicity has been shown previously to occur at the onset of secondary structural transitions.[18]

This analysis proves the claim of LeBret[6] that a transition to writhing of the type described by Eq. (15) will occur. It disproves a conjecture by Vologodskii and Frank-Kamenetskii[19] that smooth torsional deformations do not occur at stressed equilibrium, a claim that was known to be false on other grounds.

CONFORMATIONAL FLUCTUATIONS IN NICKED MOLECULES

When a circular duplex DNA molecule is nicked, the twist angles between neighbor base pairs fluctuate independently. This implies that the variance in the total twist along a nicked domain of length L is [20]

$$V(T) = \frac{LkT}{4\pi^2 C} . \tag{16}$$

Inserting into this expression the measured torsional stiffness C of DNA, one finds that the root-mean-square fluctuations in twist angle between neighboring base pairs at room temperature is ±4°. This is almost 15% of the unstressed twist angle of the B form.

Although the minimum energy conformation of a nicked molecule is unwrithed and torsionally undeformed, if its length L is sufficient, random thermal fluctuations of the twist angles can give rise to a total torsional deformation (and hence a linking difference) exceeding the threshold for the onset of writhing. If a population of such molecules is treated with a ligating agent, then a fraction of the molecules will be closed into conformations that are writhed. From Eqs. (15) and (16) one can determine the length L_{crit} at which the fluctuations in $\mathcal{T}$ have root-mean-square value equal to the threshold needed for onset of writhing

$$L_{\text{crit}} = \frac{12\pi^2 A^2}{CkT} . \tag{17}$$

Using the values of the elastic parameters given above, this length is calculated to be 20 500 base pairs at $T = 310$ K.

Similar calculations of the exchange of stability between the straight and the helically writhed conformations have been performed by Manning for linear polymers.[21] The dimensional dependence of the exchange of stability between unwrithed and writhed conformations was found to be qualitatively the same in linear and circular polymers. In both cases the threshold total twist for onset of writhing was found to be independent of polymer length. However, Manning found that in the linear case this threshold total twist was 0.163 turns, an order of magnitude less than that found here in the circular case. Also, the threshold length for this exchange of stability to be driven by rms torsional fluctuations in the linear case was approximately equal to the polymer persistence length, which is about 300 base pairs for DNA. One may interpret the present result as concluding that the effective persistence length for circularly constrained, nicked polymers vastly exceeds that for linear, unconstrained ones. This demonstrates the immense effect circularity has on the stability of the unwrithed conformation.

In nicked circular molecules the total twist and writhe may fluctuate freely. There is no topological coupling between these quantities, nor is there any energetic coupling according to present understandings of the mechanical properties of duplex DNA. However, the exchange of stability described here constitutes a mechanical coupling between these two modes of deformation. We emphasize that it is torsional deformations alone which drive writhing in this case.

DISCUSSIONS AND CONCLUSIONS

This paper examines the mechanical equilibria of slightly supercoiled DNA. As the linking difference is varied from zero in a polymer molecule with the geometric and mechanical properties that are ascribed to DNA, an exchange of stability has been theoretically predicted to occur that corresponds to the onset of writhing. This provides a positive correlation between $\mathcal{T}$ and $\mathcal{W}$ in nicked circular molecules. That is, surpassing the twist threshold for transition in either direction drives a change in $\mathcal{W}$ of the same sign, as Eq. (9) shows. This demonstrates that the assumption of statistical independence of torsional and flexural deformations in nicked molecules is inconsistent with the known mechanical properties of DNA.

This analysis describes only the mechanical equilibria of the domain, which are states of least action. It does not give direct information about the mixture of low energy microstates that comprises thermodynamic equilibrium.

Several simplifying assumptions have been made regarding the mechanical and conformational properties of DNA. First, the bending and torsional stiffnesses are regarded as constant along the molecule. Although these values are known to vary with base sequence,[17,22] the use of a sequence-averaged value does not affect the present results significantly. It is possible to account for these sequence effects explicitly. The local analysis which resulted in Eq. (15) may be amended to use the sequence-averaged bending and torsional stiffnesses within each subsequence of length j. However, the resulting equations require numerical computation to solve. Rigorous analyses based on this approach are very difficult. However, preliminary results of this type indicate that sequence variations of mechanical properties have minimal influence on our conclusions regarding the onset of writhing. Second, unstressed DNA is regarded as being straight. Naturally curved DNA is not explicitly considered. However, the mechanical analysis presented here extends trivially to structures having natural curvature by simply reinterpreting the curvature κ and torsional deformation τ of Eq. (1) as deviations from their unstressed values. Provided the relaxed state is ascribed its natural curvature, the analysis proceeds precisely as before, and all conclusions [especially Eq. (15)] are unchanged.

This analysis demonstrates the existence of two regimes of linking difference, within which the writhed and the unwrithed equilibrium conformations are stable, respectively. In the transition between these regimes the conformational energetics and the partitioning of incremental amounts of linking undergo qualitative changes. This work, in conjunction with a previous study which reached a similar conclusion concerning the onset of superhelical transitions of secondary structure,[18] suggest that the energetics of superhelicity is likely to be complex. In regimes where transitions of secondary structure occur, the energetics of residual linking has been shown previously to have a different functional form than elsewhere.

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