The Role of the Stress Resultant in Determining Mechanical Equilibria of Superhelical DNA

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INTRODUCTION

Duplex DNA has the mechanical properties of a hyperelastic symmetric rod.[1,2] Each secondary structure has an unstressed shape, which is usually straight with a helical twist characteristic of the secondary structure involved. Because DNA is hyperelastic, a free energy density W is associated to deformations away from the unstressed conformation. These deformations are expressed in terms of $\kappa(s)$, the curvature at position s along the molecule, and $T(s)$, the local deviation from the unstressed helicity measured in radians per unit length. Because DNA is mechanically symmetric, bending has no preferred direction and the hyperelastic energy density is a function of three variables[3]: $W = W(\kappa, T, s)$.

In most analyses of DNA superhelical tertiary structure the molecule is regarded as linearly elastic, homogeneous, and mechanically symmetric with constant cross section,[2,4] so that W has the form

$$W = \tfrac{1}{2}\left(A\kappa^2(s) + CT^2(s) \right) \tag{1}$$

Here A and C are the bending and torsional stiffnesses of the molecule, respectively, which have fixed values at all positions s. In fact, this quadratic expression is only a lowest order approximation to what is probably a much more complex relationship. The elastic assumptions made in this paper encompass this linear case as well as many others. In particular, nonlinear elastic laws are included in which free energy relations of types other than the quadratic expression above occur. Also, the effective stiffnesses to twisting and bending are permitted to vary with position s. This is known to occur in DNA due to sequence effects and transitions between secondary structures.[5,6] The assumption of mechanical symmetry is probably quite accurate. This is because the shell of hydration, which surrounds the molecule in solution and participates in its motions, imparts an effectively mechanically symmetric cross section to it.[7,8]

When the molecule is deformed away from its unstressed shape, internal forces arise that are resolvable into a torque $\mathbf{M}(s)$ and a stress resultant $\mathbf{N}(s)$ acting at the center of each cross section.[9] In order for the molecule to be in equilibrium, these internal stresses and torques must be in balance. This condition of equilibrium is expressed in the two equations[10]

$$\left(\frac{d\mathbf{N}}{ds} \right)_{\text{space}} + \mathbf{p}(s) = 0 \tag{2a}$$

and

$$\left(\frac{d\mathbf{M}}{ds} \right)_{\text{space}} + \mathbf{t}(s) \times \mathbf{N}(s) + \mathbf{q}(s) = 0 \tag{2b}$$

Here $\mathbf{p}$ and $\mathbf{q}$ are the imposed force and torque per unit length, respectively, while $\mathbf{t}$ is

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the local unit tangent vector at position s pointing in the direction of increasing s. All derivatives here are taken with respect to a fixed set of axes in space. These two equations give the necessary and sufficient conditions for mechanical equilibrium. They state that the distribution of internal stresses is in balance, so that neither local rotational or translational accelerations, nor dynamic deformations, occur. The imposition of constraints limits the family of accessible conformations among which equilibria are sought. But constraints do not alter the conditions expressed by eqs. (2a, b) that must be satisfied in order for a conformation to be at equilibrium.

If there are no externally imposed forces or torques, then the equilibrium condition on the stress resultant implies that $N(s)$ is a constant vector in the space coordinate system,

$$\left(\frac{d\mathbf{N}}{ds}\right)_{\text{space}} = \mathbf{0} \tag{3a}$$

while $M(s)$ obeys

$$\left(\frac{d\mathbf{M}}{ds}\right)_{\text{space}} + \mathbf{t}(s) \times \mathbf{N} = \mathbf{0} \tag{3b}$$

These conditions for equilibrium may be expressed in terms of the analogous action S,[3] which is given by

$$S = K - V = \int_0^L W[\kappa(s), T(s), s] \, ds - \int_0^L \mathbf{N} \cdot \mathbf{t}(s) \, ds \tag{4}$$

where L is the length of the molecule. The elastic deformation strain free energy is K, the integral of the hyperelastic energy density W. The other integral is V, the analogous potential energy arising from the stress resultant N. The action S, as the difference between two energies, is itself an energy. Mechanical equilibria occur in conformations in which S is extremal, within the collection of all conformations consistent with the imposed constraints. Stable equilibria occur when the governing action S is minimized.

Superhelical DNA is constrained by the constancy of its linking number, and usually also by smooth closure into a topologically circular molecule. In the absence of self-contacts or externally imposed forces, stable superhelical equilibria are the conformations of least action within the family of all (usually smoothly closed) conformations having a fixed value of the linking number.[8]

Some analyses of superhelical tertiary structure posit that equilibria occur in conformations that extremize the elastic deformation strain free energy K consistent with constraints, while the potential energy V is not considered.[11-13] Because it is the extrema of the action that correctly determine equilibria, this is equivalent to implicitly assuming, not just that $V = 0$ in all configurations so that $S = K$ overall, but also that the integrand $\mathbf{N} \cdot \mathbf{t}(s)$ of the analogous potential energy vanishes identically at all points in all accessible conformations. The reason for this local condition is that, for the structure as a whole to be in equilibrium, every piece of it must be in equilibrium. The same condition that governs equilibrium for the whole domain must also hold in every part of the domain. Thus, for equilibrium to be determined by extremals of the deformation strain energy K, $dV = \mathbf{N} \cdot \mathbf{t}(s) \, ds$ must integrate to zero over every connected subset of the domain, which can happen only if $\mathbf{N} \cdot \mathbf{t}(s) = 0$. This can occur in either of two ways: Either N is perpendicular to the unit tangent vector everywhere in all accessible conformations, or $N = 0$. The former case requires that all conformations accessible to the molecule are planar, which does not occur in

problems of interest related to the tertiary structure of superhelical DNA. Hence the assumption that equilibria occur at extremals of K requires that $\mathbf{N} = \mathbf{0}$. In this communication we investigate the circumstances under which this assumption is correct.

MECHANICAL EQUILIBRIA IN THE FORCE-FREE CASE

The analysis of mechanical equilibria requires that a local body system of coordinates be associated to each position s on the axis of the rod. While in other circumstances it is appropriate to choose these to be the principal axes of the rod, in this case calculations are facilitated if the Frenet trihedron is chosen as the local coordinate system.[14,15] These are the mutually perpendicular triad of unit vectors $[\mathbf{t}(s), \mathbf{n}(s), \mathbf{b}(s)]$, the unit tangent, normal, and binormal vectors of differential geometry. The relationship between derivatives computed in the fixed space axes and those found using the local system associated to each position s is expressed by [16]

$$\left(\frac{d\mathbf{V}}{ds} \right)_{\text{space}} = \dot{\mathbf{V}} + \omega \times \mathbf{V} \tag{5a}$$

where $\mathbf{V}$ is any vector-valued function of s and the dot denotes differentiation with respect to s in the local body coordinate system. For the choice of the Frenet trihedron as the local system of axes, the vector ω expressing the local rotation of the body system relative to the space system is the Darboux vector,[15] which is

$$\omega = \tau(s)\mathbf{t}(s) + \kappa(s)\mathbf{b}(s) \tag{5b}$$

where $\tau(s)$ and $\kappa(s)$ are the differential-geometric torsion and curvature at position s, respectively.

When $\mathbf{N} = \mathbf{0}$ Eq. (3b), expressing the equilibrium condition on the torque, becomes

$$\left(\frac{d\mathbf{M}}{ds} \right)_{\text{space}} = \mathbf{0} \tag{6}$$

so the torque vector $\mathbf{M}(s)$ is fixed in magnitude and direction in space. Using Eq. (5a), this equilibrium condition is expressed in terms of the local body coordinate system as

$$\dot{\mathbf{M}} = \mathbf{M} \times \omega \tag{7}$$

We decompose $\mathbf{M}$ into its components in the local coordinate system:

$$\mathbf{M} = m_t\mathbf{t}(s) + m_n\mathbf{n}(s) + m_b\mathbf{b}(s) \tag{8}$$

Then

$$\mathbf{M} \times \omega = \kappa(s)m_n\mathbf{t}(s) + \left[\tau(s)m_b - \kappa(s)m_t \right]\mathbf{n}(s) - \tau(s)m_n\mathbf{b}(s) \tag{9}$$

Inserting this expression into Eq. (7), expressing the equilibrium condition on the torque in the force-free case and equating coefficients gives three equations:

$$\dot{m}_t = \kappa(s)m_n \tag{10a}$$

$$\dot{m}_n = \tau(s)m_b - \kappa(s)m_t \tag{10b}$$

$$\dot{m}_b = -\tau(s)m_n \tag{10c}$$

The assumption of mechanical symmetry implies that the torque $\mathbf{M}$ points in a direction orthogonal to the normal vector[3]:

$$\mathbf{M} = \gamma(s)\mathbf{t}(s) + \beta(s)\mathbf{b}(s) \tag{11a}$$

It follows that

$$m_t = \gamma(s) \qquad m_n = 0 \qquad m_b = \beta(s) \tag{11b}$$

Here $\gamma(s)$ is the component of the torque that torsionally deforms the molecule, while $\beta(s)$ is the part that bends it. Using these values, Eqs. (10a–c) become:

$$\dot{\beta} = 0 \qquad \dot{\gamma} = 0 \qquad \beta\tau(s) = \gamma\kappa(s) \tag{12}$$

If $\beta = 0$, then the only deformations are torsional and the rod remains straight. In this case, which is not available to a closed circular DNA molecule, the local system of axes must be reinterpreted as principal axes of the cross section. In all other cases the bending moment β is nonzero at all cross sections, so the curvature $\kappa(s)$ never vanishes. (In particular, the Frenet trihedron is well determined.) The equations in (12) above together imply that the ratio of the differential geometric torsion to the curvature satisfies

$$\frac{\tau(s)}{\kappa(s)} = \frac{\gamma}{\beta} = \text{constant} \tag{13}$$

The only space curves for which the ratio of torsion to curvature is a constant are generalized helices. These are curves for which the tangent vector $\mathbf{t}(s)$ makes a fixed angle with a particular axis in space.[14] The tangent indicatrix of a generalized helix [i.e., the curve on the unit sphere described by the collection of tangent vectors $\mathbf{t}(s)$ when they are all translated to a common origin] lies entirely on a single circle of latitude about the fixed axis.[10] If the rod has homogeneous elastic properties and constant cross section, this curve will be a standard helix. If the angle that $\mathbf{t}(s)$ makes with the distinguished axis of a generalized helix is not $\pi/2$, then the curve cannot be closed.[17] This is because it can penetrate any plane perpendicular to the distinguished axis in one direction only, so it can never turn around to close on itself. Conversely, if this angle is $\pi/2$, then the curve lies entirely within one plane perpendicular to the space axis.

SUPERHELICAL EQUILIBRIA OF CLOSED CIRCULAR DNA

If follows from the analysis presented above that the only equilibrium structures available to a closed circular molecule in the force-free case are planar, and hence have $Wr = 0$. Moreover, the differential-geometric torsion of a planar curve is identically zero, so from Eq. (13), $\gamma = 0$. This means that the molecule also experiences no deformations of twist. The relaxed conformation is the only equilibrium structure accessible to a closed circular molecule in which there is no stress resultant, $\mathbf{N} = 0$. The imposition of superhelicity on a closed circular DNA molecule always induces a nonzero stress resultant $\mathbf{N}$, which must be taken into account in the analysis of tertiary structure. The mechanical equilibria of closed circular, superhelically stressed DNA do not occur as extremals of the deformation strain energy K alone.

The mechanical equilibrium conformations accessible to a superhelically constrained closed circular molecule of DNA in the absence of imposed forces and torques all have internal stress resultant $\mathbf{N} \neq \mathbf{0}$ that is constant at every position s. One may choose

the fixed space system of coordinates so that the origin coincides with the center of the cross section at $s = 0$, and the space Z-axis points in the direction of the stress resultant $\mathbf{N}$. With this choice the analogous potential energy V is given by

$$V = \int_0^L \mathbf{N} \cdot \mathbf{t}(s)\, ds = |\mathbf{N}| \int_0^L \cos\theta(s)\, ds \tag{14}$$

where $\theta(s)$ is the Euler angle between the space Z-axis and the local tangent. The Z-coordinate of any position s on the molecular central axis is[10]

$$Z(s) = \int_0^s \cos\theta(s)\, ds \tag{15}$$

In a closed circular molecule $Z(0) = Z(L)$, so that

$$0 = \int_0^L \cos\theta(s)\, ds \tag{16}$$

When this result is inserted into Eq. (14), we see that $V = 0$ in this case. For a closed circular DNA domain in which there are no imposed forces, the analogous action S equals the deformation strain free energy $S = K$ in all equilibrium conformations. But it remains incorrect to conclude that these equilibria occur as extremals of K, as the following example from linear elasticity shows.

When DNA is regarded as being homogeneous, symmetric, and linearly elastic with constant cross section, the following conservation law has been shown to hold at every position s at equilibrium[8, 10]:

$$\frac{dK}{ds} + \frac{dV}{ds} = \tfrac{1}{2}\left(A\kappa^2(s) + CT^2(s)\right) + \mathbf{N} \cdot \mathbf{t}(s) = \text{constant} \tag{17}$$

When a closed circular domain is not relaxed, its stress resultant $\mathbf{N}$ is nonzero. If its tertiary structure is not planar, then $\mathbf{N} \cdot \mathbf{t}(s)$ will vary with s, so neither dK/ds nor dV/ds is constant. If equilibria were to occur at extremals of K, this conservation law would have the form $dK/ds = \text{constant}$. The equilibrium conformations predicted in this case clearly must have different curvatures $\kappa(s)$ and twist deformations $T(s)$—hence different tertiary structures—than when the stress resultant is included. This example shows that, even though the analogous potential energy V vanishes for closed circular molecules at equilibrium, the stress resultant still has an important influence on conformation.[8] The correct analysis of mechanical equilibrium tertiary structure extremizes the action S, which must include the potential energy term V.

INTERWOUND SUPERHELICAL EQUILIBRIA

In the analysis presented in this section, DNA is regarded as having the mechanical properties of a homogeneous, symmetric, linearly elastic rod of constant cross section. The equilibrium conformations available to such a DNA domain constrained by the constancy of its linking number in the absence of imposed forces or torques have been determined previously.[8] It was shown that these equilibria exhibit two orders of superhelicity.[8-10, 18] The tangent indicatrix oscillates approximately sinusoidally between two circles of latitude. [Precisely, $\cos\theta(s)$ oscillates between two extreme values u_1 and u_2 as the square of the Jacobi elliptic function Sn.] In circular domains, one of these orders reduces to the closure of the molecular central axis, so the equilibria approximate toroidal helices. The results of small-angle x-ray scattering experiments

indicate that this is the conformation in which superhelical circular DNA usually is found in solution.[19]

If the molecule is allowed to assume conformations in which self-contacts occur, then another class of equilibria become possible. These are interwound structures, where the duplex winds helically on itself. This is the form of DNA superhelical tertiary structure usually seen in electron micrographs.[20] There is also some x-ray evidence suggesting that the interwound form can occur in solution.[21]

Interwound superhelical conformations are stabilized by forces of self-contact. This fact may be demonstrated informally with macroscopic analogs, or proven rigorously by showing that interwound structures do not satisfy the equations that govern equilibrium in the absence of imposed forces. To verify this conclusion, consider the tangent indicatrix of an interwound superhelix. As the ascending helical portion of this structure is traversed, the tangent indicatrix curve rotates in a circle on the unit sphere a number of times equal to the number of interwound turns present. When the loop joining the ascending and descending portions of the superhelix is encountered, the tangent indicatrix moves to the opposite hemisphere. As the descending part of the interwound structure is traversed, the tangent indicatrix rotates an equal number of times about the symmetrically placed circle, then it closes by returning to the upper hemisphere at the lower loop region. The tangent indicatrix of a branched interwound structure is composed of several parts having this general description, one for each branch. In no interwound conformation does the tangent indicatrix oscillate periodically between two circles of latitude according to the relationship

$$\theta(s) = \cos^{-1}\left[u_1 + (u_2 - u_1)Sn^2(qs)\right] \qquad (18)$$

which is the behavior that occurs at equilibrium in the absence of imposed forces.[8] It follows that, in order for interwound conformations to be equilibria, they must have nonzero imposed force per unit length $\mathbf{p}(s)$ at the sites of self-contact.

To analyze interwound superhelices, these contact forces must be included in the governing equilibrium Eqs. (2a, b). In particular, the internal stress resultant $\mathbf{N}(s)$ of an interwound conformation is not constant at all positions along the domain. Instead, it experiences discontinuous changes at point contacts, and continuous variations within more extensive regions of self-contact. $\mathbf{N}(s)$ is constant in those portions of the domain between points of self-contact, but its value will not be the same in every such region. Because $\mathbf{N}(s)$ is not constant throughout, the analogous potential energy V generally does not vanish in an interwound structure, even within a closed circular domain. Hence, interwound equilibria also do not occur as extremals of the deformation strain energy K. Because forces of self-contact have not been included in the treatments of interwound superhelices that have been published to date,[11-13, 22, 23] a correct analysis of this type of equilibrium tertiary structure has yet to be performed.

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