Geometry and Mechanics of DNA Superhelicity

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Synopsis

This paper analyzes the elastic equilibrium conformations of duplex DNA constrained by the constancy of its molecular linking number, Lk. The DNA is regarded as having the mechanical properties of a homogeneous, linearly elastic substance with symmetric cross section. Integral representations of the writhing number Wr and of Lk are developed, in terms of which the equilibria are given as solutions to an isoperimetric problem. It is shown that the Euler angles defining equilibrium conformations must obey equations identical to those governing unconstrained equilibria. A scaling law is developed stating that molecules supercoiled the same amount ΔLk will have geometrically similar elastic equilibria regardless of their length. Thus, comparisons among molecules of properties related to their large-scale tertiary structure should be referred to differences in ΔLk rather than to their superhelix densities. Specific conditions on the elastic equilibrium conformations are developed that are necessary for ring closure. The equilibrium superhelical conformations accessible to closed-ring molecules are shown to approximate toroidal helices. Questions relating to the stability and nonuniqueness of equilibria are treated briefly. A comparison is made between these toroidal conformations and interwound configurations, which are shown to be stable, although they are not equilibria in the present sense. It is suggested that entropic factors are responsible for favoring the toroidal conformation in solution.

INTRODUCTION

The mechanical properties of duplex DNA are known to be important determinants of its large-scale, tertiary structure. For linear molecules in dilute solution, the effective bending stiffness A (suitably averaged) is linearly related to the molecular persistence length. This parameter in turn measures the scale of the random coil assumed by a segment under conditions where it acts like a wormlike coil.[1] The other mechanical attribute of the duplex that is important in treating large-scale structure, its torsional stiffness C, has been measured by fluorescence depolarization.[2,3] These stiffnesses endow duplex DNA with the mechanical properties of a symmetric, linearly elastic, slender rod. When stresses are imposed on such a segment, the resulting mechanical equilibrium conformations are those that extremize the elastic deformation strain action, consistent with constraints.

As was first suggested by Fuller,[4] this approach may be applied to the treatment of superhelically stressed molecules. In this analysis, the DNA is regarded as having stiffnesses that remain constant along the length of the segment. When a force **N** and torque **M** are applied at one cross sec-

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tion, the resulting stressed equilibrium conformations have been shown to involve two orders of periodicity (i.e., supercoiling).[5,6] Although covalently closed ring molecules were not explicitly considered in that initial treatment, it was noted that the larger order can close into a circle to form a stressed ring.

The physically important constraint on closed circular DNA is the constancy of the molecular linking number Lk, which is not related in any simple way to the forces and torques present. Lk may be continuously partitioned between twisting (Tw = total twist) and bending (through the parameter Wr, the molecular writhing number) according to the equation[4]

$$Lk = Tw + Wr \tag{1}$$

For any fixed value of Lk there are an infinite number of ways of performing this partitioning.

Le Bret[7] has developed an elastic analysis of closed circular molecules that is fundamentally different from the present isoperimetric treatment. Although his methods produced several important results, he lacked specific analytic criteria for ring closure. He relied on numerical simulations in which circular structures were approximated by linear conformations whose ends were sufficiently close in space.

Before proceeding with our analysis, we consider the extent to which actual duplex DNA conforms to the properties ascribed to it. These are that the duplex is mechanically symmetric (i.e., having a single bending stiffness—see Ref. 6) and linearly elastic with bending and torsional stiffnesses that are constant along the length of the molecule. Actual DNA is a highly polymorphic substance. Although mechanical stiffnesses have only been measured for the B-form structure to date, it is reasonable to suppose that these parameters assume different values for different conformations. If a molecule contains multiple conformations, then these stiffnesses will vary accordingly. Although the theory may be modified to accommodate this variation,[6] the present analysis tacitly assumes a uniform secondary structure throughout the molecule. Highly supercoiled DNA is known to experience local transitions to alternative conformations.[8–10] This limits the strict applicability of the present analysis to cases of low superhelix density, where the molecule is known to be in the B-form under physiologically reasonable solution conditions.[11] Although the elastic properties of the B-form may not be strictly linear, the deformations at low superhelix density are sufficiently gradual that the linear contributions dominate. Drew et al.[12] have obtained a crystal structure for a dodecameric segment of B-form DNA. Viewed on a large scale, this structure was uniformly bent in response to crystal forces. If the B-form were substantially nonsymmetric, one would expect bending to occur nonuniformly at sites where it is energetically least costly. Although this evidence is not conclusive, it does suggest that the B-form is approximately symmetric mechanically. One explanation for this symmetry is that or-

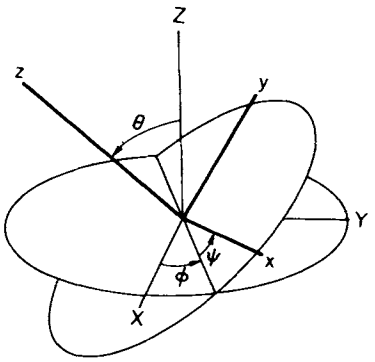

Fig. 1. The three Euler angles θ, ϕ, and ψ. These specify the orientation of the local system of axes $x(s)$, $y(s)$, $z(s)$, defined at each distance s along the rod, relative to fixed space axes X, Y, Z. The local $z(s)$ axis points in the tangent direction to the central axis of the rod.

dered water may fill one or both of the grooves, imparting a more circular cross section to the structure as a whole.

Finally, we note that the analysis of equilibrium properties of superhelical DNA actually involves the minimization of free energy, of which the elastic energy is one contribution. Although the influence of entropic factors is difficult to evaluate with precision, the considerations presented below suggest that they can be important in determining which of several competing stable conformations is favored under given circumstances.

MECHANICAL EQUILIBRIUM THEORY

Geometry of Ring Molecules

Consider a segment of duplex DNA as a slender rod. At each distance s along the molecular central axis, this rod has a perpendicular cross section. A local system of axes $[x(s),y(s),z(s)]$ is found at each distance s by letting $x(s)$ and $y(s)$ be the principal axes of this cross section, with $z(s)$ tangent to the rod in the direction of increasing s. The orientation of this local system relative to fixed-space (X,Y,Z)-axes is given by the Euler angles θ, ϕ, ψ as shown in Fig. 1. As the rod twists and bends θ, ϕ, and ψ vary with the axial distance s. The unit tangent vector $\mathbf{T}(s)$ to the rod may be expressed in the fixed space (X,Y,Z) system as $\mathbf{T}(s) = (\sin\theta \sin\phi, -\sin\theta \cos\phi, \cos\theta)$. Hence, the shape of the central axis curve of the rod in space may be found from the Euler angles to be

$$X(s) = \int_0^s \sin\theta(s) \sin\phi(s)\, ds \tag{2a}$$

$$Y(s) = -\int_0^s \sin\theta(s) \cos\phi(s)\, ds \tag{2b}$$

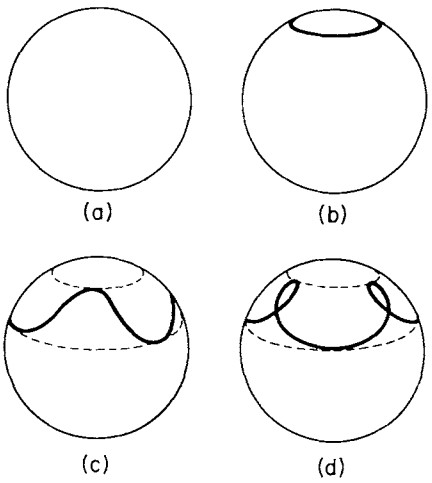

Fig. 2. When the unit tangent vectors along the rod are all translated to a common origin, they trace a curve on the unit sphere called the tangent indicatrix. This figure shows the tangent indicatrices of four curves. Parts (a) and (b) give the indicatrices of a straight line and a helix, respectively. The Euler angle θ is given by the angle of latitude between a point on the sphere and the north pole, while ϕ is the angle of longitude. Parts (c) and (d) show two tangent indicatrices having properties of elastic equilibria. These curves oscillate between parallels of latitude given by $u_1 = \cos\theta_1$ and $u_2 = \cos\theta_2$. The apsidal angle, Φ, is the angle of longitude traversed as the indicatrix passes once from the lower limit curve u_1 to the upper limit curve u_2. In (c), the Euler angle ϕ increases monotonically, while in (d), $\dot{\phi}$ changes sign.

$$Z(s) = \int_0^s \cos\theta(s)\, ds \tag{2c}$$

When the unit tangent vectors $\mathbf{T}(s)$ are translated to a common origin in space, their end points all lie on the unit sphere. The spherical curve that they determine is called the tangent indicatrix of the central axis curve of the rod. For instance, the tangent indicatrix of a straight rod is a single point on the sphere (see Fig. 2.) A helix has a tangent indicatrix that is a circle of latitude. A circle (or any other closed planar curve) has the equatorial great circle for tangent indicatrix. As these examples show, many different curves may be constructed that have the same tangent indicatrix if the parametrization rate is varied. The indicatrix of a space curve is unchanged by the addition of straight segments to the curve, provided this is done so that the tangents patch smoothly. Although a closed smooth curve must have closed indicatrix, the converse is not true. A curve need not be closed just because its tangent indicatrix is, as the example of the helix shows. A spherical curve must intersect *every* great circle for there to exist a closed space curve having that spherical curve for tangent indicatrix.[13] Two closed curves having the same tangent indicatrix need not have identical writhing numbers, although these can only differ by an integer multiple of two.[14]

The writhing number of a smooth closed curve of length L may be computed from its unit tangent vector field $\mathbf{T}(s)$ using the integral representation of Fuller.[14] If the curve $[X(s),Y(s),Z(s)]$, $0 \le s \le L$, can be deformed through a family of nonself-intersecting curves $[X_\zeta(s),Y_\zeta(s),Z_\zeta(s)]$ into the circle of equal length $(k \cos ks, k \sin ks, 0)$ in such a way that the unit tangent vector $\mathbf{T}_\zeta(s) \neq (\sin ks, -\cos ks, 0)$ for any s or ζ, then its writhing number is

$$Wr = \frac{1}{2\pi} \int_0^L \Omega(s)\, ds \tag{3a}$$

where

$$\Omega(s) = \frac{\dot{\phi} \sin\theta \cos\theta \cos(ks - \phi) - k \cos\theta - \dot{\theta} \sin(ks - \phi)}{1 - \sin\theta \cos(ks - \phi)} \tag{3b}$$

Here, $k = 2\pi/L$, L is the total length of the structure, and the dot denotes differentiation with respect to contour length s. The total twist Tw of a segment is found by integrating the local twist rate $2\pi d(Tw)/ds = \dot{\phi} \cos\theta + \dot{\psi}$:

$$Tw = \frac{1}{2\pi} \int_0^L \dot{\phi} \cos\theta + \dot{\psi}\, ds \tag{4}$$

It follows that the linking number Lk may also be expressed as an integral involving Euler's angles and their derivatives:

$$Lk = Tw + Wr = \frac{1}{2\pi} \int_0^L \Lambda(s)\, ds \tag{5a}$$

where

$$\Lambda = \dot{\psi} + \frac{(\dot{\phi} - k) \cos\theta - \dot{\theta} \sin(ks - \phi)}{1 - \sin\theta \cos(ks - \phi)} \tag{5b}$$

We note that these integral expressions for Lk, Tw, and Wr may be constructed regardless of whether the structure is closed. (In the following sections, we choose our local coordinates so that the unstressed structure is untwisted. Thus, the torsional deformation rate will be $\tau = \dot{\phi} \cos\theta + \dot{\psi}$.)

Mechanical Equilibria and Kirchhoff's Analogy

At each distance s along a slender rod, one side of the cross section acts on the other with a system of forces that is resolvable into a force $\mathbf{N}(s)$ and a torque $\mathbf{M}(s)$. In order for the rod to be in equilibrium in the absence of externally imposed force and torque densities along the rod, these vector-valued functions must satisfy the conditions[5]

$$\dot{\mathbf{N}}(s) + \kappa(s) \times \mathbf{N}(s) = 0 \tag{6a}$$

$$\dot{\mathbf{M}}(s) + \kappa(s) \times \mathbf{M}(s) + \mathbf{T}(s) \times \mathbf{N}(s) = 0 \tag{6b}$$

Here, $\kappa(s)$ is the curvature vector, $\mathbf{T}(s)$ is the unit tangent, and all derivatives are taken with respect to distance s.[6] The components of the curvature vector in the local system are $\kappa(s) = [\kappa_x(s),\kappa_y(s),\tau(s)]$, where κ_x and κ_y are the curvatures in the principal directions, while $\tau(s)$ is the torsional deformation rate. (In general, this is the difference between the actual molecular twist rate and its unstressed twist rate.)

Kirchhoff[15] noted that the equations of equilibrium governing a slender rod of uniform cross section are formally analogous to the equations of motion of a top spinning about a fixed point in a gravitational field. That is, the manner in which the local axes of the rod rotate relative to the fixed-space axes as we traverse the rod (increasing s) is governed by equations identical to those describing the rotation of axes fixed in a top as time passes, provided the parameters involved are appropriately identified. The force $\mathbf{N}$ and torque $\mathbf{M}$ on the rod are analogous to the force of gravity and angular momentum vector on the top. The bending and torsional stiffnesses are analogous to the corresponding moments of inertia, while the curvature vector $\kappa(s)$ of the rod corresponds to the angular velocity of the top. The slender rod (resp. top) is symmetric if its bending stiffnesses in the principal x and y directions (resp. moments of inertia I_x, I_y) are equal.

Kirchhoff's kinetic analogy permits problems relating to equilibria of slender rods to be formulated within the framework of Lagrangian mechanics. That is, equilibria correspond to extremals of the analogous action S, with stability at conformations that (locally) minimize this action. For a symmetric, linearly elastic rod, this analogous action, defined as the integral of the governing Lagrangian, is found in terms of Euler's angles to be[6]

$$S = \int_0^L \frac{A}{2}(\dot{\phi}^2 \sin^2\theta + \dot{\theta}^2) + \frac{C}{2}(\dot{\phi}\cos\theta + \dot{\psi})^2 - N\cos\theta\, ds \quad (7)$$

The expression multiplying the bending stiffness A is the squared curvature $(\kappa_x^2 + \kappa_y^2)$, and $N\cos\theta$ is the force component in the tangent direction, which is analogous to the potential energy of the top ($N = |\mathbf{N}|$).

CONSTRAINED EQUILIBRIA

The equilibrium conformations accessible to our slender rod when constrained in some manner are given by the extremals of the action S consistent with the imposed conditions. One important class of constraints involves the imposition of a force $\mathbf{N}(0)$ and a torque $\mathbf{M}(0)$ at one cross section. In this case, the analysis of elastic equilibria reduces to the solution of an initial value problem.[6] Because of Kirchhoff's analogy, this is equivalent to solving for the subsequent motion of a symmetric top spinning in a constant gravitational force field, with conservation of angular momentum and energy. The theory of spinning tops has been studied ex-

tensively.[16–18] In the symmetric case, exact solutions are known. This approach to the analysis of stable conformations of stressed duplex DNA has been developed elsewhere.[5,6]

At present, we study the mechanical equilibria of a closed circular duplex molecule constrained by the constancy of its linking number Lk. These equilibrium conformations are given by the extremals of the action integral [Eq. (7)], restricted to conformations whose linking integral [Eq. (5)] has a particular fixed value. This formulates our question as an isoperimetric problem that may be treated by classical variational techniques.[19]

Briefly, the extremals of one integral,

$$F = \int_0^L f[s,x_1(s),\dot{x}_1(s),\ldots,x_n(s),\dot{x}_n(s)]\,ds \tag{8a}$$

subject to the constancy of another,

$$G = \int_0^L g[s,x_1(s),\dot{x}_1(s),\ldots,x_n(s),\dot{x}_n(s)]\,ds \tag{8b}$$

may be found as follows. To each function $x_i(s)$ appearing in these integrands, there is associated the Euler equation

$$\frac{d}{ds}\left(\frac{\partial h}{\partial \dot{x}_i}\right) - \frac{\partial h}{\partial x_i} = 0 \tag{8c}$$

Here, $h = f + \lambda g$, with λ an introduced Lagrange multiplier. The extremals of this system (which in our case correspond to mechanical equilibrium conformations) are given by the solution of this set of Euler equations. Stable equilibria occur at local minima.

Several important problems may be formulated in this manner. These include the determination of elastic equilibria constrained by the constancy of Lk and (neglecting twist) the evaluation of equilibrium bending deformations subject to the constancy of Wr. As both of these problems yield formally similar solutions, we concentrate here on analyzing cases involving fixed linking number. The results obtained for problems involving Wr will be described elsewhere.

Elastic equilibria occur at conformations yielding extremals of the analogous action S constrained by the constancy of Lk. (In order to minimize the number of constants involved, we formally find extremals of $2S/A$ subject to the constancy of $2\pi Lk$, but this does not alter the problem or its solution.) Using the integral expressions for S and Lk given above, the quantity h appearing in Euler's equation is evaluated to be

$$h = \dot{\phi}^2 \sin^2\theta + \dot{\theta}^2 + \gamma(\dot{\phi}\cos\theta + \dot{\psi})^2 - \eta\cos\theta + \lambda\left[\dot{\psi} + \frac{(\dot{\phi}-k)\cos\theta - \dot{\theta}\sin(ks-\phi)}{1-\sin\theta\cos(ks-\phi)}\right] \tag{9}$$

Here, $\gamma = C/A$ and $\eta = 2N/A$. This expression involves three generalized

coordinates, θ, ϕ, ψ and/or their derivatives, so three corresponding Euler equations result:

$$\frac{d}{ds}\left(\frac{\partial h}{\partial \dot{\theta}}\right) - \frac{\partial h}{\partial \theta} = 0 \tag{10a}$$

$$\frac{d}{ds}\left(\frac{\partial h}{\partial \dot{\phi}}\right) - \frac{\partial h}{\partial \phi} = 0 \tag{10b}$$

$$\frac{d}{ds}\left(\frac{\partial h}{\partial \dot{\psi}}\right) - \frac{\partial h}{\partial \psi} = 0 \tag{10c}$$

For the expression for h given above, these three equations may be computed to be

$$\ddot{\theta} - \dot{\phi}^2 \sin\theta \cos\theta + \gamma\dot{\phi} \sin\theta(\dot{\phi} \cos\theta + \dot{\psi}) - (\eta/2) \sin\theta = 0 \tag{11a}$$

$$\ddot{\phi} \sin^2\theta + 2\dot{\phi}\dot{\theta} \sin\theta \cos\theta - \gamma\dot{\theta} \sin\theta(\dot{\phi} \cos\theta + \dot{\psi}) + \gamma \cos\theta[\ddot{\phi} \cos\theta + \ddot{\psi} - \dot{\phi}\dot{\theta} \sin\theta] = 0 \tag{11b}$$

$$\frac{d}{ds}[2\gamma(\dot{\phi} \cos\theta + \dot{\psi}) + \lambda] = 0 \tag{11c}$$

Because ψ is a cyclic coordinate (that is, $\dot{\psi}$ appears in h but ψ does not), the last equation has a simple form, which integrates directly to

$$\dot{\phi} \cos\theta + \dot{\psi} = \alpha_1 = \text{constant}. \tag{12}$$

Substitution of this expression into the Euler equation for ϕ gives

$$\ddot{\phi} \sin^2\theta + 2\dot{\theta}\dot{\phi} \sin\theta \cos\theta - \gamma\alpha_1\dot{\theta} \sin\theta = 0 \tag{13a}$$

which integrates to

$$\dot{\phi} = (\alpha_2 - \gamma\alpha_1 \cos\theta)/\sin^2\theta \tag{13b}$$

Finally, substitution of these expressions in the Euler equation for θ yields

$$\ddot{\theta} + \frac{\alpha_2 - \gamma\alpha_1 \cos\theta}{\sin^2\theta}\left(\frac{\gamma\alpha_1 - \alpha_2 \cos\theta}{\sin\theta}\right) - \frac{\eta}{2} \sin\theta = 0 \tag{14a}$$

Multiplication of this expression by $\dot{\theta}$ permits direct integration to

$$\frac{\dot{\theta}^2}{2} + \frac{\eta}{2} \cos\theta + \frac{(\alpha_2 - \gamma\alpha_1 \cos\theta)^2}{2 \sin^2\theta} = \alpha_3 \tag{14b}$$

Therefore, the extremals of the analogous action occur for conformations that, when expressed using Euler angles as coordinates, satisfy

$$\dot{\theta}^2 = 2\alpha_3 - \eta \cos\theta - \frac{(\alpha_2 - \gamma\alpha_1 \cos\theta)^2}{\sin^2\theta} \tag{15a}$$

$$\dot{\phi} = \frac{\alpha_2 - \gamma\alpha_1 \cos\theta}{\sin^2\theta} \tag{15b}$$

$$\dot{\psi} = \alpha_1 - \frac{\alpha_2 - \gamma\alpha_1 \cos\theta}{\sin^2\theta} \cos\theta \tag{15c}$$

The first differential equation may be simplified by the substitution $u = \cos\theta$ to become

$$\dot{u}^2 = (2\alpha_3 - \eta u)(1 - u^2) - (\alpha_2 - \gamma\alpha_1 u)^2 \tag{16}$$

The right-hand side of this equation is a cubic in u, with the physically significant range $-1 \leq u = \cos\theta \leq 1$. If there is no force **N**, then $\eta = 0$ and the cubic reduces to the quadratic

$$\dot{u}^2 = 2\alpha_3(1 - u^2) - (\alpha_2 - \gamma\alpha_1 u)^2 \tag{17}$$

Two important conclusions follow from this analysis. First, these equations governing equilibria constrained by constancy of Lk are identical to those previously found assuming instead a constant force and torque at a cross section.[5,6] Thus, all results derived in those previous papers apply directly to the present case. In particular, the solution to this system of differential equations involving elliptic integrals and elliptic functions has been given in Ref. 6. Second, the parameter λ does not appear in these equations. This means that the constancy of the linking number does not explicitly alter the equilibrium conditions. Although this result may seem surprising, reflection shows that it is actually quite reasonable: the linking number is a topological invariant that is not affected by continuous changes of shape. Once Lk is specified, further deformations do not influence it, so it will not appear explicitly in the equilibrium conditions.

We note that the calculations presented in this section do not require the curve involved to be closed.

SCALING

Before treating the solutions to the equilibrium equations, we consider how these conformations depend on the length of the molecule. Consider two molecules of lengths L_1 and L_2, each constrained so that its linking number deviates from the relaxed value by the same amount ΔLk. Then,

$$\Delta Lk = \Delta Tw_1 + Wr_1 = \Delta Tw_2 + Wr_2 \tag{18}$$

Whatever tertiary structure molecule 1 is in, let molecule 2 occur in the geometrically similar conformation. Because the writhing number does not change with dilations,[14] $Wr_1 = Wr_2$ in this case. It follows that $\Delta Tw_1 = \Delta Tw_2$ also. The curvature at any point on molecule 2 is related to the curvature at the corresponding point [i.e., $s_2 = (L_2/L_1)s_1$] on molecule 1 by

$$\kappa(s_2) = (L_1/L_2)\kappa(s_1) \tag{19a}$$

Because the total torsional deformation is the same for both structures, we also have that

$$L_2\tau(s_2) = L_1\tau(s_1) \tag{19b}$$

The action associated with the conformation of molecule i ($i = 1$ or $i = 2$) is

$$S_i = \int_0^{L_i} \left[\frac{A}{2} \kappa^2(s_i) + \frac{C}{2} \tau^2(s_i) - N \cos \theta(s_i) \right] ds_i \tag{20}$$

For a closed circular molecule, it will be shown below that

$$\int_0^{L_i} \cos \theta(s_i)\, ds_i = 0 \tag{21}$$

It follows that the actions associated with these conformations of our two molecules (now assumed to be closed circular) are simply proportional: $S_1/S_2 = L_2/L_1$. Therefore, any shape that gives an extremal of the action S_1 will also yield an extremal of the action S_2 (after dilation).

To summarize, the elastic equilibrium conformations of a molecule depend on superhelicity ΔLk, but *not* on length. This conclusion contrasts with the implicit assumption of many workers in the field, who often display their results as functions of superhelix density. The results described below relating to the onset of superhelical deformations provide an example of this scaling law. (We note in passing that other properties of superhelical molecules, such as the onset of transitions in secondary structure,[8] do scale with superhelix density rather than with ΔLk alone. This is one reason why the formulation of a theory encompassing secondary and tertiary structural elastic deformations and conformational transitions is so difficult.)

SOLUTIONS OF THE EQUILIBRIUM EQUATIONS

Two distinct classes of solutions exist, depending on whether the force **N** is identically zero. If $N \neq 0$, the differential equation for $u = \cos \theta$ [Eq. (16)] contains a cubic expression in u. As this case has been solved in Ref. 6, we simply summarize those results here. In the force-free case $N = 0$, so $\eta = 0$ and the lead coefficient in this cubic vanishes, reducing it to a quadratic [Eq. (17)].

$\boldsymbol{N \neq 0}$

In this case, the differential equation for $u = \cos \theta$ is

$$\begin{aligned} \dot{u}^2 &= (2\alpha_3 - \eta u)(1 - u^2) - (\alpha_2 - \gamma \alpha_1 u)^2 \\ &= \eta \left(u^3 + \left[\frac{-2\alpha_3 - \gamma^2 \alpha_1^2}{\eta} \right] u^2 + \left[\frac{2\gamma \alpha_1 \alpha_2}{\eta} - 1 \right] u \right. \\ &\quad \left. + \frac{2\alpha_3 - \alpha_2^2}{\eta} \right) \\ &= \eta (u - u_1)(u - u_2)(u - u_3) \end{aligned} \tag{22a}$$

where the roots u_1, u_2, and u_3 are all real and satisfy

$$-1 \leq u_1 \leq u_2 \leq 1 < u_3 \tag{22b}$$

The physical range of motion is confined to the interval $u_1 \leq u \leq u_2$ (which is where the cubic is nonnegative, so that $\dot{u}$ is real). The solution to this differential equation has been shown previously to be[6]

$$u = \cos\theta = u_1 + (u_2 - u_1)sn^2\left[\frac{s[\eta(u_3 - u_1)]^{1/2}}{2} + \frac{Q(z_0)}{2}\right] \tag{23}$$

If we choose the point $s = 0$ to occur where $u = u_1$, then the initial value $Q(z_0)/2 = 0$. This solution may be substituted into the differential equations for ϕ and ψ, which then are solved by quadratures. The solution for $\phi(s)$ is given in Ref. 6.

The primary conclusion of this analysis is that the equilibrium conformations involve two orders of periodicity if the roots satisfy $u_1 \neq u_2$. The functions expressing this solution are not trigonometric, but instead involve Jacobi elliptic functions and elliptic integrals. The change in ϕ experienced during one semiperiod of θ is called the apsidal angle, hereafter denoted by Φ.

For a closed structure, both θ and ϕ must undergo integral numbers of periods, n_θ and n_ϕ. In this case, the apsidal angle is given by $\Phi = \pi n_\phi / n_\theta$. The equilibrium conformation resembles a toroidal helix that winds n_θ times around the thickness of the torus and n_ϕ times around the central hole. According to the criterion of Ref. 13, a necessary condition for ring closure is that the tangent indicatrix intersects every closed hemisphere. It follows that $u_1 \leq 0$ and $u_2 \geq 0$ are required. (This criterion will be considerably sharpened below.)

Force-Free Case, $N = 0$

Now the differential equation for $u = \cos\theta$ is

$$\begin{aligned}\dot{u}^2 &= 2\alpha_3(1 - u^2) - (\alpha_2 - \gamma\alpha_1 u)^2 \\ &= au^2 + bu + c\end{aligned} \tag{24a}$$

where

$$a = -2\alpha_3 - \gamma^2\alpha_1^2 \tag{24b}$$

$$b = 2\alpha_1\alpha_2\gamma \tag{24c}$$

$$c = 2\alpha_3 - \alpha_2^2 \tag{24d}$$

In order that $\dot{u}$ be real, $\dot{u}^2$ must be nonnegative. It follows that $\alpha_3 \geq 0$ must occur. If $\alpha_3 = 0$, then the quadratic expression in Eq. (24a) never assumes positive values, so $\dot{u} = 0$ at $u = \alpha_2/\gamma\alpha_1$ is the only physical solution. It follows that $\dot{\phi} = 0$ and $\dot{\psi} = \alpha_1$. Therefore, when $\alpha_3 = 0$, the solution is a straight conformation that twists at a constant rate.

We now consider the case where $\alpha_3 > 0$. The discriminant of Eq. (24a) is

$$\delta = b^2 - 4ac = 8\alpha_3(2\alpha_3 - \alpha_2^2 + \alpha_1^2\gamma^2) \tag{25}$$

If $\delta = 0$, then the quadratic portion of Eq. (24a) is nonpositive, so $\dot{u} = 0$ is the only physical solution. The constant value of u is $u = \cos\theta = \alpha_1\alpha_2\gamma/(2\alpha_3 + \gamma^2\alpha_1^2)$.

Finally, if $\delta > 0$, the conformation assumes a range of values of u. Equation (24a) may be integrated using the relationship[20]

$$\int \frac{du}{(au^2 + bu + c)^{1/2}} = \frac{-1}{(-a)^{1/2}} \sin^{-1}\left(\frac{2au + b}{(b^2 - 4ac)^{1/2}}\right) \tag{26}$$

The solution is

$$u = \frac{-b}{2a} - \frac{(b^2 - 4ac)^{1/2}}{2a} \sin[(-a)^{1/2}(s - Q)] \tag{27a}$$

If $u = u_1$ when $s = 0$, then

$$Q = \sin^{-1}\left(\frac{2au_1 + b}{(b^2 - 4ac)^{1/2}}\right) \Big/ (-a)^{1/2} \tag{27b}$$

In this case, $u = \cos\theta$ varies sinusoidally with distance s along the structure. The differential equations for ϕ and ψ may be integrated analytically after substitution of this expression for $u = \cos\theta$. In particular, the Euler angle ϕ is given by

$$\phi(S) = \left(\frac{E}{T} - \frac{FG}{TH}\right) \frac{2}{(G^2 - H^2)^{1/2}} \tan^{-1}\left[\frac{G\tan(T(s - Q)/2) + H}{(G^2 - H^2)^{1/2}}\right] + \left(\frac{E}{T} + \frac{FL}{TH}\right) \frac{2}{\sqrt{L^2 - H^2}} \tan^{-1}\left[\frac{L\tan(T(s - Q)/2) - H}{(L^2 - H^2)^{1/2}}\right] \tag{28}$$

where

$$E = \alpha_2/2 + \gamma\alpha_1 b/4a, \quad F = \delta\gamma\alpha_1/4a$$
$$T = \sqrt{-a}, \quad G = 1 + b/2a$$
$$H = \delta/2a, \quad L = 1 - b/2a$$

and a, b, and δ are given above.

RING CLOSURE

The solutions presented in the previous section do not require that the structure be closed circular. We now consider the constraints imposed by the condition of ring closure.

Once the equations of equilibrium are solved so that the Euler angles $\theta(s)$, $\phi(s)$, and $\psi(s)$ are known as functions of position s along the molecule, one may determine the equilibrium tertiary structure in terms of fixed coordinates $[X(s), Y(s), Z(s)]$ using Eqs. (2a)–(2c) above. For the molecule to

be smoothly closed into a circle, it is necessary that $X(0) = X(L)$, $Y(0) = Y(L)$, $Z(0) = Z(L)$, and that the tangent indicatrix also be smoothly closed. The latter condition requires that both θ and ϕ experience integral numbers of periods with $\theta(0) = \theta(L)$ and $\phi(0) = \phi(L)$. One is not free to choose n_θ and n_ϕ independently, for the apsidal angle $\Phi = \pi n_\phi / n_\theta$ is determined by the solution of Eqs. (15a)–(15c). The requirement that $Z(0) = Z(L)$ implies that

$$Z(L) - Z(0) = \int_0^L \cos\theta(s)\, ds = 0 \tag{29}$$

It follows that the term $N \cos\theta$ appearing in the action, which is analogous to the potential energy of the top, makes no contribution to the action of a closed structure. In this case, the action integral becomes the total elastic deformation strain energy associated to the structure. (In general, one cannot delete the force term $N \cos\theta$ from this analysis, however, despite the fact that it does not contribute to the integral being extremized. The force-free situation represents a special case.)

Now we consider the requirements that $\theta(0) = \theta(L)$ while θ experiences n_θ complete periods as the structure is traversed. We treat the general case first, where the expression for $u = \cos\theta$ at equilibrium is found from Eq. (23) to be

$$u(s) = u_1 + (u_2 - u_1)sn^2\,[s(\eta(u_3 - u_1))^{1/2}/2] \tag{30a}$$

There are two ways in which $u(0) = u(L)$ can occur. First, if $u_1 = u_2$, then $u = \cos\theta$ has a constant value everywhere. In this case, the only closed equilibrium structure is circular, for which $\cos\theta = 0$. Alternatively, one could have that

$$sn[L(\eta(u_3 - u_1))^{1/2}]/2 = sn(0) = 0 \tag{30b}$$

so that

$$L[(\eta(u_3 - u_1))]^{1/2}/2 = 2Kj \tag{30c}$$

where j $(= n_\theta)$ is an integer and K is the real quarter-period of the Jacobi elliptic function sn.

Next, consider the condition expressed by Eq. (29). Substitution of the expression for $u = \cos\theta$ into this integral gives[20]

$$0 = \int_0^L u_1 + (u_2 - u_1)sn^2\left(\frac{2Kjs}{L}\right) ds$$

$$= u_1 L + \frac{(u_2 - u_1)L}{2Kj}\left(\frac{-E(2Kj)}{m} + \frac{2Kj}{m}\right) \tag{31a}$$

Here, $E(t)$ is the incomplete elliptic integral of the second kind with parameter $m = (u_2 - u_1)/(u_3 - u_1)$. From this expression it follows that

$$u_2/u_1 = 1 + mK/(E - K) \tag{31b}$$

is a necessary condition for ring closure, where E is the complete elliptic integral of the second kind. Because both E and K depend on the parameter $m = (u_2 - u_1)/(u_3 - u_1)$, and the roots u_1, u_2, and u_3 are themselves functions of the parameters η, γ, and the constants of integration α_i [see Eq. (22a)], it follows that Eq. (31b) expresses a relationship among the quantities α_i, γ, η whose satisfaction is necessary for ring closure. Using descending Landen transformations, one can show that $mK/(E - K) < -1$, so that u_1 and u_2 have opposite signs as required by the closure condition of Ref. 13. (This result follows from formula 17.5.10 of Ref. 20.)

In the force-free case, $u = \cos\theta$ varies sinusoidally along the structure, as shown by Eq. (27a). If u experiences n_θ complete periods, it follows that

$$(-a)^{1/2} = 2\pi n_\theta / L \tag{32a}$$

As before, the condition $Z(L) = Z(0)$ requires that

$$0 = \int_0^L u \, ds = -b \frac{L}{2a} \tag{32b}$$

Therefore, a necessary condition for ring closure in this case is $b = 2\alpha_1\alpha_2\gamma = 0$. Consequently, the roots u_1 and u_2 satisfy $u_2 = -u_1$, so that the tangent indicatrix oscillates between symmetric parallels of latitude. This condition for closure also constitutes a restriction on the constants of integration α_i, that either $\alpha_1 = 0$ or $\alpha_2 = 0$. If $\alpha_1 = 0$, then there is no torsional deformation (as $\alpha_1 = \tau$).

Two conditions have been shown to be necessary for ring closure. These are given by a relationship among the roots u_i (and hence also among the constants of integration α_i) and the requirement that the tangent indicatrix be smoothly closed so that both θ and ϕ experience an integral number of periods. We conjecture that these conditions are also sufficient for closure. (In general, the task of proving a curve is closed, given only that it satisfies a certain differential equation, is very difficult.[21]) Note that these conditions may be viewed as pertaining to the tangent indicatrix of the curve, rather than to the curve itself.

Unfortunately, specification of the tangent indicatrix of a smoothly closed, elastic equilibrium conformation does not uniquely determine the conformation. To see this, consider a case where $u_1 = u_2 = 0$, $n_\phi = 3$, so that the tangent indicatrix winds three times around the equator of the sphere before closing. Three distinct curves can have this tangent indicatrix, depending on how the closure is performed. These curves have writhing numbers $Wr = 2, 0, -2$, depending on how the projected crossings are performed. This example is illustrated in Fig. 3.

MULTIPLICITY AND STABILITY OF EQUILIBRIA

The equations expressing the elastic equilibrium conformations involve three constants of integration α_1, α_2, α_3 in addition to the mechanical parameter $\gamma = C/A$ and (in the general case) the force parameter $\eta = 2N/A$.

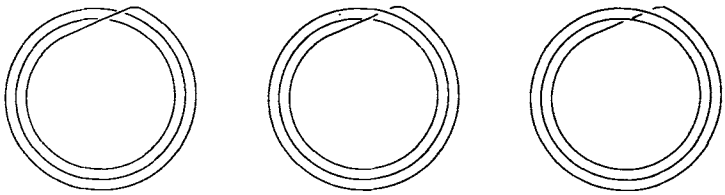

Fig. 3. Three curves are shown, each of which has a tangent indicatrix that passes three times around the equator before closing. The writhing numbers of the curves depend on their relative numbers of overcrossings and undercrossings as shown. As a closed, non-self-intersecting space curve is passed through itself, its writhing number changes by ±2.

These α_i correspond to properties of the conformation involved. Specifically, α_1 determines the twist rate at which the structure is deformed away from its unstressed shape, α_2 gives the analogous momentum conjugate to the cyclic coordinate ϕ in the Lagrangian, while α_3 is related to the deformation strain energy density due to bending. If one fixed the values of these parameters, the equilibrium behavior of the Euler angles $\theta(s)$, $\phi(s)$, and $\psi(s)$ is uniquely determined. To be closed a relationship among these parameters must be satisfied. This is given by Eq. (31b) in the general case, by $\alpha_1\alpha_2 = 0$ in the force-free case. The linking number of a specific closed circular conformation may be computed by integration of Eq. (5) above.

Multiple closed equilibrium conformations may have the same value of the linking number, however. This occurs because Lk can be partitioned continuously between twist and writhe. The total twist is

$$Tw = \frac{1}{2\pi}\int_0^L \dot{\phi}\cos\theta + \dot{\psi}\, ds = \frac{\alpha_1 L}{2\pi} \tag{33}$$

so specification of Tw fixes α_1. This leaves one more degree of freedom in the choice of the constants α_2 and α_3 that may be used to specify Lk. It follows that there are several *equilibrium* conformations having the same value of Lk but differing in their partitioning between Tw and Wr.

One must consider how these multiple equilibria compete. There are several aspects to this question. The equilibria that have been determined in the previous sections represent extremals of the analogous action S. Only those solutions that (locally) minimize S will be stable. If there is more than one stable conformation, one may ask which among them has the lowest elastic energy and what barriers (if any) exist for transitions between the equilibria. We consider an example.

Onset of Nonplanar Bending

Consider the onset of superhelical deformation in a circular molecule that is assumed to be nicked so that the linking number may vary continuously. Let the ends of the nicked strand be held so that one may be wound (or

unwound) about the helix axis relative to the other. As this winding angle is varied from its relaxed value, one may ask what conformation the structure assumes at equilibrium. When relaxed, the structure of lowest energy is geometrically circular with no torsional deformation, $\tau = 0$. As the strands are wound, a critical value of τ will be encountered at which the structure begins to bend out of its plane. Le Bret[7] has calculated that the nonplanar bending must occur if

$$|\Delta Tw| > 3^{1/2}A/C \tag{34}$$

For a mechanically symmetric elastic rod, the ratio of the bending and torsional stiffnesses is related to its Poisson ratio σ by[22]

$$A/C = 1 + \sigma \tag{35}$$

As σ is a mechanical parameter whose value satisfies $0 < \sigma < 0.5$,[23] it follows that the circular conformation must buckle when twisted an amount between 1.7 and 2.5 turns. This threshold value does not depend on either the thickness or the length of the rod involved. (The invariance with length illustrates the scaling law enunciated above.) Perhaps more surprisingly, the threshold also does not depend on the Young's modulus of the material.

Apsidal Angles

In order to be closed, the apsidal angle Φ of a conformation must be a rational fraction of 2π. The multiplicity of conformations that are possible under given circumstances obviously depends on the range of values that Φ can assume. Diaz and Metcalf[24] have shown that this range depends critically on the ratio $r = \alpha_2/\gamma\alpha_1 = \alpha_2/\gamma\tau$. If $r \geq 1$, then Eq. (15b) shows that $\dot{\phi}$ never changes sign, so that ϕ varies monotonically along the structure. In this case, $\Phi \geq \pi/2$ necessarily,[24] so that the only possible equilibrium conformations have either $n_\phi > 1$ or $n_\phi = 1$ with $n_\theta = 2$. If $n_\phi > 1$, then the conformation approximates a toroidal helix that wraps several times around the central hole of the torus. For example, the structures of Fig. 3 have $n_\phi = 3$. The nonplanar conformation of the infinitesimally warped circle has $n_\phi = 1$ and $n_\theta = 2$.

If the structure is sufficiently stressed that $\gamma\tau > \alpha_2$ (i.e., $r < 1$ in the notation of Ref. 24), then the apsidal angle may have any value in the range $0 < \Phi \leq \pi/2$. It follows that closed conformations can occur that approximate toroidal helices winding any integer number of times (>1) around the thickness of the torus while passing once around the hole.

NONEQUILIBRIUM CONFORMATIONS

There is another class of structures that a superhelical molecule can assume that are not solutions to the equilibrium equations. These are typified by the interwound conformation of Fig. 4. If this structure has a total of N turns, then its writhing number is $Wr \simeq N$, the approximation getting

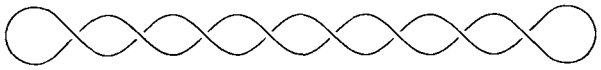

Fig. 4. A prototypical interwound configuration. As the radius of the helical regions decreases, its curvature (and hence its elastic energy of deformation) decreases, other factors remain fixed. This is why self-contacting interwound configurations are stable.

better as the radius decreases.[4] The curvature of this helix is $\kappa = 4\pi^2 r/\lambda$, where r is the radius and λ is the length of one turn of the helix. The bending energy associated to this structure is

$$E = E(\text{ends}) + 8\pi^4 NAr^2/\lambda \tag{36}$$

where $E(\text{ends})$ is the energy associated with the ends. If we hold N and λ fixed while decreasing r, then $E(\text{ends})$ is approximately constant and $E \to E(\text{ends})$ as $r \to 0$.

It follows that a molecule of any fixed superhelicity ΔLk may assume an interwound conformation in which $\Delta Lk = Wr = N$ and $\Delta Tw = 0$. The only elastic energy associated with such a structure arises from bending, as there is no torsional deformation. If the radius of the interwound helix is very small relative to its pitch, then this energy is $E \simeq E(\text{ends})$. In the limit $r \to 0$, $E = E(\text{ends})$, so any amount of superhelicity may be absorbed at essentially the same energetic cost.

As a DNA molecule has finite thickness, the radius of an interwound helix can only decrease until self-contact occurs. Further shrinkage is opposed by the contact forces that arise, resulting in deformations of the cross section.[25] Although this conformation is stable, it is not a solution to the equilibrium equations presented above because the latter do not take into account forces of self-contact.

Under appropriate circumstances, this class of interwound conformations could be energetically favored over the equilibrium structures of the previous sections. For once an interwound supercoil is formed, it may absorb incremental superhelicity at little energetic cost (provided r/p remains small). In contrast, a highly supercoiled toroidal structure of the type analyzed above must be either substantially bent or twisted (or both). This suggests that sufficiently supercoiled elastic structures should occur in the interwound form in preference to the alternatives. Indeed, this is the behavior exhibited by macroscopic analogs such as torsionally deformed rubber hoses or telephone cords. Superhelical molecules viewed by electron microscopy do occur in the interwound form. However, this need not be representative of their conformation in solution. The observed interwound structure could be an artifact of the drastic procedures involved in the preparation of samples.

COMPARISON WITH EXPERIMENT

This paper presents a theoretical analysis of stressed equilibria in closed circular molecules constrained by the constancy of their linking number, Lk. We have shown that a competition exists between elastic equilibria

approximating toroidal helices and alternative interwound configurations. Recently, natively supercoiled COP608 plasmic DNA in solution has been shown to scatter x-rays in a manner consistent with a toroidal conformation but *not* with an interwound one.[26] The reason why the toroidal structure is preferred probably arises from entropic contributions to the governing free energy. Unfortunately, the entropy associated with a superhelical conformation is difficult to estimate with any accuracy. The molecule clearly does not act as a wormlike coil or a sequence of jointed segments. The constancy of Lk is a global constraint on a closed molecule, so different regions are not free to move independently. Further, twisting and bending are mechanically coupled through Eq. (1), so fluctuations in either may affect the other. These factors complicate the analysis of the entropic contributions to superhelical free energies beyond the scope of the present paper. However, qualitative estimates may be made based on considerations of scale.

We assume that the entropy associated with a toroidal conformation is given by an expression analogous to the entropy of an unconstrained loop,[27]

$$S = k(aL - c \ln L + b) \tag{37}$$

which scales approximately linearly with loop length L. As shown above, the elastic equilibria of two molecules having the same ΔLk will be geometrically similar. Therefore, the mechanical energy associated with a given superhelical conformation scales as $1/L$, decreasing as the length of the molecule increases. (This is because the curvature κ and torsional deformation τ both scale as $1/L$—see Scaling.) One sees that the free energy of a toroidal conformation becomes increasingly favorable with molecular length, other factors remaining fixed. If the mechanical energy of the toroidal configuration is small (because L is large), then the mechanical energy of the interwound conformation, although possibly less, cannot be much less because it must be positive. Therefore, the entropic terms become relatively significant in the comparison of free energies. In the interwound conformation, those portions of the molecule that are helically wound are held relatively rigid; hence, they would be expected to contribute little to the entropy regardless of their lengths. For this reason, entropic factors favor the toroidal conformation over the interwound.

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