A Theory of Thermal Fluctuations in DNA Miniplasmids

Irwin Tobias
Department of Chemistry, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854 USA

ABSTRACT A recent analysis of the normal modes of vibration of a ring formed by bringing together and sealing, with or without the addition of twist, the ends of rods that are straight when stress free is taken as the basis for a theory of the statistical thermodynamics of a canonical ensemble of DNA minicircles with specified linking number difference ΔL_k and number N of base pairs. It is assumed that N corresponds to a circumference in the range of one or two persistence lengths. For such an ensemble, the theory yields an expression for the average writhe $\langle W_r \rangle$, which can be employed to calculate the free energy, entropy, and enthalpy of supercoiling, ΔG_{sc}, ΔS_{sc}, and ΔH_{sc}. The results obtained for the dependence of ΔG_{sc} on ΔL_k and N are in accord with experimental observations of equilibrium distributions of topoisomers of plasmids with $N \approx$ 200 bp.

INTRODUCTION

Colleagues and I recently developed a theory of small-amplitude vibrations of circular elastic rings formed from naturally straight, inextensible, isotropic, elastic rods, with the possible addition of twist (Coleman et al., 1996). Here the methods of classical statistical mechanics are applied to the normal modes of vibration of such rings to obtain an expression for the free energy of a ring as a function of its linking number difference. This free energy of supercoiling ΔG_{sc} is the quantity measured in experiments on thermally fluctuating nicked DNA plasmids (Pulleyblank et al., 1975; Depew and Wang, 1975; Shore and Baldwin, 1983; Horowitz and Wang, 1984). In so far as a DNA plasmid behaves like an elastic ring, our results are relevant to those experiments. Because we treat only the case of small deviations from circularity, application of the results we present is limited to plasmids having ~200 bp and linking differences of magnitude not in excess of one. Measurements on such miniplasmids have been made by Shore and Baldwin (1983) and by Horowitz and Wang (1984). These experiments have inspired theoretical work by Shimada and Yamakawa (1984, 1985), who gave an analytical treatment based on an expression for ring-closure probability, and by Frank-Kamenetskii et al. (1985), who gave a numerical treatment.

In the next section, we assemble the mathematical concepts employed to describe the nearly circular configurations of a straight rod that has been twisted and bent to have its ends joined. A discussion of the dynamical equations of the Kirchhoff-Clebsch theory of rods (see, e.g., Dill, 1992; Coleman et al., 1993), the form they assume upon linearization, and the nature of the normal modes implied by the linearized equations follows. Given these modes, we are able to generate all ring configurations in which the rod axis is slightly deformed from a circle. That makes it possible, once we identify the energy associated with each normal mode, to employ the methods of classical statistical mechanics to determine the canonical ensemble average of any quantity of interest. Here we concentrate on the canonical ensemble average of the writhe $\langle W_r \rangle$ of a DNA topoisomer as a function of its linking difference ΔL_k. We find that for each value of ΔL_k, $\langle W_r \rangle$ is proportional to the absolute temperature and to the number of base pairs N in the plasmid. Our methods permit the derivation of an exact relation between the variance in the writhe, $\langle W_r^2 \rangle - \langle W_r \rangle^2$, and $\partial \langle W_r \rangle / \partial \Delta L_k$, which we use to obtain an explicit formula for $\langle W_r^2 \rangle$ for a plasmid with $\Delta L_k = 0$.

The fact that the free energy, entropy, and enthalpy of supercoiling, ΔG_{sc}, ΔS_{sc}, and ΔH_{sc}, can all be expressed in terms of $\langle W_r \rangle$ enables us to calculate these thermodynamic quantities as functions of ΔL_k. In the last section, we compare the results of our theory with experimental measurements of the free energy of supercoiling of miniplasmids of DNA. We find that the theory is in accord with the assumed quadratic dependence of the free energy on ΔL_k, and agrees with the dependence of ΔG_{sc} on N implied by experimental data obtained by Horowitz and Wang (1984) for plasmids with $N \approx 200$ bp.

THE CONFIGURATION OF NEARLY CIRCULAR RINGS

At each time t the points on the axis of the rod making up the ring form a closed curve $\mathbf{C}(t)$, which we describe by giving the location of its points in space as a function $\mathbf{x}(s, t)$ of t and arc length s. The unit tangent vectors for $\mathbf{C}(t)$ are $\mathbf{t} = \mathbf{t}(s, t) = \partial \mathbf{x} / \partial s \equiv \mathbf{x}_s$. The configuration of the ring at a given time is defined by specifying the axial curve $\mathbf{C}(t)$, and a quantity $\gamma(s, t)$, which we refer to as the *excess twist density*. Imagine that there is imbedded in the rod, at each point along the axis, a vector $\mathbf{d}(s, t)$ perpendicular to the axis. If $\tilde{\mathbf{d}}(s)$ is this vector in the stress-free linear configu-

Received for publication 7 August 1997 and in final form 25 October 1997.
Address reprint requests to Dr. Irwin Tobias, Department of Chemistry, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854. Tel.: 908-445-2622; Fax: 908-445-5958; E-mail: tobias@rutchem.rutgers.edu.

0006-3495/98/05/2545/09 $2.00

ration, then $\gamma(s, t)$ is defined by the function

$$\gamma(s, t) = \frac{1}{2\pi}[\mathbf{d}(s, t) \times \mathbf{d}_s(s, t) \cdot \mathbf{t} - \tilde{\mathbf{d}}(s) \times \tilde{\mathbf{d}}_s(s) \cdot \tilde{\mathbf{t}}], \quad (1)$$

i.e., it is the difference between tangential components of the rate of rotation with respect to movement along **C** of the imbedded vectors in the configuration in question, and this rate of rotation in the stress-free configuration. The integral of the excess twist density over the entire length L of the rod is called the *excess twist* ΔT_w,

$$\Delta T_w = \int_0^L \gamma(s, t)\mathrm{d}s. \quad (2)$$

To define the *linking number difference*, ΔL_k (also called *delta link*), for a closed rod formed from a rod that is straight when stress-free, let us suppose that such a rod has been brought into a configuration in which the axial curve **C** lies in a plane, and that its two end points have been brought together in such a way that $\mathbf{x}(0) = \mathbf{x}(L)$, $\mathbf{t}(0) = \mathbf{t}(L)$, with $\gamma(s) = 0$ for $0 \le s \le L$. If one then rotates the end with $s = L$ through φ turns about $\mathbf{t}(L)$, while continuing to hold the ends of the rod coincident with the tangents there parallel, and then cements the ends together, one produces a closed rod with $\Delta L_k = \varphi$. By convention, ΔL_k is taken to be positive when the rotation of the cross section at $s = L$ appears counterclockwise to an observer toward whom $\mathbf{t}(L)$ is pointing. It follows from a now familiar theorem (White, 1969) that the sum of the excess twist ΔT_w for a configuration, and a quantity called the writhe, W_r, which depends only on the shape of the curve **C** for that configuration, is always equal to ΔL_k,

$$\Delta L_k = \Delta T_w + W_r. \quad (3)$$

The writhe W_r of a closed curve **C**, to which we shall return in a later section, can be defined as the number obtained by averaging, over all orientations of a plane P, the sum of the signed self-crossings occurring in the planar curves resulting from the perpendicular projection of **C** on P (Fuller, 1971). In the case of a DNA plasmid, for ΔL_k to be constant, it must be assumed that the temperature remains constant, and that the electrostatic and chemical environment of the DNA do not change.

The configurations in which the axial curve **C** is a circle $\mathbf{C}^o$, and, in addition, the excess twist density γ^o is constant, i.e., is independent of s, is of particular importance, for each of them is always an equilibrium configuration. We shall refer to them collectively as the *reference configuration*.

When $\gamma(s, t)$ is given, the function $(1/2\pi)[\mathbf{d}(s, t) \times \mathbf{d}_s(s, t) \cdot \mathbf{t}]$ depends on the arbitrary choice of $(1/2\pi)[\tilde{\mathbf{d}}(s) \times \tilde{\mathbf{d}}_s(s) \cdot \tilde{\mathbf{t}}]$. For convenience, we choose the orientation of the vectors $\tilde{\mathbf{d}}(s)$ so that $(1/2\pi)[\tilde{\mathbf{d}}(s) \times \tilde{\mathbf{d}}_s(s) \cdot \tilde{\mathbf{t}}]$ is independent of s. Given that the excess twist density is constant in the reference configuration, and that we have chosen $(1/2\pi)[\tilde{\mathbf{d}}(s) \times \tilde{\mathbf{d}}_s(s) \cdot \tilde{\mathbf{t}}]$ to be constant, it follows that $(1/2\pi)[\mathbf{d}(s) \times \mathbf{d}_s(s) \cdot \mathbf{t}]$ in the reference configuration (call it $(1/2\pi)[\mathbf{d}^o(s) \times \mathbf{d}_s^o(s) \cdot \mathbf{t}^o]$) is also constant. We choose this constant to be zero, i.e., in the reference configuration the **d** vectors do not twist about the axial curve. Then, from Eqs. 1 and 2, we get for ΔT_w^o the value of ΔT_w in the reference configuration,

$$\Delta T_w^o = -\frac{1}{2\pi}\int_0^L (\tilde{\mathbf{d}}(s) \times \tilde{\mathbf{d}}_s(s) \cdot \tilde{\mathbf{t}})\mathrm{d}s, \quad (4)$$

and, because a circle has zero writhe, from Eq. 3,

$$\Delta T_w^o = \Delta L_k. \quad (5)$$

It follows from Eqs. 4 and 5 that the constant value of $\tilde{\mathbf{d}}(s) \times \tilde{\mathbf{d}}_s(s) \cdot \tilde{\mathbf{t}}$ in the stress-free configuration is now fixed at $-\Delta L_k/R$, where R is the radius of $\mathbf{C}^o$.

The linking number difference ΔL_k; the radius R of the elastic ring in the reference configuration; and Ω, the ratio of the coefficient of torsional rigidity C of the rod to its coefficient of flexural rigidity A, completely parameterize the closed elastic rod. We shall be concerned with how the values of these parameters affect the time evolution of the configurations through which the ring passes. It is well known that the elastic rings we are considering have stable reference configurations, as long as $-\sqrt{3}/\Omega < \Delta L_k < \sqrt{3}/\Omega$ (Zajac, 1962). Here we shall only consider rings for which ΔL_k falls within this range.

The motions we treat are such that for all s and t, the differences $\mathbf{x}(s, t) - \mathbf{x}^o(s)$ and $\mathbf{d}(s, t) - \mathbf{d}^o(s)$ are sufficiently small in magnitude to permit linearization about the reference configuration of the governing partial differential equations. We write $\mathbf{u}(s, t)$ for the small deviation $\mathbf{x}(s, t) - \mathbf{x}^o(s)$, at time t, of the rod axis from a place on the circle having an arc length s. It will be convenient to work with the components of $\mathbf{u}(s, t)$, and of other vectors, along the directions of the right-handed Frenet-Serret triad, ($\mathbf{n}^o$, $\mathbf{b}^o$, $\mathbf{t}^o$), the principal normal, binormal, and tangent associated with the reference configuration, unit vectors which are, of course, functions of s, but not of t. We orient the reference configuration so that the plane of the circle is perpendicular to a space-fixed z axis, which then becomes parallel to the binormal $\mathbf{b}^o$. Thus,

$$\mathbf{u} = u^r\mathbf{n}^o + u^z\mathbf{b}^o + u^\theta\mathbf{t}^o. \quad (6)$$

The derivative of $\mathbf{u}(s, t)$ with respect to arc length, $\mathbf{u}_s(s, t)$, in terms of its components, is

$$\mathbf{u}_s = N\mathbf{n}^o + B\mathbf{b}^o + T\mathbf{t}^o, \quad (7)$$

where $N = u_s^r + k^o u^\theta$, $B = u_s^z$, and $T = u_s^\theta - k^o u^r$, because the Frenet-Serret triad obeys the equations

$$\mathbf{t}_s^o = k^o\mathbf{n}^o \qquad \mathbf{n}_s^o = -k^o\mathbf{t}^o \qquad \mathbf{b}_s^o = \mathbf{0}. \quad (8)$$

with $k^o(= 1/R)$ the constant geometric curvature of the circular rod axis in the reference configuration. As $\mathbf{t} = \mathbf{x}_s$,

$$\mathbf{u}_s = \mathbf{t} - \mathbf{t}^o, \quad (9)$$

and it can be shown (Coleman et al., 1996) that there is a vector $\mathbf{w}$ such that, to within terms of order $|\mathbf{w}|^2$,

$$\mathbf{t} - \mathbf{t}^{\mathrm{o}} = \mathbf{w} \times \mathbf{t}^{\mathrm{o}} \tag{10}$$

and

$$\mathbf{d} - \mathbf{d}^{\mathrm{o}} = \mathbf{w} \times \mathbf{d}^{\mathrm{o}}. \tag{11}$$

Equations 9 and 10 imply that the component of $\mathbf{u}_{\mathrm{s}}$ along $\mathbf{t}^{\mathrm{o}}$ vanishes, and by Eq. 7, that

$$u_{\mathrm{s}}^{\theta} - k^{\mathrm{o}} u^{\mathrm{r}} = 0. \tag{12}$$

Equations 7, 9, and 10 imply, further, that $\mathbf{w}$ can be written in the form

$$\mathbf{w} = -B\mathbf{n}^{\mathrm{o}} + N\mathbf{b}^{\mathrm{o}} + \beta\mathbf{t}^{\mathrm{o}}, \tag{13}$$

where $\beta = \mathbf{w} \cdot \mathbf{t}^{\mathrm{o}}$. It can be shown that β, the component of $\mathbf{w}$ along $\mathbf{t}^{\mathrm{o}}$, is a measure, in a certain sense, of a torsion angle, i.e., of the angular rotation of the rod about the tangent to its axial curve. In the present linear theory, a deformation from the reference configuration is characterized by β and $\mathbf{u}$.

EQUATIONS OF MOTION

In the Kirchhoff-Clebsch theory of the dynamics of rods, the equations of motion, expressing balance of linear and angular momentum, are

$$\begin{aligned} \mathbf{F}_{\mathrm{s}} &= \rho S \mathbf{x}_{\mathrm{tt}} \\ \mathbf{M}_{\mathrm{s}} + \mathbf{t} \times \mathbf{F} &= \rho I[\mathbf{d} \times \mathbf{d}_{\mathrm{tt}} + (\mathbf{d} \times \mathbf{t}) \times (\mathbf{d} \times \mathbf{t})_{\mathrm{tt}}], \end{aligned} \tag{14}$$

where ρ is the density of the material of which the rod is composed, S is the cross-sectional area of the rod, I is the moment of inertia of a cross section, and $\mathbf{M}$ and $\mathbf{F}$ are the resultant moments and stresses acting on a cross section. We use units expressed in terms of ρ, S, I, and the coefficient of flexural rigidity A such that $(I/S)^{1/2}$ is the unit of length, $I(\rho/SA)^{1/2}$ is the unit of time, and (SA/I) is the unit of force. (In Appendix A the units of length, frequency, and energy so defined are evaluated for the case of a rod modeling a DNA molecule.) In these units the equations of motion (Eq. 14) become

$$\begin{aligned} \mathbf{F}_{\mathrm{s}} &= \mathbf{x}_{\mathrm{tt}} \\ \mathbf{M}_{\mathrm{s}} + \mathbf{t} \times \mathbf{F} &= \mathbf{d} \times \mathbf{d}_{\mathrm{tt}} + (\mathbf{d} \times \mathbf{t}) \times (\mathbf{d} \times \mathbf{t})_{\mathrm{tt}}. \end{aligned} \tag{15}$$

The moment $\mathbf{M}$ has components only along the binormal $\mathbf{b}$ and the tangent $\mathbf{t}$, and, in the new units, Kirchhoff's constitutive relation for an inextensible, isotropic, elastic rod becomes

$$\begin{aligned} \mathbf{M} = k(s,t)\mathbf{b}(s,t) + \Omega(\mathbf{d}(s,t) \times \mathbf{d}_{\mathrm{s}}(s,t) \\ \cdot \mathbf{t}(s,t) + k^{\mathrm{o}}\Delta L_{\mathrm{k}})\mathbf{t}(s,t), \end{aligned} \tag{16}$$

where $k(s,t)$ is the curvature of the axial curve $\mathbf{C}(t)$, and Ω, defined previously, is the ratio, C/A, of the coefficient of torsional rigidity C of the rod to its coefficient of flexural rigidity A.

Coleman et al. (1996) have shown that the dynamical equations (Eq. 15) can be linearized about an arbitrary equilibrium reference configuration. Here, with a circular reference configuration, they take the form

$$\mathbf{w}_{\mathrm{s}} \times \mathbf{F}^{\mathrm{o}} + \Delta\mathbf{F}_{\mathrm{s}} = \mathbf{u}_{\mathrm{tt}} \tag{17}$$

$$\begin{aligned} \mathbf{w}_{\mathrm{s}} \times \mathbf{M}^{\mathrm{o}} + \Delta\mathbf{M}_{\mathrm{s}} + \mathbf{t}^{\mathrm{o}} \times \Delta\mathbf{F} \\ = \mathbf{n}^{\mathrm{o}} \times (\mathbf{w}_{\mathrm{tt}} \times \mathbf{n}^{\mathrm{o}}) + \mathbf{b}^{\mathrm{o}} \times (\mathbf{w}_{\mathrm{tt}} \times \mathbf{b}^{\mathrm{o}}), \end{aligned} \tag{18}$$

where $\mathbf{F}^{\mathrm{o}}$ and $\mathbf{M}^{\mathrm{o}}$, the stress resultant and the resultant moment in the circular reference configuration are

$$\mathbf{F}^{\mathrm{o}} = \Omega(k^{\mathrm{o}})^2 \Delta L_{\mathrm{k}} \mathbf{b}^{\mathrm{o}}, \tag{19}$$

$$\mathbf{M}^{\mathrm{o}} = k^{\mathrm{o}}\mathbf{b}^{\mathrm{o}} + \Omega k^{\mathrm{o}} \Delta L_{\mathrm{k}} \mathbf{t}^{\mathrm{o}}, \tag{20}$$

and

$$\Delta\mathbf{M} = -(B_{\mathrm{s}} - k^{\mathrm{o}}\beta)\mathbf{n}^{\mathrm{o}} + N_{\mathrm{s}}\mathbf{b}^{\mathrm{o}} + \Omega(\beta_{\mathrm{s}} + k^{\mathrm{o}}B)\mathbf{t}^{\mathrm{o}}. \tag{21}$$

Equation 12, the condition of inextensibility, and Eqs. 17 and 18 the equations to be solved for the seven unknowns, u^{r}, u^{z}, u^{θ}, β, and the three components ΔF^{r}, ΔF^{z}, and ΔF^{θ} of $\mathbf{F} - (\mathbf{F}^{\mathrm{o}} + \mathbf{w} \times \mathbf{F}^{\mathrm{o}})$, the vector denoted by $\Delta\mathbf{F}$ in Eqs. 17 and 18.

The six components along $(\mathbf{n}^{\mathrm{o}}, \mathbf{b}^{\mathrm{o}}, \mathbf{t}^{\mathrm{o}})$ of Eqs. 17 and 18 are

$$\begin{aligned} -\Omega(k^{\mathrm{o}})^2\Delta L_{\mathrm{k}}(\beta_{\mathrm{s}} + k^{\mathrm{o}}B) + \Delta F_{\mathrm{s}}^{\mathrm{r}} + k^{\mathrm{o}}\Delta F^{\theta} &= u_{\mathrm{tt}}^{\mathrm{r}} \\ \Delta F_{\mathrm{s}}^{\mathrm{z}} &= u_{\mathrm{tt}}^{\mathrm{z}} \\ -\Omega(k^{\mathrm{o}})^2\Delta L_{\mathrm{k}}(B_{\mathrm{s}} - k^{\mathrm{o}}\beta) + \Delta F_{\mathrm{s}}^{\theta} - k^{\mathrm{o}}\Delta F^{\mathrm{r}} &= u_{\mathrm{tt}}^{\theta} \\ \Omega k^{\mathrm{o}}\Delta L_{\mathrm{k}} N_{\mathrm{s}} - (B_{\mathrm{ss}} - \Omega k^{\mathrm{o}}\beta_{\mathrm{s}}) - (k^{\mathrm{o}})^2(1-\Omega)B - \Delta F^{\mathrm{z}} &= -B_{\mathrm{tt}} \\ \Omega k^{\mathrm{o}}\Delta L_{\mathrm{k}}(B_{\mathrm{s}} - k^{\mathrm{o}}\beta) + N_{\mathrm{ss}} + \Delta F^{\mathrm{r}} &= N_{\mathrm{tt}} \\ \Omega(\beta_{\mathrm{ss}} + k^{\mathrm{o}}B_{\mathrm{s}}) &= 2\beta_{\mathrm{tt}}. \end{aligned} \tag{22}$$

Equation 12, in effect, determines u^{r} in terms of u^{θ}. We thus need consider only the above six equations after u^{r} has been eliminated from them. One can also eliminate the components of $\Delta\mathbf{F}$ from the equations. We are then left with three equations for the three unknowns, u^{z}, β, and u^{θ},

$$\begin{aligned} (u_{\theta\theta}^{\mathrm{z}} + (1-\Omega)u^{\mathrm{z}})_{\theta\theta} - \Omega\Delta L_{\mathrm{k}}(u_{\theta\theta}^{\theta} + u^{\theta})_{\theta\theta} - R\Omega\beta_{\theta\theta} \\ = R^2(u_{\theta\theta}^{\mathrm{z}} - R^2 u^{\mathrm{z}})_{\mathrm{tt}} \\ \Omega\Delta L_{\mathrm{k}}(u_{\theta\theta}^{\mathrm{z}} + u^{\mathrm{z}})_{\theta\theta} + (u_{\theta\theta\theta\theta}^{\theta} + 2u_{\theta\theta}^{\theta} + u^{\theta})_{\theta\theta} \\ = R^2(u_{\theta\theta\theta\theta}^{\theta} + (2 - R^2)u_{\theta\theta}^{\theta} + (1 + R^2)u^{\theta})_{\mathrm{tt}} \\ \Omega(R\beta_{\theta\theta} + u_{\theta\theta}^{\mathrm{z}}) = 2R^3\beta_{\mathrm{tt}}, \end{aligned} \tag{23}$$

with the angle $\theta = s/R$ replacing the independent variable s.

NORMAL MODES OF VIBRATION OF THE ELASTIC RING

First of all, we make the general observation that when $\Delta L_k = 0$, the motion of the ring in the plane containing the circle $\mathbf{C}^o$, i.e., the (r, θ) plane, is independent of the motion perpendicular to that plane. Twisting the rod, before joining its ends, serves to couple the in-plane and out-of-plane motions. In fact, when $\Delta L_k \neq 0$, there are no modes with $n \geq 2$ that are purely in-plane or out-of-plane vibrations; each of the modes acquires a chiral aspect.

We now seek normal mode solutions of the linear differential equations (Eq. 23). Without loss of generality, we take these solutions to be traveling waves; they are of the form

$$\begin{aligned} u^z &= A^z(\pm)\cos(n\theta \pm (\omega t + \alpha)) \\ u^\theta &= A^\theta(\pm)\cos(n\theta \pm (\omega t + \alpha)) \\ \beta &= A^\beta(\pm)\cos(n\theta \pm (\omega t + \alpha)), \end{aligned} \tag{24}$$

where $A^z(\pm)$, $A^\theta(\pm)$, $A^\beta(\pm)$ are constant amplitudes; α is a constant phase; ω is the frequency; and n is zero or any positive integer related to the wavelength λ by the expression

$$\lambda = 2\pi R/n. \tag{25}$$

Substitution of Eq. 24 into Eq. 23 yields three linear, homogeneous, algebraic equations in the three unknown amplitudes A^z, A^θ, A^β:

$$\begin{aligned} a_{11}A^z + a_{12}A^\theta + a_{13}A^\beta &= 0 \\ a_{21}A^z + a_{22}A^\theta + a_{23}A^\beta &= 0 \\ a_{31}A^z + a_{32}A^\theta + a_{33}A^\beta &= 0. \end{aligned} \tag{26}$$

The coefficients a_{ij} of the unknowns are functions of Ω, R, ΔL_k, ω, and n. They are listed in Appendix B.

The equation obtained when the 3×3 determinant whose elements are a_{ij} is set equal to zero is the dispersion relation, for it can be solved for the frequency ω when Ω, R, ΔL_k, and the wavelength, as given by the integer, n, are specified. In this problem, the determinant of the coefficients turns out to be a cubic polynomial in ω^2, i.e., the dispersion relation is of the form (Coleman et al., 1996; Goriely and Tabor, 1997)

$$M_3\omega^6 + M_2\omega^4 + M_1\omega^2 + M_0 = 0. \tag{27}$$

(Appendix B also shows explicitly the coefficients M_i.)

We only consider cases for which the roots ω^2 of the polynomial of Eq. 27 are positive. Generally, for each mode number n, there are three distinct frequencies ω_{ni}, where $i = 1$, 2, or 3. We establish the convention that $\omega_{n1} < \omega_{n2} < \omega_{n3}$. One can label any mode, therefore, by specifying the two integers (n, i). Each mode so specified is doubly degenerate; the traveling wave can propagate in either of two directions around the circle. These two degenerate modes are distinguished, when necessary, with the $\pm$ notation introduced in Eq. 24.

The cases of $n = 0$ and $n = 1$ are special. When $n = 0$, there is no oscillatory motion, but the ring can show a uniform torsional motion, while the rod axis remains circular, that is, the angle β is independent of s, but can increase or decrease linearly with time. This type of motion, which is excluded from consideration in the present context, because we have limited β to small values, we recently discussed in great detail for the case of a ring made from an intrinsically curved rod when the torsional motion becomes oscillatory (Tobias et al., 1996). There is only one type of mode with $n = 1$: the rod axis still remains circular, but the circle undergoes an oscillatory rocking motion. During this rocking motion, the excess twist density γ is no longer constant—it varies with both arc length and time (Coleman, et al., 1996). However, the excess twist, ΔT_w, remains fixed at $\Delta T_w^o = \Delta L_k$. This mode, like the other modes of smaller wavelength (greater n), is doubly degenerate.

When $n \geq 2$, the modes are flexural: the axial curve no longer remains circular during the motion. The term on the left-hand side of Eq. 27, which is independent of the frequency, is

$$M_0 = -\Omega n^6(n^2 - 1)^2(n^2 - 1 - (\Omega\Delta L_k)^2). \tag{28}$$

We see that when $n = 2$, M_0 approaches zero as $\Omega\Delta L_k$ approaches the critical buckling values of $\pm\sqrt{3}$. Thus one of the three modal frequencies associated with $n = 2$ approaches zero as the linking number difference approaches $\pm\sqrt{3}/\Omega$.

In the case of miniplasmids of DNA, n may not be allowed to increase without limit. There must be a maximum value of n, call it n_m, that corresponds to a wavelength on the order of magnitude of the separation Δs^o between base pairs of the DNA. If the wavelength is set exactly equal to Δs^o, then n_m, this cut-off value of n, equals N, the number of base pairs in the ring. It turns out that our final results are quite insensitive to the precise value chosen for n_m.

Of course, Eq. 24 also determines the relative magnitudes of the amplitudes associated with a given mode (n, i). The amplitudes A_{ni}^θ and A_{ni}^β, in terms of A_{ni}^z, are

$$A_{ni}^\theta = r_{ni}^\theta A_{ni}^z$$

with

$$r_{ni}^\theta = \frac{\Omega\Delta L_k n^2(n^2 - 1)}{n^2(n^2 - 1)^2 - \omega_{ni}^2 R^2((n^2 - 1)^2 + R^2(n^2 + 1))}, \tag{29}$$

and

$$A_{ni}^\beta = r_{ni}^\beta A_{ni}^z$$

with

$$r_{ni}^\beta = \frac{\Omega n^2}{R(2\omega_{ni}^2 R^2 - \Omega n^2)}. \tag{30}$$

It is convenient to classify the three modes for a given wavelength according to the nature of that mode in the limit of zero linking number difference. In that limit, the motion

associated with one of the modes is nontorsional and entirely in the plane of the circular reference configuration, i.e., $A^z = 0$, $A^\beta = 0$, $A^\theta \neq 0$, whereas the other two have a torsional component and are entirely of the out-of-plane type, i.e., $A^z \neq 0$, $A^\beta \neq 0$, $A^\theta = 0$. The two out-of-plane modes further distinguish themselves in that for one, $|A^z| \gg |A^\beta|$, which means that between bending and torsion, bending is predominant, whereas for the other, torsion is predominant, because $|A^\beta| \gg |A^z|$.

ENERGY OF THE DEFORMED ANNULAR ROD

If one integrates along the entire length of the rod the scalar product of Eq. 18 with the vector $\mathbf{w}_t$, and use is made of Eq. 17, it can be shown (M. Lembo, private communication) that for any solution of those equations, a quantity H, defined as follows, is time independent:

$$H = K_e + \Delta V, \tag{31}$$

where

$$K_e = \frac{1}{2}\oint ds[\mathbf{u}_t \cdot \mathbf{u}_t + |\mathbf{w}_t \times \mathbf{b}^o|^2 + |\mathbf{w}_t \times \mathbf{n}^o|^2], \tag{32}$$

and

$$\Delta V = \frac{1}{2}\oint ds[\mathbf{w}_s \cdot (\Delta\mathbf{M} + \mathbf{w} \times \mathbf{M}^o) - (\mathbf{w} \cdot \mathbf{t}^o)(\mathbf{w} \cdot \mathbf{F}^o)]. \tag{33}$$

We can interpret H as the constant energy associated with the time-dependent deformation from the reference configuration. It is the sum of the kinetic energy K_e and the potential energy change ΔV from that, V^o, in the reference configuration, where

$$V^o = \pi k^o + \pi k^o \Omega (\Delta L_k)^2. \tag{34}$$

The first term in V^o is the bending energy in the reference configuration, and the second term is the twist energy there. The total elastic energy E is thus $V^o + H$.

The energies ΔV and K_e, written out in full in terms of the displacements and the torsion angle β, are

$$\Delta V = \frac{1}{2}\oint [(N_s)^2 + (B_s)^2 - (k^o B)^2 - 2\Omega k^o \Delta L_k N B_s + \Omega(\beta_s + k^o B)^2]ds \tag{35}$$

$$K_e = \frac{1}{2}\oint [\mathbf{u}_t \cdot \mathbf{u}_t + (N_t)^2 + (B_t)^2 + 2(\beta_t)^2]ds. \tag{36}$$

The collection of three terms in the total potential energy $V^o + \Delta V$ of the configuration, which are proportional to the ratio Ω, make up the twist energy of the configuration, the remaining terms, the bending energy. The various contributions to the twist energy can be further distinguished. The term $\pi k^o \Omega (\Delta L_k)^2$ in V^o is the twist energy of the reference state. The twist energy term in ΔV that is proportional to ΔL_k arises from that part of the excess twist density that is independent of arc length, whereas the remaining term is associated with that part of the excess twist density that is changing with arc length. (See the next section for further discussion of this point.)

The energy H ($= E - V^o$) associated with an arbitrary deformation from the reference configuration can be expressed, it turns out, as twice the time average of either the potential energy ΔV or the kinetic energy K_e. (For the calculations carried out here, the former was chosen.) In addition, H is in the form of a sum of separate contributions from each normal mode,

$$H = \sum_{n=1}^{n_m}\sum_{i=1}^{3} h_{ni}[(A^z_{ni}(+))^2 + (A^z_{ni}(-))^2], \tag{37}$$

where one can show with the help of Eqs. 24, 29, 30, and 35 that

$$h_{ni} = \frac{\pi n^2}{R^3}[(n^2 - 1) + (n^2 - 1)^2 (r^\theta_{ni})^2 - 2\Omega\Delta L_k (n^2 - 1) r^\theta_{ni} + \Omega(1 + R r^\beta_{ni})^2]. \tag{38}$$

We pointed out above that as $\Omega\Delta L_k$ approaches $\pm\sqrt{3}$, the frequency ω_{21} approaches zero. Here we note that, given the significance of the buckling point, in this same limit, h_{21} must and, it may be readily verified, does, indeed, also approach zero.

WRITHE OF THE DEFORMED ANNULAR ROD

The writhe is a property of a closed curve, here the axial curve $\mathbf{x}(s, t)$ of the ring. We defined it in an earlier section of the paper. It can also be defined as a Gauss integral,

$$W_r(t) = \frac{1}{4\pi}\oint\oint \frac{\mathbf{t}(s, t) \times \mathbf{t}(s', t) \cdot [\mathbf{x}(s, t) - \mathbf{x}(s', t)]}{|\mathbf{x}(s, t) - \mathbf{x}(s', t)|^3} ds\, ds'. \tag{39}$$

In a later section we calculate its ensemble average $\langle W_r \rangle$. To proceed with such a calculation, it will be necessary to express $W_r(t)$ in terms of the variables introduced in the first section. These variables, it will be recalled, lead to expressions correct to terms linear in the vector $\mathbf{w}$. We are immediately faced with a problem, for it can be shown that the first term in an expansion of the writhe about the reference configuration is of order $\mathbf{w}^2$. To find the appropriate second-order expression for the writhe, we examine that part of the total potential energy, $V^o + \Delta V$ (see Eqs. 34 and 35), which

corresponds to the twist energy V_T,

$$V_T = \pi k^o \Omega (\Delta L_k)^2 - \Omega k^o \Delta L_k \oint NB_s ds + \frac{1}{2}\Omega \oint (\beta_s + k^o B)^2 ds. \quad (40)$$

The significance of each term is clear. The first two are the first two terms in the expansion

$$\pi k^o \Omega (\Delta T_w)^2 = \pi k^o \Omega (\Delta L_k - W_r)^2 \approx \pi k^o \Omega (\Delta L_k)^2 - 2\pi k^o \Omega \Delta L_k W_r \quad (41)$$

of the expression for the twist energy of a ring in a configuration with constant excess twist density. The third term in Eq. 40 is the contribution to the energy associated with that part of the excess twist density that varies with arc length. Comparison of Eqs. 40 and 41 implies that

$$W_r = \frac{1}{2\pi} \oint NB_s ds. \quad (42)$$

We have already mentioned that when $\Delta L_k = 0$, each normal mode is either an oscillation purely in the plane of the circle $\mathbf{C}^o$ defined by the reference configuration, or an oscillation purely perpendicular to that plane. It is evident from the expression for the writhe as given in Eq. 42 that during the motion associated with the excitation of any such mode, W_r is always zero.

It will also prove useful to have an expression for the writhe analogous to Eq. 37 for the energy, i.e., in the form of a sum over normal modes. In the case of the energy, each mode makes a time-independent contribution to the total. The situation is a bit more complicated here in that time-dependent cross-terms appear. The equation for the writhe analogous to Eq. 37 is

$$W_r(t) = \frac{1}{2R^2} \sum_{n=2}^{n_m} n^2(n^2-1) \sum_{i,j=1}^{3} r_{nj}^{\theta} \{[A_{ni}^z(+)A_{nj}^z(+) + A_{ni}^z(-)A_{nj}^z(-)]\cos[(\omega_{ni} - \omega_{nj})t + \alpha_{ni} - \alpha_{nj}] + [A_{ni}^z(+)A_{nj}^z(-) + A_{ni}^z(-)A_{nj}^z(+)] \cdot \cos[(\omega_{ni} + \omega_{nj})t + \alpha_{ni} + \alpha_{nj}]\}. \quad (43)$$

THERMAL FLUCTUATIONS: THE ENSEMBLE AVERAGE OF THE WRITHE

We have now assembled the apparatus needed to apply the methods of equilibrium statistical mechanics to a canonical ensemble of elastic rings undergoing small-amplitude vibrations. We are formally dealing here with a collection of linear harmonic oscillators, one for each of the normal modes. Classical, as opposed to quantum equilibrium, mechanics may be used for any mode of frequency ω as long as the ratio $\hbar\omega/k_B T$, where k_B is Boltzmann's constant, $\hbar$ is Planck's constant divided by 2π, and T is the temperature (not to be confused with the symbol T introduced in Eq. 7), is small compared to 1. Because, in the present case, for the mode of highest frequency, found among those with $n = n_m$, this ratio is $\sim$0.2 when $T = 300$ K, we use the classical formalism.

When the energy H, in ordinary units, is expressed as a function of all of the canonical momenta and coordinates, a pair P_{ni}, Q_{ni} for each normal mode, the ensemble average $\langle f \rangle$, at the temperature T, of any function f of these canonical variables is calculated according to the formula

$$\langle f \rangle = \frac{\int\int f(P,Q)\exp[-H(P,Q)/k_B T]\,dP\,dQ}{\int\int \exp[-H(P,Q)/k_B T]\,dP\,dQ}. \quad (44)$$

Although in Eq. 44 we have indicated integration only over a single pair of canonical variables, it is understood that in the actual calculation, one must integrate over all such pairs. The limits of integration depend on the specific choice of P and Q.

When one is dealing with a linear harmonic oscillator, a convenient choice of canonical momentum is one that is proportional to the square of the amplitude of the oscillation. The corresponding coordinate is the phase $\omega t + \alpha$ (see, e.g., Goldstein, 1981). Then the energy depends linearly on P and is independent of Q. The constant of proportionality in the definition of P must be chosen so that $H = \omega P$. Because, as we have already mentioned, the normal modes of the elastic ring are formally identical to a collection of linear harmonic oscillators, the same choice of canonical variables can be made. In terms of these canonical momenta, Eq. 37 becomes

$$H = \sum_{n=1}^{n_m} \sum_{i=1}^{3} \omega_{ni}(P_{ni}(+) + P_{ni}(-)), \quad (45)$$

where

$$P_{ni}(\pm) = \frac{h_{ni}}{\omega_{ni}} (A_{ni}^z(\pm))^2, \quad (46)$$

with h_{ni} as in Eq. 38. As expected, the average energy of each normal mode,

$$\langle \omega_{ni} P_{ni} \rangle = \frac{\int_0^\infty \int_0^{2\pi} \omega_{ni} P_{ni} \exp[-\omega_{ni} P_{ni}/k_B T]\,dP_{ni}\,dQ_{ni}}{\int_0^\infty \int_0^{2\pi} \exp[-\omega_{ni} P_{ni}/k_B T]\,dP_{ni}\,dQ_{ni}}, \quad (47)$$

is $k_B T$.

The dependence of the writhe on the canonical coordinates is seen from Eq. 43 to be

$$W_r(t) = \frac{1}{2R^2}\sum_{n=2}^{n_m}\sum_{i,j=1}^{3} n^2(n^2-1)r_{nj}^{\theta}\{[A_{ni}^{z}(+)A_{nj}^{z}(+) + A_{ni}^{z}(-)A_{nj}^{z}(-)]\cos(Q_{ni}-Q_{nj}) + [A_{ni}^{z}(+)A_{nj}^{z}(-) + A_{ni}^{z}(-)A_{nj}^{z}(+)]\cos(Q_{ni}+Q_{nj})\}. \quad (48)$$

During the computation of the ensemble average of the writhe, one integrates over these coordinates. The result of that integration is zero for many of the terms. In fact, it is not difficult to deduce that after the integration over all of the Q's, Eq. 48 for the writhe is of the form

$$\bar{W}_r = \sum_{n=2}^{n_m}\sum_{i=1}^{3} w_{ni}[(A_{ni}^{z}(+))^2 + (A_{ni}^{z}(-))^2], \quad (49)$$

where

$$w_{ni} = \frac{n^2(n^2-1)}{2R^2}r_{ni}^{\theta}. \quad (50)$$

When the canonical momenta are introduced into Eq. 49, it becomes

$$\bar{W}_r = \sum_{n=2}^{n_m}\sum_{i=1}^{3}(\omega_{ni}w_{ni}/h_{ni})[P_{ni}(+) + P_{ni}(-)]. \quad (51)$$

Finally, after integrating over the momenta, we obtain for the ensemble average of the writhe,

$$\langle W_r\rangle = 2k_BT\sum_{n=2}^{n_m}\sum_{i=1}^{3}\frac{w_{ni}}{h_{ni}}. \quad (52)$$

We find that in addition to being proportional to the absolute temperature, $\langle W_r\rangle$ is also proportional to the radius of the ring R, or, equivalently, to the number of base pairs N in the DNA plasmid. Furthermore, $\langle W_r\rangle$ is an odd function of the product $\Omega\Delta L_k$. It vanishes only when $\Delta L_k = 0$. That is, $\langle W_r\rangle$ is not zero, even when the stable equilibrium state of lowest energy, for the value of ΔL_k considered, is circular, and hence of zero writhe.

When $|\Omega\Delta L_k| \ll 1$, we have found that

$$\langle W_r\rangle = \frac{\sigma(n_m)}{\pi}\frac{k_BT}{A}R\Omega\Delta L_k, \quad (53)$$

where

$$\sigma(n_m) = \sum_{n=2}^{n_m}(n^2-1)^{-1} = \frac{3}{4} - \frac{2n_m+1}{2n_m(n_m+1)}.$$

(See, for example, Jolley, 1925.) Because the ratio A/k_BT is the persistence length a of DNA (~500 Å at room temperature; Hagerman, 1988), we may write Eq. 53 in the form

$$\langle W_r\rangle = \frac{\sigma(n_m)}{2\pi^2}\left(\frac{\Delta s^o}{a}\right)N\Omega\Delta L_k, \quad (54)$$

where $\Delta s^o = 3.4$ Å is the separation between adjacent base pairs.

As $\Omega\Delta L_k$ approaches the critical buckling values of $\pm\sqrt{3}$, h_{21} approaches zero, whereas w_{21} does not vanish. It follows that Eq. 52 predicts that in this limit, $\langle W_r\rangle$ increases without bound, clearly a nonphysical result. This is not unexpected, because the present theory is limited to the treatment of nearly circular configurations, and so cannot be used to describe the buckling phenomenon correctly.

In Fig. 1 we have plotted $\langle W_r\rangle$ versus $\Omega\Delta L_k$ (Eq. 52) for values of $\Omega\Delta L_k$ between -1.6 and $+1.6$ at a temperature of 298 K, with $N = 200$, and with $n_m = 20$. The straight broken line in the figure is a graph of $\langle W_r\rangle$ as given by Eq. 54, which is valid when $|\Omega\Delta L_k| \ll 1$.

In the sum over n in Eq. 52, the terms decrease in magnitude sufficiently rapidly that it matters little whether the maximum value of n, n_m, is chosen to be infinity, or 20, as has been done here, corresponding to a minimum wavelength of 10 Δs^o. When $|\Omega\Delta L_k| \ll 1$, the values of $\langle W_r\rangle$ for these two cases differ by ~7%, given that $\sigma(20) = 0.701$ and $\sigma(\infty) = 3/4$. With increasing values of $\Omega\Delta L_k$, the percentage difference can only be less because then, in the sum over n, the single term with $n = 2$ and $i = 1$ increases in magnitude and dominates.

We also note that it follows from the form of Eq. 44, and from the nature of the dependence of the elastic energy H on ΔL_k, that

$$\langle W_r\rangle = \frac{N}{4\pi^2\Omega}\left(\frac{\Delta s^o}{a}\right)\left(\frac{\partial \ln z}{\partial \Delta L_k}\right), \quad (55)$$

where

$$\ln z = \ln\prod_{n=2}^{n_m}\prod_{i=1}^{3}\left(\int_0^{\infty}\exp[-\omega_{ni}P_{ni}/k_BT]\,dP_{ni}\right)^2.$$

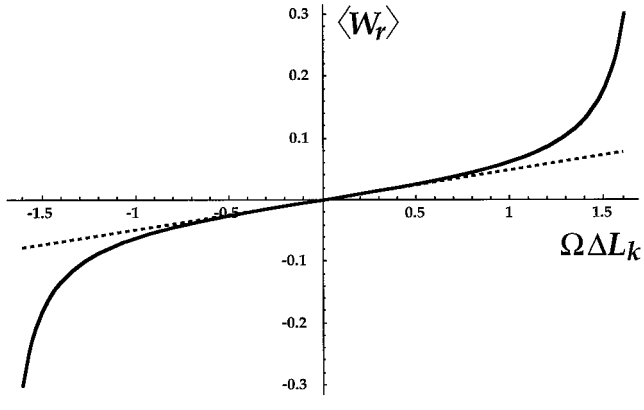

FIGURE 1 Graph of the ensemble average of the writhe $\langle W_r\rangle$ plotted versus $\Omega\Delta L_k$ for a miniplasmid of 200 bp at a temperature of 298 K. The straight broken line is a graph of $\langle W_r\rangle$ as given by Eq. 54, which is valid when $|\Omega\Delta L_k| \ll 1$.

Differentiating Eq. 55 with respect to ΔL_k yields an equation for the variance of the writhe:

$$\langle W_r^2\rangle - \langle W_r\rangle^2 = \frac{N}{4\pi^2\Omega}\left(\frac{\Delta s^o}{a}\right)\frac{\partial\langle W_r\rangle}{\partial\Delta L_k}. \tag{56}$$

Hence, for the miniplasmid that is torsionally relaxed in its equilibrium circular reference configuration ($\Delta L_k = 0$), by Eqs. 54 and 56, the root mean square writhe is given by

$$\langle W_r^2\rangle^{1/2} = \frac{1}{2\pi^2}\sqrt{\frac{\sigma(n_m)}{2}}\left(\frac{\Delta s^o}{a}\right)N. \tag{57}$$

THE THERMODYNAMIC FUNCTIONS OF SUPERCOILING

It has been shown that the Gibbs free energy of supercoiling, ΔG_{sc}, defined as the free energy of a plasmid having a given delta link, relative to the free energy of that plasmid when delta link is zero, has the property that its derivative with respect to the delta link is simply related to the ensemble average of the writhe (Gebe et al., 1995),

$$\frac{\partial\Delta G_{sc}}{\partial\Delta L_k} = 4\pi^2\Omega\left(\frac{a}{\Delta s^o}\right)\frac{k_BT}{N}(\Delta L_k - \langle W_r\rangle). \tag{58}$$

(We are again using conventional units for the energy here.) Equation 58 implies that each of the thermodynamic functions of supercoiling, ΔG_{sc}, as well as the entropy of supercoiling, ΔS_{sc}, and the enthalpy of supercoiling, ΔH_{sc}, are also known once $\langle W_r\rangle$ is known. That is,

$$\Delta G_{sc} = 4\pi^2\Omega\left(\frac{a}{\Delta s^o}\right)\frac{k_BT}{N}\left(\frac{1}{2}(\Delta L_k)^2 - \int_0^{\Delta L_k}\langle W_r\rangle d\Delta L_k\right) \tag{59}$$

$$\Delta S_{sc} = 4\pi^2\Omega\left(\frac{a}{\Delta s^o}\right)\frac{k_BT}{N}\int_0^{\Delta L_k}\frac{\partial\langle W_r\rangle}{\partial T}d\Delta L_k \tag{60}$$

$$\Delta H_{sc} = \Delta G_{sc} + T\Delta S_{sc}. \tag{61}$$

In the present case, because the average writhe is proportional to the temperature, we have for the entropy of supercoiling,

$$\Delta S_{sc} = 4\pi^2\Omega\left(\frac{a}{\Delta s^o}\right)\frac{k_B}{N}\int_0^{\Delta L_k}\langle W_r\rangle d\Delta L_k, \tag{62}$$

and, hence, for the enthalpy of supercoiling,

$$\Delta H_{sc} = 2\pi^2\Omega\left(\frac{a}{\Delta s^o}\right)\frac{k_BT}{N}(\Delta L_k)^2. \tag{63}$$

From Eq. 54 it follows that when $|\Omega\Delta L_k| \ll 1$,

$$\Delta S_{sc} = k_B\sigma(n_m)(\Omega\Delta L_k)^2. \tag{64}$$

COMPARISON OF THEORY AND EXPERIMENT

The various experimental studies of thermal fluctuations in nicked DNA plasmids lead to values for the free energy of supercoiling ΔG_{sc}, which is often written in the form

$$\Delta\hat{G}_{sc} = K(\Delta L_k)^2. \tag{65}$$

with $\Delta\hat{G}_{sc} = \Delta G_{sc}/k_BT$, and with K assumed to be independent of ΔL_k. Certainly in the case of plasmids with many base pairs, the data confirm that K is independent of ΔL_k. For the smallest plasmids studied, those having ~200 bp, it is more difficult to measure directly the dependence of K on delta link. We have found that with $N = 200$ bp and $\Omega = 1.4$, over the range $|\Delta L_k| \le 1$, the theory predicts K to be constant to within 3%. Shimada and Yamakawa (1985) similarly found the distribution of ΔL_k to be close to Gaussian for small N.

For plasmids having many base pairs N, all measurements indicate that NK is virtually independent of N. For small values of N, the case for which the present theory is valid, there has been some disagreement about the observed dependence of NK on N. The data of Horowitz and Wang (1984) imply that, in this case, NK decreases with increasing N, whereas those of Shore and Baldwin (1983) have NK independent of N. The present theory supports the observations of Horowitz and Wang (1984). Of the two terms making up the free energy of supercoiling ΔG_{sc}, Eq. 59, the first is positive and varies inversely with N, whereas the second, which is negative, is independent of N, given that $\langle W_r\rangle$ is itself proportional to N. It follows that the theory predicts that NK ($= N\Delta G_{sc}/(\Delta L_k)^2$) decreases linearly with increasing N. For the case $|\Omega\Delta L_k| \ll 1$ (see Eq. 64),

$$NK = 2\pi^2\Omega\left(\frac{a}{\Delta s^o}\right) - \sigma(n_m)\Omega^2N. \tag{66}$$

The theory of Frank-Kamenetskii et al. (1985), valid for all values of N, also has NK decreasing with increasing N when N is small. Equation 66 becomes identical to the expression given by Shimada and Yamakawa (1985) for NK, correct to terms linear in N, when $\sigma(n_m)$ is set equal to ¾, its maximum value. The latter workers note that the decrease in NK with increasing N when N is small "arises from the fluctuation in W_r." The formalism of this paper suggests that the phenomenon can also be related to the difference in the N dependence of the enthalpic and entropic contributions to the free energy of supercoiling. As Eqs. 63 and 64 show, the enthalpy of supercoiling varies inversely with N, whereas the entropy of supercoiling is independent of N. Hence one can also say that it is the increase in $T\Delta S_{sc}$ relative to ΔH_{sc} as N is increased that accounts for the decrease in NK when N is small.

Among the smallest of the plasmids studied by Horowitz and Wang (1984) was one with $N = 210$ bp. With this value of N, and the values for the elastic moduli A and C assumed by the experimenters ($\Omega = 1.4$, $a = 500$ Å), Eq. 66 predicts

that NK is 3.8×10^3 at room temperature. The corresponding measured value is 3910.

We also note that NK in the limit of N becoming zero, $(NK)_0$, is the first term of Eq. 66, i.e.

$$(NK)_0 = 2\pi^2\Omega\left(\frac{a}{\Delta s^o}\right), \tag{67}$$

which, with $\Omega = 1.4$, $a = 500$ Å, and $\Delta s^o = 3.4$ Å, equals 4.1×10^3.

APPENDIX A

To present the equations of motion in the form of Eq. 15, we chose $(I/S)^{1/2}$ as the unit of length, $I\,(\rho/SA)^{1/2}$ as the unit of time, and (SA/I) as the unit of force. Here we give the values of the units of length, frequency, $I^{-1}(SA/\rho)^{1/2}$, and energy, $A(S/I)^{1/2}$, for the case of a DNA molecule modeled as an elastic rod.

For any rod having a circular cross section of radius r, the cross-sectional area S is, of course, πr^2, and the corresponding moment of inertia I is $(\pi/4)r^4$. Our unit of length for this type of rod is thus $r/2$, which, in the case of DNA, is ~5 Å. When the rod is closed to form a ring, the dimensionless radius R of the ring must satisfy the inequality $R \geq 2$. The Kirchhoff theory employed here is valid only for thin rods, so that R should, in fact, be considerably larger than 2.

To compute the unit of frequency, we must have a value for ρ, the mass density of the DNA molecule. The sum of the atomic weights of all of the atoms contained in a volume $\pi r^2\Delta s^o$, where $\Delta s^o = 3.4$ Å is the separation between adjacent base pairs, is ~660 amu. Furthermore, the bending modulus A can be written as a product $A = ak_BT$ of the persistence length a, Boltzmann's constant k_B, and the absolute temperature T. At a temperature of 298 K, a is usually taken to be 500 Å. With these numbers substituted, one finds that the unit of frequency, $I^{-1}(SA/\rho)^{1/2}$, has a value of 1×10^{12} Hz.

The unit of energy, $A(S/I)^{1/2}$, is readily found to be $(2a/r)k_BT = 100k_BT$.

APPENDIX B

The nine coefficients a_{ij} that appear in Eq. 26 are

$$a_{11} = n^2(1 - \Omega - n^2) + \omega^2R^2(n^2 + R^2)$$

$$a_{12} = \Omega\Delta L_k n^2(n^2 - 1)$$

$$a_{13} = -n^2R\Omega$$

$$a_{21} = -\Omega\Delta L_k n^2(n^2 - 1)$$

$$a_{22} = n^2(n^2 - 1)^2 - \omega^2R^2((n^2 - 1)^2 + R^2(n^2 + 1))$$

$$a_{23} = 0$$

$$a_{31} = \Omega n^2$$

$$a_{32} = 0$$

$$a_{33} = R(\Omega n^2 - 2R^2\omega^2).$$

The four coefficients M_i in Eq. 27 are

$$M_3 = 2R^6(R^2 + n^2)((n^2 - 1)^2 + R^2(n^2 + 1))$$

$$M_2 = -(((n^2 - 1)^2 + R^2(n^2 + 1))(2(n^2 - 1 + \Omega) + \Omega(R^2 + n^2)) + 2(R^2 + n^2)(n^2 - 1)^2)n^2R^4$$

$$M_1 = ((n^2 - 1)^2(2(n^2 - 1 + \Omega) + \Omega(R^2 + n^2)) + \Omega(n^2 - 1)((n^2 - 1)^2 + R^2(n^2 + 1)) - 2(\Omega\Delta L_k)^2 \cdot (n^2 - 1)^2)n^4R^2$$

$$M_0 = -\Omega n^6(n^2 - 1)^2(n^2 - 1 - (\Omega\Delta L_k)^2).$$

The author is grateful to Bernard Coleman, Marzio Lembo, and David Swigon for reading the manuscript and making valuable suggestions. This paper was a research contribution presented at the DIMACS/MBBC/PMMB Workshop on DNA Topology, Rutgers University, April 1997. This research was supported by the United States Public Health Service under grant GM34809.

REFERENCES

Coleman, B. D., E. H. Dill, M. Lembo, Z. Lu, and I. Tobias. 1993. On the dynamics of rods in the theory of Kirchhoff and Clebsch. *Arch. Rational Mech. Anal.* 121:339–359.

Coleman, B. D., M. Lembo, and I. Tobias. 1996. A new class of flexure-free vibrations of annular rods. *Meccanica.* 31:565–575.

Depew, R. E., and J. C. Wang. 1975. Conformational fluctuations of DNA helix. *Proc. Natl. Acad. Sci. USA.* 72:4275–4279.

Dill, E. H. 1992. Kirchhoff's theory of rods. *Arch. Hist. Exact Sci.* 44:1–23.

Frank-Kamenetskii, M. D., A. V. Lukashin, V. V. Anshelevich, and A. V. Vologodskii. 1985. Torsional and bending rigidity of the double helix from data on small DNA rings. *J. Biomol. Struct. Dyn.* 2:1005–1012.

Fuller, F. B. 1971. The writhing number of a space curve. *Proc. Natl. Acad. Sci. USA.* 68:815–819.

Gebe, J. A., S. A. Allison, J. B. Clendenning, and J. M. Schurr. 1995. Monte Carlo simulations of supercoiling free energies for unknotted and trefoil knotted DNAs. *Biophys. J.* 68:619–633.

Goldstein, H. 1981. Classical Mechanics. Addison-Wesley Publishing Company, Reading, MA.

Goriely, A., and M. Tabor. 1997. Nonlinear dynamics of filaments. I. Dynamical instabilities. *Physica D.* 105:20–44.

Hagerman, P. J. 1988. Flexibility of DNA. *Annu. Rev. Biophys. Biophys. Chem.* 17:265–286.

Horowitz, D. S., and J. C. Wang. 1984. Torsional rigidity of DNA and length dependence of the free energy of DNA supercoiling. *J. Mol. Biol.* 173:75–91.

Jolley, L. 1925. Summation of Series. Chapman and Hall, London.

Pulleyblank, D. E., M. Shure, D. Tang, J. Vinigrad, and H. P. Vosberg. 1975. Action of nicking-closing enzyme on supercoiled and nonsupercoiled closed circular DNA: formation of a Boltzmann distribution of topological isomers. *Proc. Natl. Acad. Sci. USA.* 72:4280–4284.

Shimada, J., and H. Yamakawa. 1984. DNA topoisomer analysis on the basis of the helical worm-like chain. *Biopolymers.* 23:853–857.

Shimada, J., and H. Yamakawa. 1985. Statistical mechanics of DNA topoisomers. The helical worm-like chain. *J. Mol. Biol.* 184:319–329.

Shore, D., and R. L. Baldwin. 1983. Energetics of DNA twisting. II. Topoisomer analysis. *J. Mol. Biol.* 170:983–1007.

Tobias, I., B. D. Coleman, and M. Lembo. 1996. A class of exact dynamical solutions in the elastic rod model of DNA with implications for the theory of fluctuations in the torsional motion of plasmids. *J. Chem. Phys.* 105:2517–2526.

White, J. 1969. Self-linking and the Gauss-integral in higher dimensions. *Am. J. Math.* 91:693–728.

Zajac, E. E. 1962. Stability of two planar loop elasticas. *J. Appl. Mech. Trans. ASME Ser. E.* 29:136–142.