Catastrophic Variation of Twist and Writhing of Circular DNAs with Constraint?

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Synopsis

The number of constraint turns is defined as the number of bound unwinding ligands converted in turns necessary to relax the energy stored in a supercoiled DNA. It is equal to the sum of the twist and of the writhing and is interpreted geometrically. The twist is shown to be related to the derivative of the energy of superhelix formation with respect to the constraint turns. This leads to a semiempirical evaluation of the variation of the conformational energy with the writhing and of the writhing number. The bending contribution to the conformational energy is estimated independently using first-order elasticity. The variation of the twist and of the writhing with the constraint is, in this model, catastrophic. In particular, the writhing number jumps between intervals of allowed values.

INTRODUCTION

How the tertiary structure of DNA changes under applied constraints is a problem which must be solved to understand its packing in phages or in the chromatin. Circular, double-stranded DNAs are particularly interesting, since (1) the applied constraint is experimentally well controlled by the amount of bound unwinding ligands such as ethidium and (2) their tertiary structure has been extensively studied using electron microscopy,[1–3] x-ray diffraction,[4] viscosimetry,[5,6] and sedimentation[7–11] techniques. The number of constraint turns c is defined as the amount of DNA expressed in turns of bound unwinding ligands necessary to relax all the energy stored in the supercoiled DNA. It is equal to the sum of the writhing number[12] w and of the twist $\Delta\beta$ of the DNA. $\Delta\beta$ is the difference between the number of secondary turns β in the supercoiled conformation and its value, β_0, in the relaxed conformation. For a given constraint, the writhing results form a balance[12] between the twisting free energy, which (using a dimensionless formalism) may be written $(\Delta\beta)^2$, and the minimum of the free energy necessary to set the tertiary structure of the DNA into a conformation of given writhing $2eg_{ap}(w)$. The balance depends on the ratio $2e$ of the bending to the twisting elastic parameters determined previously.[13] If the secondary structure of the DNA were independent of the constraint (if the DNA shear modulus was infinitely larger than its Young modulus) as is usually assumed, the number of tertiary turns (the writhing number) would be exactly equal to the number of constraint turns.[14] If the shear

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modulus were infinitely less than the Young modulus, the twist would be much more easily modified than the bending and no writhing would occur. This is the case of single-stranded DNAs. Natural, double-stranded DNAs are an intermediate case: tertiary turns are formed and their secondary structure changes as can be inferred[13] from the torsional Brownian motion of linear DNA[15] and from the heterogeneity of supercoiled DNAs obtained by closing a nicked DNA by ligase.[16,17] Therefore, in order to compute the twisting and the writhing contributions of a given constraint, we must know the values of the function $g_{ap}(w)$. According to Fuller,[12] the twist $\Delta\beta$ is equal to $eg'_{ap}(w)$. As the number of constraint turns c is equal to the sum of the twist and of the writhing number, it may be written as a function of w only, using $g'_{ap}(w)$. As a corollary, w may be deduced from c once $g'_{ap}(w)$ is known. The function $g_{ap}(w)$ is involved in other problems. The fluctuations of w and β about their average values $\overline{w}$ and $\overline{\beta}$ are equal, since the sum $\beta + w$ is a constant[18] (the linking number α of the two DNA strands). The fluctuations of w depend on the second derivative of $g_{ap}(w)$ computed at $\overline{w}$.[13] As the conformational fluctuations of the DNA about a stable conformation should be finite, the value of $g''_{ap}(\overline{w})$ indicates whether an equilibrium conformation of given $\overline{w}$ is stable.

Because of the importance of the function $g_{ap}(w)$ in the understanding of the properties of circular DNAs, we attempt here to give its numerical values. It will first be evaluated from a semiempirical approach, then from a completely theoretical one. It was, however, impossible using the theoretical approach to take into account all the parameters which are known to play a role in the conformation of circular DNAs: the bending parameter, ionic environment, temperature, pH, hydration, density of states, and entropy. When only the elastic properties are taken into account, we find a function $g(w)$ which is the minimum of the bending energy which must be given to a relaxed DNA to bend it into a conformation of a given writhing number. Nevertheless, this simplification is interesting, since it corresponds to Fuller's original definition[12] and since such a model has been proposed by Benham.[19]

SEMIEMPIRICAL DETERMINATION OF $g_{ap}(w)$

Here we deduce the value of an apparent function $g_{ap}(w)$ by identifying the theoretical free energy of supercoiling to its experimental value. The number of constraint turns, the writhing number and the twist are related by

$$c = w + \Delta\beta \tag{1}$$

When c is fixed, the free energy of supercoiling $U(\Delta\beta,w)$ is minimum when its first partial derivatives relative to β and w are equal. As c varies, the variation of the minimum of the free energy is equal to the partial derivatives of U computed at the equilibrium values $\overline{\Delta\beta}$ and $\overline{w}$:

$$\frac{dU}{dc} = U'_{\beta} = U'_{w} \tag{2}$$

The free energy is actually a minimum if ΔU is positive:

$$\Delta U = U(\Delta\beta,w) - U(\Delta\bar{\beta},\bar{w}) \sim 0.5\,(w - \bar{w})^2(U''_{w2} - 2U''_{w\beta} - U''_{\beta 2}) \quad (3)$$

Differentiating Eq. (2) and reporting into Eq. (3),

$$\Delta U \sim 0.5\,(w - \bar{w})^2(U''_{\beta 2} - U''_{w\beta})\,\frac{dc}{d\bar{w}} \quad (4)$$

The free energy of supercoiling is assumed to have two independent contributions—the twisting and the conformational ones:

$$U = (\kappa RT/L)[(\Delta\beta)^2 + 2eg_{ap}(w)] \quad (5)$$

κ is the twisting elastic parameter; R is the perfect gas constant; T is the absolute temperature; L is the number of base pairs; and $2e$ is the bending to twisting ratio. The free energy of supercoiling measured by Bauer and Vinograd[7] should be corrected for the now accepted value of the unwinding angle of ethidium.[20] It is written as

$$U = (\kappa RT/L)(ac^2 - 2bc^3) \quad (6)$$

The derivative of Eq. (6) is equated to the partial derivatives of Eq. (5):

$$ac - 3bc^2 = c - w = eg'_{ap}(\bar{w}) \quad (7)$$

If the parameter of superhelix formation is not equal to the twisting parameter κ (if $a \neq 1$); then

$$w = (1 - a)c \quad (8)$$

$$2eg_{ap}(w) \sim aw^2/(1 - a) \quad (9)$$

As the equilibrium should be stable, a is necessarily less than 1. A helical elastic model for supercoiled DNA in solution has recently been proposed.[21] The pitch angle χ of the helix would be independent from w if the elasticity parameters are assumed to be constant. However, a slight discrepancy appears between their result and ours ($\chi = tg^{-1}[2e(1 - a)/a]^{1/2}$) because the radius of the helix was assumed constant in the minimization equation and variable in the other parts of their computations. On the other hand, if a is equal to 1, as we suggested,[13] Eq. (7) gives

$$w = 3bc^2 \quad (10)$$

$$2eg_{ap}(w) = 4w^{3/2}(27b)^{-1/2} - w^2 \quad (11)$$

Therefore, the function $g_{ap}(w)$ may increase less rapidly than w^2. In the following parts of this work, we show that the minimum of the bending energy $g(w)$ increases at most as $|w|^{2/3}$. Moreover, the relationship between c and w is more subtle than expressed in Eqs. (8) or (10).

GEOMETRICAL INTERPRETATION OF *c* AND *w*

From elasticity theory[22] the shape of a rod, submitted to external forces and to couples at its ends only, may easily be determined. However, the Euler angles θ, ϕ, ψ, relative to the fixed reference **I, J, K** of the moving reference made by the inertia axes of the cross section and the tangent **t** to the central axis Γ are easier to compute as a function of the curvilinear abscissa s than the coordinates of Γ. That is why we must relate the writhing number, the twist, and the constraint to the Euler angles (Fig. 1).

The rate of twist $\tilde{\omega}$ of a moving reference centered on Γ and defined by the unit vectors $\tilde{\mathbf{i}}(s)$ and $\mathbf{t}$ is equal to $(\mathbf{t} \times \tilde{\mathbf{i}})\, d\tilde{\mathbf{i}}/ds$. It has two contributions[23]: the first one is equal to $\cos\theta\, d\phi/ds$. It depends only on Γ and not on the field $\tilde{\mathbf{i}}(s)$. The second one, $d\tilde{\psi}/ds$, is the rate of rotation of the reference **i, t** about the reference **u,t.** If the end of the vectors $\tilde{\mathbf{i}}(s)$ describes a closed curve, the difference between the linking numbers of the strips $(\Gamma,\tilde{\mathbf{i}})$ and $(\Gamma,\mathbf{u})$ is the variation of $\tilde{\psi}/2\pi$ along Γ, which is an integer written $[\tilde{\psi}/2\pi]$. In the unconstrained state the rod is neither twisted nor writhed. Therefore, the closed line described by the end of the inertia axis of the cross section of the unconstrained closed rod is not linked with its central axis. If $\tilde{\mathbf{i}}(s)$ stands for the inertia axis of the cross section in the rod in the twisted and writhed conformation, the linking number of the strip $(\Gamma,\tilde{\mathbf{i}})$ is zero. As the vectors **t,K,u** are coplanar, the linking numbers of the strips $(\Gamma,\mathbf{u})$ and $(\Gamma,\mathbf{K})$ are equal. Their common value is the directional writhing number $\alpha(\mathbf{K})$ of Γ in the direction **K**. Therefore,

$$[\tilde{\psi}/2\pi] = \alpha(\mathbf{K}) \tag{12}$$

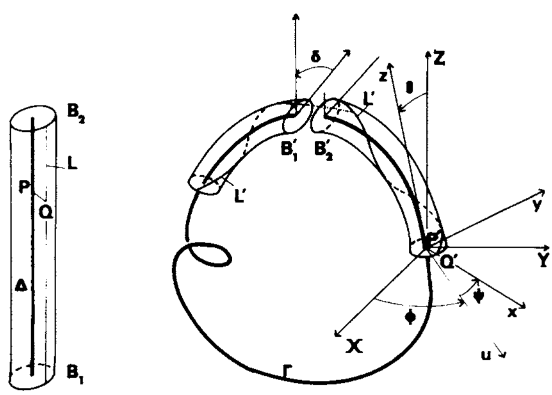

Fig. 1. The straight rod shown on the left is closed twisted and writhed on the right. The two end bases B_1, B_2 of the straight rod are actually in contact in the supercoiled conformation. However B_1' and B_2' are drawn slightly separated for clarity. The central line Δ of the straight rod is deformed into Γ. The whole curve Γ is drawn, but only a segment of the deformed rod is shown. The direction of the triple XYZ is fixed, θ is the angle $(0 < \theta < \pi)$ between the tangent $P'z$ to Γ and $P'Z$. $P'u$ is perpendicular to the plane Zz and the triple (Z,z,u) is right handed. ϕ is the angle of $P'u$ with $P'X$ $(-\pi < \phi < \pi)$. The point Q of the straight rod lies in Q' in the deformed conformation. $P'Q'$ is perpendicular to $P'z$. ψ is the angle of $P'Q'$ with $P'u$ $(-\pi < \psi < \pi)$. Q' describes L'. Within an integer the angle δ is equal to $-c$. L' is not in general closed, as is the dotted line.

We assume that infinitely thin unwinding dyes are intercalated between the two end bases B_1' and B_2' of the rod. The direction of the inertia axis of the rod cross section is $\mathbf{i}(s)$. Since the number of constraint turns is not necessarily an integer, the line L' described by the end of $\mathbf{i}(s)$ is not usually closed. However, the variation $[\psi/2\pi]$ corresponding to the field $\mathbf{i}(s)$ satisfies

$$[\psi/2\pi] - c = [\tilde{\psi}/2\pi]$$

Therefore,

$$c = [\psi/2\pi] + \alpha(\mathbf{K}) \tag{13}$$

The twist $\Delta\beta$ of the rod is

$$\Delta\beta = [\psi/2\pi] + \int_\Gamma \cos\theta \frac{d\phi}{2\pi}$$

$$\Delta\beta = [\psi/2\pi] + [\phi/2\pi] - \int_\Gamma d\phi \int_0^{\theta(\phi)} \sin\theta \frac{d\theta}{2\pi} \tag{14}$$

The double integral in Eq. (14) is equal to the surface $\Omega(K)$ computed from the extremity $\mathbf{K}$ of the hodograph of Γ (that is, the line described by the unit tangents $\mathbf{t}$ to Γ reported to a fixed point). Because of Eq. (1),

$$w = -\int_\Gamma \cos\theta \frac{d\phi}{2\pi} + \alpha(\mathbf{K}) \tag{15}$$

$$w = -\,\Omega(\mathbf{K}) = \alpha(\mathbf{K}) - [\phi/2\pi] \tag{16}$$

Therefore, the difference $w - \Omega(\mathbf{K})$ is an integer. In general, as Γ is deformed, it does not change its value because w and $\Omega(\mathbf{K})$ vary continuously. However, if Γ cuts itself during the deformation, w jumps by two.[24] If the opposite of $\mathbf{K}$ cuts the hodograph during the deformation, $\Omega(\mathbf{K})$ jumps by two.[24] As a consequence, Eq. (16) is either an odd or an even integer. For a circle it is equal to -1; and therefore for any closed curve, it is an odd integer. A similar result has recently been found by Fuller.[25] In Eqs. (13) and (15), $\alpha(\mathbf{K})$ may be computed by inspection if the shape of Γ is known or deduced within an odd integer from the variation of the Euler angle ϕ.

If the tangent $\mathbf{t}$ to Γ is not continuous, the twist is not defined.[13] If the angular point of Γ is replaced by an arbitrary smooth curve, the value of $\Omega(\mathbf{K})$ is as arbitrary and so is w. However, if the angular points of Γ are conventionally replaced by an arc of a circle of any small radius, the discontinuity in the hodograph is always replaced by the same smaller arc of a great circle. Therefore, a conventional writhing number may be computed for a polygonal line made of a finite number n of sides. This conventional writhing number is the average on all the directions $\mathbf{K}$ of the directional writing numbers $\alpha(\mathbf{K})$. If two adjacent sides numbered i and $i + 1$ of the polygonal line having the Euler angles θ_i,ϕ_i and θ_{i+1},ϕ_{i+1} ($0 < \theta_i < \pi$; $-\pi < \phi_i < \pi$) are perpendicular to the direction of Euler angles ζ_i,η_i ($0 < \zeta_i < \pi/2$; $-\pi < \eta_i < \pi$), Eq. (15) gives

$$2\pi[w - \alpha(\mathbf{K})] = \sum_{i=1}^{n} \sin^{-1}[\sin \zeta_i \sin(\phi_{i+1} - \eta_i)] - \sin^{-1}[\sin \zeta_i \sin(\phi_i - \eta_i)]$$

As usual, $\sin^{-1}x$ is the angle comprised between $-\pi/2$ and $\pi/2$, the sine of which is equal to x. Since the linking number of the two DNA strands is defined even if the DNA axis has a polygonal conformation, a conventional value of β may be derived from White's formula.[18]

COMPUTATION OF $g(w)$ FOR $|w| < 1$

We want to compute $g(w)$ the minimum of

$$J = L \int_{\Gamma} \rho^2 \frac{ds}{4\pi^2} - 1 \tag{17}$$

for a closed rod of constant length L having the writhing number w. In Eq. (17), ρ is the curvature of the central axis Γ of the rod. In the mechanical equilibrium state, the internal stresses at the abscissa s of the rod are resolvable into a force $\mathbf{N}(s)$ and a torque $\mathbf{M}(s)$ which, in the absence of external constraints and to the first order, should satisfy[22]

$$\frac{d\mathbf{N}}{ds} = 0 \tag{18}$$

$$\frac{d\mathbf{M}}{ds} + \mathbf{t} \times \mathbf{N} = 0 \tag{19}$$

where the vectors $\mathbf{N}$, $\mathbf{M}$ are reported to a fixed reference $\mathbf{I}$,$\mathbf{J}$,$\mathbf{K}$. The curvilinear abscissa of a given cross section in the supercoiled rod is assumed to be constant and equal to the curvilinear abscissa in the linear conformation. Because of Eq. (18), $\mathbf{N}$ is a fixed vector of length N and unit vector $\mathbf{K}$. The curvature ρ, the torsion τ, the tangent $\mathbf{t}$, the normal $\mathbf{n}$, and the binormal $\mathbf{b}$ are related by

$$\begin{aligned} \frac{d\mathbf{t}}{ds} &= \rho\mathbf{n} \\ \frac{d\mathbf{n}}{ds} &= -\rho\mathbf{t} + \tau\mathbf{b} \\ \frac{d\mathbf{b}}{ds} &= -\tau\mathbf{n} \end{aligned} \tag{20}$$

As usual in elasticity, the stress is assumed to be proportional to the deformation. If A and κ are the bending and the twisting parameters (their ratio is written $2e$), the relevant expression for a cylindrical rod with no preferred side is

$$\mathbf{M} = A\rho\mathbf{b} + \kappa\omega\mathbf{t} \tag{21}$$

where ω is the rate of twist of the rod. Both scalar products $\mathbf{M}\cdot\mathbf{t}$ and $\mathbf{M}\cdot\mathbf{K}$

are constant, as may easily be shown using Eqs. (19)–(21). Since ω is constant, the twist $\Delta\beta$ is equal to $\omega L/2\pi$. The scalar product $\mathbf{M}\cdot\mathbf{K}$ is called p. We recall some classical results[22,23]: Eqs. (22)–(26). If E is a constant,

$$A\rho^2 = 2(E - Nu) \tag{22}$$

If the curvature vanishes at some point of Γ and if the parameter $Np - E\kappa\omega$ is not zero, the torsion is infinite, since

$$A\tau = \kappa\omega/2 - (Np - E\kappa\omega)/A\rho^2 \tag{23}$$

If u stands for $\cos\theta$,

$$\left(\frac{du}{ds}\right)^2 = f(u) = \frac{2}{A}(E - Nu)(1 - u^2) - \frac{(p - \kappa\omega u)^2}{A^2} \tag{24}$$

$$\frac{d\phi}{ds} = \frac{p - \kappa\omega u}{A(1 - u^2)} \tag{25}$$

$$\frac{d\psi}{ds} = \omega - u\frac{d\phi}{ds} \tag{26}$$

Now $f(u)$ should be a positive cubic polynomial. Its roots u_1, u_2, u_3 should satisfy

$$-1 < u_1 < u < u_2 < 1 < u_3$$

Moreover, the roots of $f(u)$ are related to the parameters $p,\kappa,N,$ and ω by

$$\begin{aligned}
p\kappa\omega/AN &= 1 + u_1u_2 + u_2u_3 + u_3u_1\\
p^2/AN &= u_1 + u_2 + u_3 + u_1u_2u_3 + \epsilon[(u_1^2 - 1)(u_2^2 - 1)(u_3^2 - 1)]^{1/2}\\
(\kappa\omega)^2/AN &= u_1 + u_2 + u_3 + u_1u_2u_3\\
&\quad - \epsilon[(u_1^2 - 1)(u_2^2 - 1)(u_2^2 - 1)]^{1/2}\\
2E/N &= u_1 + u_2 + u_3 - u_1u_2u_3 + \epsilon[(u_1^2 - 1)(u_2^2 - 1)(u_3^2 - 1)]^{1/2}
\end{aligned} \tag{27}$$

where ϵ is either $+1$ or -1. The integral of Eq. (24) is a periodic elliptic function. The hodograph of Γ is formed by m arcs, which are deduced from each other by a rotation. m may be interpreted as the number of toroidal turns. The variable u increases from u_1 to u_2, then decreases from u_2 to u_1 for an elementary arc. The number m is well defined if u_2 and u_1 differ and is otherwise undetermined. The length of an elementary arc l may be computed if Eq. (24) is integrated using the variable change $u = u_1 + (u_2 - u_1)\sin^2 x$:

$$L = ml = \pi m[2A/N(u_3 - u_1)]^{1/2}F_1(k^2) \tag{28}$$

where

$$k^2 = (u_2 - u_1)/(u_3 - u_1) \tag{29}$$

and for any positive or negative integer n

$$F_{2n-1}(k^2) = \frac{2}{\pi}\int_0^{\pi/2}(1 - k^2\sin^2 x)^{-(2n-1)/2}\,dx \tag{30}$$

The integral J defined in Eq. (17) may, using Eq. (22), be written

$$J = 2EL^2 - 2NL^2 \langle u \rangle / m \tag{31}$$

where the signs $\langle\ \rangle$ stand for an integral extended over one of the m elementary arcs of Γ with the density ds/l. For instance,

$$\langle u \rangle = 2 \int_{u_1}^{u_2} u \frac{ds}{l} \tag{32}$$

For any positive integer n,

$$\left\langle \left(\frac{u}{u_3} \right)^n \right\rangle = \sum_{q=0}^{n} (-1)^q \frac{n!}{(n-q)!q!} \left(1 - \frac{u_1}{u_3} \right)^q \frac{F_{1-2q}(k^2)}{F_1(k^2)} \tag{33}$$

We must find conditions on u_1, u_2, u_3 and m so that the curve Γ is closed. We start from the equation

$$\int_\Gamma \mathbf{t}\, ds = 0 \tag{34}$$

Projecting Eq. (34) on $\mathbf{K}$,

$$\langle u \rangle = 0 \tag{35}$$

Because of Eq. (33), we find a relationship between u_1, k^2, and u_3:

$$u_1 = u_3[1 - F_1(k^2)/F_{-1}(k^2)] \tag{36}$$

Because of Eq. (22), u_2 is related to k^2 and u_3 only by

$$u_2 = [1 + (k^2 - 1)F_1(k^2)/F_{-1}(k^2)]u_3 \tag{37}$$

The length L of the rod is

$$L^2 = 2m^2\pi^2 F_1(k^2) F_{-1}(k^2)/Nu_3 \tag{38}$$

The closure of the curve Γ is not ensured if Eq. (35) alone is satisfied. The total variation $[\phi/2\pi]$ should be an integer. However if m is larger or equal to 2 and if $[\phi/2\pi]$ is equal to 1, the projections of Eq. (34) on $\mathbf{I}$ and $\mathbf{J}$ are equal to zero by symmetry. Now,

$$\frac{2[\phi/2\pi]^2}{mF_1(k^2)F_{-1}(k^2)} = \frac{p^2}{ANu_3} \left\langle \frac{1}{1-u^2} \right\rangle^2 - \frac{2p\kappa\omega}{ANu_3} \left\langle \frac{1}{1-u} \right\rangle \left\langle \frac{u}{1-u} \right\rangle + \frac{(\kappa\omega)^2}{ANu_3} \left\langle \frac{u}{1-u^2} \right\rangle^2 \tag{39}$$

We may now explain how J, U, w, $\Delta\beta$, and c have been computed for given values of k^2, m, and $[\phi/2\pi]$:

1. The integers m and $[\phi/2\pi]$ are chosen.
2. A value is given to k^2. The Legendre complete elliptic integrals[26] $F_1(k^2)$ and $F_{-1}(k^2)$ and the ratios u_1/u_3, and u_2/u_3 are computed.
3. A value is given to u_3. As a consequence, the roots of $f(u)$ are known, and the quantities on the left-hand side of Eq. (27) are easily computable. The integrals $\langle 1/(1-u^2) \rangle$ and $\langle u/(1-u^2) \rangle$ are evaluated using

$$F_1(k^2)\,\langle 1/(1 \pm u)\rangle = [(1 \pm u_1)(1 \pm u_2)]^{-1/2} + \int_0^{\pi/2} [-1 + (1 - k^2 \sin^2 x)^{-1/2}] \frac{d(2x/\pi)}{1 \pm u_1 \pm (u_2 - u_1)\sin^2 x} \quad (40)$$

The integral lying in the right-hand side of Eq. (40) is estimated using the trapezoidal rule.

4. Step 3 is reiterated with another value of u_3. The value of u_3 for which both members of Eq. (39) are equal within 10^{-10} is found by an interpolation procedure.

5. pL/A, $\kappa\Delta\beta/A$, w, and J are computed from

$$(\kappa\Delta\beta/A)^2 = [(\kappa\omega)^2/2ANu_3]m^2F_1(k^2)F_{-1}(k^2) \quad (41)$$

$$(pL/2A)^2 = (p^2/2ANu_3)m^2F_1(k^2)F_{-1}(k^2) \quad (42)$$

$$w = pL/2\pi A\langle u/(1 - u^2)\rangle - \kappa\Delta\beta/A\langle u^2/(1 - u^2)\rangle + \alpha(\mathbf{K}) \quad (43)$$

$$J = m^2F_1(k^2)F_{-1}(k^2)E/Nu_3 - 1 \quad (44)$$

$g(w)$ is the lowest value taken by J for a given value of w when m and $[\phi/2\pi]$ are varied. Note that the algorithm simultaneously gives an estimation of $g(w)$ and of its derivative.

When u_1,u_2, and k^2 are equal to zero, as they are in the circular conformation, the length L is not given by Eq. (38). However, as long as u_1 and u_2 differ from 0, the corresponding conformation is not planar but tends to the circle as k^2 tends to 0. At the limit case, the length is given by Eq. (39). We now compute the critical number of constraint turns over which the conformation cannot be circular. When k^2 is vanishingly small, Eq. (36) and (37) may be developed in series. Since $\langle(u/u_3)^3\rangle$ varies as k^8, $\langle u/(1 - u^2)\rangle$ may be neglected. If m and $[\phi/2\pi]$ are both equal to 1, no value of u_3 satisfies Eq. (39). If $[\phi/2\pi]$ is larger than 1, J does not tend to zero with k^2. If $[\phi/2\pi]$ is equal to 1, ϵ is equal to -1, and m is larger or equal to 2, u_3 is related to k^2 by

$$u_3^2 = (m^4/4(m^2 - 1))(1 - (2m^2 - 1)(m^2 - 2)(k^4 + k^6)/32(m^2 - 1))$$

Then

$$w = m^4\{(k^4 + k^6)/[32(m^2 - 1)]^{1/2}\} \quad (45)$$

$$J = 2(m^2 - 1)^{1/2}w - (1 + 3/2m^2)w^2 \quad (46)$$

For a given value of w, J is minimum when m is equal to 2. It is then equal to $g(w)$. The largest number of constraint turns leaving a circle as it is, is $\sqrt{3}A/\kappa$, as can be deduced from the derivative of Eq. (46).

The conformations of a linear rod submitted to forces at only its ends have been extensively studied.[23] The conformations are planar and are not closed in general. The conformation is closed when u_1 and u_3 are -1 and $+1$, respectively. The value of k^2 is the solution k_0^2 of

$$\begin{aligned} F_1(k_0^2) &= 2F_{-1}(k_0^2) \\ k_0^2 &= 0.82611476597 \end{aligned} \quad (47)$$

Then

$$u_2 = \cos(49.290089°)$$
$$g(1) = 1.8481285867$$

Since the rod is not twisted (forces are applied only at its ends), the rate of twist and therefore $g'(1)$ are equal to zero.

A 9810 A Hewlett Packard calculator was used to solve the algorithm (Table I). $g(w)$ and $g'(w)$ have the correct values when w tends to 0 or to 1. Moreover, Fuller's relationship between the twist and the derivative of $g(w)$ is correctly verified. The polynomial

$$g_p(w) = 2\sqrt{3}w - 11w^2/8 - 0.197997609403\,w^3 - 0.059664102410\,w^4 - 0.03384594250w^5 + 0.06596504601w^6 - 0.0154304201201w^7 \quad (48)$$

fits the computed values of $g(w)$ within 1.4×10^{-5}. Moreover, $g_p'(w)$ fits the computed values of $2\kappa\Delta\beta/A$ within 3.9×10^{-5}. Finally, $g_p(0)$, $g_p'(0)$, $g_p''(0)$, $g_p(1)$, and $g_p'(1)$ are exact.

VALUES OF $g(w)$ FOR INTEGRAL VALUES OF w

For values of $|w|$ larger than 1, two points of different curvilinear abscissae are in contact. The contact creates an external force of unknown value at an unknown abscissa, which makes Eqs. (24)–(26) difficult to integrate. Kirchhoff's kinetic analog may not be used in a simple way as above. In order to compute the function $g(w)$, we use a minimum principle: $g(w)$ is the minimum of the bending energy of all the closed curves of given writhing number. Of course it is not possible to consider all the curves. If curves $\Gamma_{\upsilon,\lambda}$ depending on the parameters υ and λ are chosen, $g(w)$ is certainly less or equal to the minimum of their bending energy $J_{\upsilon,\lambda}(w)$ when υ and λ are varied. The minimum of $J_{\upsilon,\lambda}(w)$ is a reasonable approximation of $g(w)$ if curves of a reasonable shape are chosen. We consider the curves shown on Fig. 2. These curves have $|w|$ self-intersections and would correspond to the conformation of infinitely thin rods of integral writhing number w. These planar curves are made by $4(w-1)$ identical sinusoidal arcs of equation

$$y = y_0 \sin(x/x_0) \quad (49)$$

where y_0 and x_0 are parameters to be fitted. At both ends the preceding sinusoids are closed, as shown in Fig. 2 by the curve (C) of equation, in polar coordinates (r,θ),

$$r = x_0\upsilon \cos^{\lambda}\theta/\lambda \quad (50)$$

here υ and λ are dimensionless parameters. Since the sinusoids and the arcs (C) must form a curve with continuous tangents,

$$y_0 = x_0 \tan \lambda\pi/2 \quad (51)$$

The parameters x_0 and y_0 are eliminated using Eq. (51). The integral, Eq. (17), giving the bending energy is

$$J_{\upsilon,\lambda}(w) = -1 + (l_S + \upsilon l_C)(J_S + J_C/\upsilon) \quad (52)$$

where l_S, l_C, J_S, and J_C depend only on w and on λ:

$$l_S = (w-1)\frac{F_{-1}(\sin^2 \lambda\pi/2}{\cos \lambda\pi/2}$$

TABLE I
Some of the Computed Values of $J(w)$ for $m = 2$ and $[\phi/2\pi] = 1$

k^2	u_1	u_2	u_3	$E/Nu_2 - 1$	$\kappa\Delta\beta/A$	$pL/2\pi A$	$J(w)$	w
0.1000	−0.06157	0.05997	1.153768	3.86	1.72765	0.99815	0.01107299431	0.0032005661
0.5000	−0.4167	0.3507	1.118042	0.13	1.54469	0.91675	0.4336636482	0.1322701729
0.5500	−0.4796	0.3933	1.107501	0.076	1.48416	0.88796	0.5579536639	0.1733028290
0.8260	−0.99967	0.6521	1.000069	10^{-10}	0.04176	0.02723	1.847256628	0.9791194193

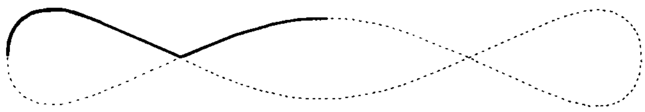

Fig. 2. The planar curve Γ used in the computations is made of four arcs of a curve (C) and $4(w-1)$ arcs of a sinusoid (S).

$$l_C = \Gamma(\lambda/2)/\Gamma[(\lambda+1)/2]$$

$$J_S = (w-1)\cos^2\lambda\pi/2(F_5(\sin^2\lambda\pi/2) - F_3(\sin^2\lambda\pi/2))$$

$$J_C = \pi^{-1/2}(1+\lambda)^2\Gamma(1-\lambda)/\Gamma(3/2-\lambda/2)$$

$$\Gamma(\lambda) = (\lambda-1)! = \int_0^\infty \exp(-t)t^{\lambda-1}\,dt$$

When v varies while w and λ are fixed,

$$\min_v J_{v,\lambda}(w) = -1 + [(l_S J_S)^{1/2} + (l_C J_C)^{1/2}]^2 \tag{53}$$

which is rewritten using classical properties of the gamma function:

$$\min_v J_{v,\lambda}(w) = -1 + \left[\left((w-1)\cos\frac{\lambda\pi}{2}F_{-1}\left(\sin^2\frac{\lambda\pi}{2}\right)\times\left(F_5\left(\sin^2\frac{\lambda\pi}{2}\right) - F_3\left(\sin^2\frac{\lambda\pi}{2}\right)\right)\right)^{1/2} + (1+\lambda)\left(\frac{2}{\pi(1-\lambda)}\tan\frac{\lambda\pi}{2}\right)^{1/2}\right]^2 \tag{54}$$

The value of λ minimizing Eq. (54) is found by trial and error. Table II shows the results. The following expression is exact within a relative error less than 0.002:

$$g(w) = \min_{v,\lambda} J_{v,\lambda(w)} = 1 + 4.5(|w|/\pi)^{2/3} - 1/(0.80 + 0.57\log_{10}|w|) \tag{55}$$

STABILITY OF THE CONFORMATIONS

According to Eq. (4), an equilibrium conformation is stable (and can be observed) if $dc/d\bar{w}$ is positive, that is, if $1 + eg''(w)$ is positive. When w varies from 0 to 1, $g''(w)$ steadily decreases from $-11/4$ to -4. The conformations are stable if e is less than 0.25. Otherwise, if $2e$ is equal to 3.5, as it probably is for DNA,[13] all the computed conformations are unstable and should not be observed. However, since $g'(1) = 0$ and since $g(2) > g(1)$, the second derivative of $g(w)$ is certainly positive in an interval within the interval [1,2]. Moreover, everyday experience on rubber tubing shows that stable conformations exist when w is larger than 2. Therefore, $1 + eg''(w)$ should be positive for some values of w larger than 2, whatever the value

TABLE II
Values of $\mathrm{Min}_{\upsilon,\lambda} J_{\upsilon,\lambda}(w)$ for the Planar Curves Γ Shown in Fig. 3 and Having w Self-Intersections[a]

w	λ	χ	υ	$\mathrm{Min}_{\upsilon,\lambda} J_{\upsilon,\lambda}(w)$
1	0.4458	80.2		1.84931
2	0.2607	46.9	1.7261	3.29852
3	0.1981	35.7	1.8335	4.42450
4	0.1537	29.5	1.9335	5.40561
5	0.1413	25.4	2.0240	6.29575
6	0.1255	22.6	2.1061	7.12125
7	0.1134	20.4	2.1820	7.8975
8	0.1040	18.7	2.2525	8.63429
9	0.0963	17.3	2.3183	9.33867
10	0.0899	16.2	2.3803	10.01561
20	0.0572	10.3	2.8639	15.83349
30	0.0439	7.9	3.2151	20.68091
100	0.0312	5.6	4.6305	45.74404
1000	0.0043	0.8	9.7281	210.47660
10,000	0.0009	0.2	20.838	934.50588

[a] χ is the angle in degrees between the tangents at the self-intersections. Using Kirchhoff's kinetic analog for $w = 1$, we would have found $\chi = 81.42°$ and $g(1) = 1.8481$.

of e. Consequently, Eq. (55) is valid only for integral values of w and cannot be interpolated.

We now go back to Kirchhoff's kinetic analog. As c continuously increases from zero, the central curve of the rod Γ is continuously deformed and takes successive stable or unstable equilibrium conformations. Hence w and $g'(w)$ vary continuously. However, $g''(w)$ may jump. The linking number of the strip made by Γ and its principal normal is α_{SL}, the self-linking number[24] of Γ:

$$\alpha_{\mathrm{SL}} = w + \int_\Gamma \tau \frac{ds}{2\pi} \tag{56}$$

According to Eq. (23), we write

$$\alpha_{\mathrm{SL}} = w + g'(w)/4 + h(w) \tag{57}$$

The parity of the integer α_{SL} does not change when Γ varies continuously, unless the torsion gets infinite at some point.[24] When w goes through 1, α_{SL} jumps from 0 to 1 and $h(w)$ jumps together. Therefore, $g''(w)$ may be discontinuous. The rubber tubing available to us remained circular and stable for $c = 1$. However, when a couple was applied to the rod in the plane of the circle while the constraint remained equal to 1, a stable 8-shaped conformation appeared. That is why we assume that although $g''(1-) = -4$, $g''(1+) = 0$. The polynomial

$$g_p(w) = 1.848 + 5.802\,(w-1)^3 - 4.351(w-1)^4 \tag{58}$$

satisfies the requirement. Moreover, the expressions (58) and (48) and their first derivatives are equal for $w = 1$. The polynomial (58) fits the estimation of $g(2)$ given on Table II.

Figure 3 shows how w varies with c, assuming the ratio $2e$ equal to 3.5, when w lies between 0 and 2. Stable (observable) states correspond to the branch with a positive derivative. The corresponding variations of the energy of superhelix formation U are shown in Fig. 4. When c is less than 1, only the circular shape is found. When c is less than 1, only the circular shape is found. When c is larger than 1, two stable states are found: the circular and the 8-shaped ones. An activation energy is necessary to

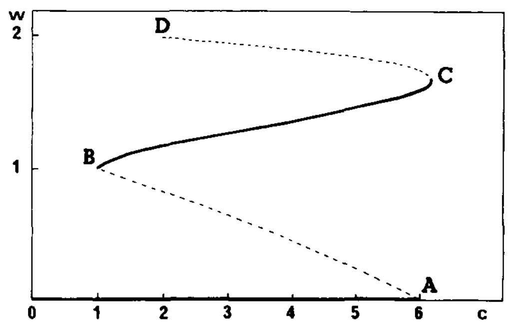

Fig. 3. Plot of w versus c for a closed elastic rod of parameter $e = A/2\kappa = \sqrt{3}$ like DNA. The bold lines (OA) and (BC) represent locally stable states.

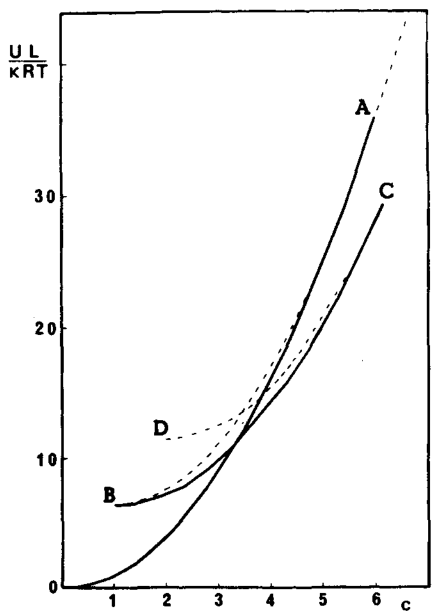

Fig. 4. The energy of superhelix formation U of an elastic rod of parameter $e = \sqrt{3}$ is plotted using the dimensionless ratio $UL/\kappa RT$ versus the number of constraint turns c. The segment of parabola OA represents locally stable equilibrium states of an unwrithed twisted circle. The line BC represents locally stable equilibrium states of an 8-shaped rod. The arcs CD and AB are unstable equilibrium states. Because of the presence of the arc AB, the circle is not stable when c is larger than 6: it will spontaneously writhe. The 8-shaped conformation is more stable than the circular one when c is larger than 3.3.

transform the circle into the 8-shaped conformation. It is approximately equal to $29.6\kappa RT/L$ when $c = 1$ and decreases when c increases up to 6. The free energy change associated with the transformation of the circle into the 8-shaped conformation varies from $+5.4$ to $-8\kappa RT/L$ when c varies from 1 to 6. For c larger than 6, the circular conformation is never stable and will spontaneously assume the 8-shaped conformation. In spite of the complexity of the formation of the first superturn, the energy of superhelix formation behaves as $\kappa RTc^2/L$ at a first approximation. Apparently, the twisting and the superhelix formation parameters are equal.

CONCLUSION

In the framework of the first-order theory of elasticity, the relationship between the number of constraint turns and the writhing number is complex. The writhing number may remain equal to zero for a finite number of constraint turns, at least when it is less than a critical value. As the number of constraint turns increases continuously, the writhing number may jump to and from allowed ranges of values. In spite of these discontinuities, the energy of superhelix formation increases like c^2, at least if c is not too large.

Such catastrophic conformational changes of DNA are difficult to observe because of the fluctuations in c. These fluctuations are first provoked by the heterogeneity[27] in the linking number of DNAs of natural origin. A second source of fluctuation is provoked by the use of ligands (mono-, bis-intercalating[28] dyes, proteins) to unwind the DNA. The ratio of bound ligand per base pair r fluctuates about its average $\bar{r}$. The fluctuation is[29]

$$\Delta r^2 = \langle (r - \bar{r})^2 \rangle = \frac{\partial \bar{r}}{\partial \ln x} \tag{59}$$

where x is the product of the intrinsic affinity by the free concentration of the ligand. The multisite binding of a ligand covering ν consecutive base pairs of a covalent double-stranded DNA satisfies to[30]

$$\frac{\bar{r}}{x} = (1 - \nu\bar{r})^{\nu}[1 - (\nu - 1)\bar{r}]^{1-\nu} \exp\left(-\frac{dU}{LRT\, d\bar{r}}\right) \tag{60}$$

If ϕ is the unwinding angle of the ligand, Eqs. (59) and (60) give

$$\frac{1}{\Delta c^2} = \frac{1}{L\phi^2\bar{r}(1 - \nu\bar{r})(1 - (\nu - 1)\bar{r})} + \frac{d^2(U/RT)}{dc^2} \tag{61}$$

which does not depend on the affinity of the ligand for DNA. When natural DNAs are set in the relaxed conformation by ethidium, $(\Delta c^2)^{1/2}$ is close to its maximum (1.6 turns for PM2 DNA). These fluctuations and the heterogeneity in the linking number smooth the apparent relationship between the number of constraint turns and the writhing number. Nevertheless, sudden large conformational changes should be observed in short DNAs.

The occurrence of toroidal tertiary turns has been qualitatively predicted using first-order elasticity theory.[16] We actually found such conformations. However, they are unstable and should not be observed in the frame of this theory.

Assuming that the twisting free energy is proportional to the square of the twist, the conformational free energy $g_{ap}(w)$ fitting the experimental data increases like w^2, or at most like $w^{3/2}$. At large values of w, the discrepancy between $g(w)$ and $g_{ap}(w)$ may be explained: the electrostatic repulsion between the parts of the DNA getting closer and the finite thickness of the DNA preventing the minimum of the bending energy from being attained. The variation of the entropy of the conformations of a given w may also play a role. But how the density of states of given w and given energy varies with w is still unknown.

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