Kink instability in circular DNA studied as Helfrich chiral chains

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The mechanism of kink instability observed in short DNA circles [W. Han *et al.*, Nature (London) **386**, 563 (1997)] has been analyzed based on the model of chiral elasticity for helical fibers proposed by Helfrich [Langmuir **7**, 567 (1991)]. It is shown theoretically that the circle is the planar solution of the equilibrium shape equations derived within this model and its stability is studied as the function of the elastic moduli of DNA. It is found that above some thresholds of the chiral modulus, k_3, the circular DNA can be deformed into elliptical, triangular, square, or other polygonal shapes. The predicted shape transformation is found in good agreement with the above kink instability observed in DNA.
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DNA is the primary genetic material of most organisms. It is a double helix in which two polynucleotide chains wind right-handedly around a common central axis. Over the past two decades, several important investigations have been done on the elastic properties of DNA chains (see, for example, Refs. [1–3]), the motivation of which being that DNA's elastic properties are very important in determining its higher-order (tertiary) structures and biological functions. In prokaryotic cells of viruses and bacteria, many circular DNA molecules are free of binding with proteins and they are often found to be supercoiled to form interwound configurations. It has been found that the particularly structural and elastic properties of DNA play a key role in this supercoiling mechanism [1]. In eukaryotic genomes of higher organisms, DNA molecules are highly packed and folded onto histone proteins to form chromosomes [4], the structure and stability of which are thought to be directly related to DNA's elasticity.

With the advent of new probing techniques, such as real-time fluorescence microscopy [5], force-measuring laser tweezers [6], and magnetic optical microscopy [7], it has become possible to study the elastic properties of a single DNA molecule directly. For a long linear DNA pulled with low or moderate external forces, force-extension experiment [7] showed that its elasticity is mainly caused by entropic effects, and the chain can be well described as a wormlike chain (WLC) [8] with a bending persistence length A of about 50 nm [2,9] and the internal energy being $E=(1/2)k_BTA\int_0^L\kappa^2 ds$, where κ and s are, respectively, the curvature and the arc length along the DNA central axis and L is its total length. However, when the external force becomes large enough [6] or if the chain is torsionally constrained (thus axially strained by local writhing [1,10–12]), the situation is much more complicated: Very recently, non-WLC elastic behaviors have been observed [6], and the DNA was shown to be a strongly chiral molecule [10]. Thus, these experiments [6,10] revealed that when the structural elasticity of a DNA chain becomes more important than its entropic elasticity caused by thermal fluctuations, it is necessary to develop a more appropriate model than the simple WLC one mentioned above to explain these non-WLC and chiral behaviors. This is especially important in chromatin studies, where between adjacent binding cites with proteins the DNA's form short loops whose entropic effects are negligible [4].

It has already been suggested by Crick and Klug [13] several years ago that chromosomal DNA in the nucleosome might be kinked while binding with histone proteins, however a very recent high-resolution x-ray structure shows that the DNA in the nucleosome is smoothly bent [4]. A direct experimental observation on such kinks was performed recently by Han *et al.* [14,15]. In their experiment [15], kink instability of short circular DNA's was indeed observed as they are axially strained. Obviously, this kink phenomenon cannot be explained under the framework of WLC theory in which only bending energy of harmonic form is contained. It is shown below that for the WLC model a planar circle is always stable with respect to small deformations. There have been a number of papers analyzing the stability of DNA models (see [16,17], for example), however among them no one considered the pseudoscalar elasticity caused by torsion-bending coupling [see, for example, Eq. (14) in [17], which is purely scalar]. The coupling is due to the chirality of the chain and is the origin for a phase transition from a straight line to a helix, first analyzed by Helfrich [18]. To understand DNA chiral elasticity and the mechanism of the kink instability (an obvious shape phase transition), it is natural that we invoke the new model for chiral polymer chains to determine whether or not the kink found in [15] is caused by the same coupling. This model may be viewed as a fourth-order Landau expansion of the elastic energy of the curvature and torsion of a string. The most important point of Helfrich's theory [18] is the mentioned bend-torsion coupling induced by chirality [the k_3 term in Eq. (1)]. Using this model we have investigated the elastic properties and deformation of short DNA circles. It is shown theoretically that the circle is the planar solution of the equilibrium shape equations derived within the model and its stability is studied as a function of the elastic moduli of DNA. It is found that above some thresholds of the chiral modulus, k_3, the circular DNA can be deformed into elliptical, triangular, square, or other polygonal shapes, respectively. The predicted shape transfor-

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mation is found to be in good agreement with the above kink instability observed in DNA [15].

We remark here that since DNA is a double-stranded molecule, twisting elastic energy as well as the topological restraint for closed DNA [16] should in general also be considered, and some authors have done investigations based on twist-bend coupling [19] or twist-stretch coupling [20]. However, in the case in which we are interested, the effect of the latter coupling is negligible since it is important only for forces >10 pN [20], and from the viewpoint of pure elasticity, energy terms related to twisting will have a remarkable effect only when the chain is topologically constrained with excess or deficit-linking numbers larger than a threshold [16]. However, considering the fact that for the two kinds of DNA circles investigated experimentally [15] the base-pair numbers, 126 and 168, respectively, are just integral multiples of 10.5, which is the well-known base-pair number per helix of the untwisted DNA duplex, we can infer that there is no remarkable difference between the factual linking number and the equilibrium one, so the kink instability may be caused mainly by axial strains, i.e., by a ring closure constraint. Thus, for us to understand the present kink phenomena, it might be appropriate to first consider bending energies, and to take into account the constraint of fixed chain length to generate axial stress.

In the Helfrich elasticity energy model of chiral chains [18], the elastic energy per unit length, g, is given by

$$g=\tfrac{1}{2}k_2\kappa^2+k_3\kappa^2\tau+\tfrac{1}{4}k_{22}\kappa^4+\tfrac{1}{2}k_4(\kappa_s^2+\kappa^2\tau^2), \tag{1}$$

where κ and τ are the curvature and torsion, respectively, $\kappa_s=d\kappa/ds$, k_2, k_{22}, k_4, and k_3 are the elastic moduli, where k_2 may be seen as $k_2=k_BTA$ in the WLC model [9], and the last one, k_3, is only characteristic of chiral materials. The equilibrium shape of the chiral chain is then determined by the minimization of the shape energy, which is

$$G=\int_0^L g\,ds+\lambda\int_0^L ds, \tag{2}$$

where λ is the Lagrange multiplier to take account of the constant length of the chiral chain, L. From the first-order variation equation, $\delta^{(1)}G=0$, the shape equations of the chiral chain have been derived by Ou-Yang and Helfrich [21],

$$\begin{aligned}&\tfrac{1}{2}k_2(\kappa^3-2\kappa\tau^2+2\kappa_{ss})-\lambda\kappa+k_3(3\kappa^3\tau-2\kappa\tau^3+6\kappa_s\tau_s+2\kappa\tau_{ss}+6\kappa_{ss}\tau)+\tfrac{1}{4}k_{22}(3\kappa^5-4\kappa^3\tau^2+24\kappa\kappa_s^2+12\kappa^2\kappa_{ss})\\&\quad+k_4(\tfrac{5}{2}\kappa^3\tau^2-\kappa\tau^4+\tfrac{1}{2}\kappa\kappa_s^2-\kappa^2\kappa_{ss}-\kappa_{ssss}+6\kappa_{ss}\tau^2+12\kappa_s\tau\tau_s+4\kappa\tau\tau_{ss}+3\kappa\tau_s^2)=0\end{aligned} \tag{3}$$

and

$$\begin{aligned}&\tfrac{1}{2}k_2(4\kappa_s\tau+2\kappa\tau_s)+k_3(6\kappa_s\tau^2+6\kappa\tau\tau_s-3\kappa^2\kappa_s-2\kappa_{sss})+\tfrac{1}{4}k_{22}(4\kappa^3\tau_s+24\kappa^2\kappa_s\tau)\\&\quad+k_4(4\kappa_s\tau^3+6\kappa\tau^2\tau_s-3\kappa^2\kappa_s\tau-\kappa^3\tau_s-4\kappa_s\tau_{ss}-6\kappa_{ss}\tau_s-4\kappa_{sss}\tau-\kappa\tau_{sss})=0,\end{aligned} \tag{4}$$

where $\kappa_{ss}=d^2\kappa/ds^2$, $\kappa_{sss}=d^3\kappa/ds^3$, $\kappa_{ssss}=d^4\kappa/ds^4$, and also for τ_s, τ_{ss}, and τ_{sss}.

It is obvious that a planar circle of radius $R=L/2\pi$ is always the solution satisfying Eqs. (3) and (4) with $\tau=0$, $\kappa=1/R$, and $\lambda=(1/2)k_2R^{-2}+(3/4)k_{22}R^{-4}$. Such a circle in the x-y plane can be described as $\mathbf{r}=(R\cos\kappa s,\ R\sin\kappa s,\ 0)$. In the experiment of Ref. [15], stable circles are indeed observed in an atomic force microscopy under appropriate ionic conditions. However, if the ionic environment is changed, in some cases the circles become unstable and change to other kinked shapes. The elastic moduli k_2, k_3, k_{22}, and k_4 may be sensitive to ionic conditions and their values are different for different environments [6]. It is possible that the kink instability is caused by this change of elastic moduli, especially k_3. Now we will discuss this in some detail.

To analyze a circle's stability, we consider a slightly distorted closed curve from the circle

$$\mathbf{r}'=\mathbf{r}+\phi(s)\mathbf{n}+\psi(s)\mathbf{b}, \tag{5}$$

where $\mathbf{n}$ and $\mathbf{b}$ are, respectively, the normal and binormal unit vectors of the original circle, and we can expand the two functions ϕ and ψ as the following Fourier series:

$$\begin{aligned}\phi&=c_0+\sum_{n=1}^{\infty}c_n\cos n\kappa s+\sum_{n=1}^{\infty}d_n\sin n\kappa s,\\ \psi&=e_0+\sum_{n=1}^{\infty}e_n\cos n\kappa s+\sum_{n=1}^{\infty}h_n\sin n\kappa s.\end{aligned} \tag{6}$$

With a lengthy calculation we have obtained the corresponding deformation energy as follows:

$$\begin{aligned}\delta^{(2)}G=&(k_2\kappa^3+3k_{22}\kappa^5)\pi c_0^2+\sum_{n=1}^{\infty}[p_nd_n^2+r_ne_n^2-2q_nd_ne_n]\\&+\sum_{n=1}^{\infty}[p_nc_n^2+r_nh_n^2+2q_nc_nh_n],\end{aligned} \tag{7}$$

with

$$\begin{aligned}p_n&=\tfrac{1}{2}\pi\kappa^3(n^2-1)^2(k_2+3k_{22}\kappa^2+n^2k_4\kappa^2),\\ r_n&=\tfrac{1}{2}\pi\kappa^3n^2(n^2-1)[k_2+k_{22}\kappa^2+(n^2-1)k_4\kappa^2],\\ q_n&=\tfrac{1}{2}\pi\kappa^4k_3n(n^2-1)(2n^2-3).\end{aligned} \tag{8}$$

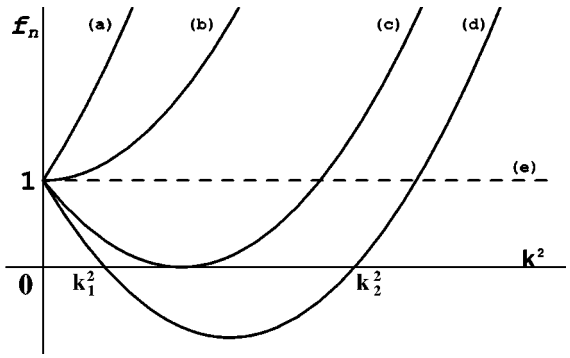

FIG. 1. The behavior of $f_n(\kappa^2)$ as a function of κ^2. The curves a, b, c, and d show, respectively, the situation when $k_3^2=0$, $b_n=0$, $\Delta\equiv b_n^2-4a_n=0$, and $\Delta>0$. For the four cases k_3^2 increases orderly. The horizental line (e) shows the behavior of $f_n(\kappa^2)$ as $k_3=k_4=k_{22}=0$, the case of WLC chains.

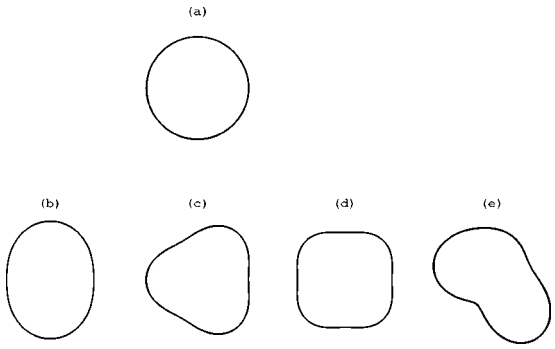

FIG. 2. Schematic illustration of polygonal shape transformation pathways of DNA circles calculated with Eq. (6) to compare the observation in experiment of the transition by cationic induction [14]. The DNA circles shown in (a) with radius $R=1$ are transformed into polygonal shapes with top views shown in (b) elliptical (with $c_2=0.15$, $d_2=0$), (c) triangular (with $c_3=0.13$, $d_3=0$), or (d) square (with $c_4=0.076$, $d_4=0$) shapes. (e) shows an irregularly deformed shape as the coactivation of modes (c_3,h_3) and (d_2,e_2) (with $c_3=0.13$, $d_2=0.29$).

In Eq. (7), apparently, the terms associated with $n=1$ are zero. This is consistent with the fact that the deformation mode of $n=1$ is only a trivial translation. We found from Eq. (7) that deformations along the normal and binormal directions are coupled with each other, i.e., d_n with e_n and c_n with h_n. In other words, Eq. (7) is a sum of the quadratic forms of (d_n,e_n) and (c_n,h_n) terms. It is clear that both quadratic forms of the n mode will be negative whenever $q_n^2-p_nr_n>0$. This leads to

$$f_n(\kappa^2)\equiv 1+b_n\kappa^2+a_n\kappa^4<0, \quad n\geqslant 2, \tag{9}$$

where

$$a_n=\left(n^2\frac{k_4}{k_2}+3\frac{k_{22}}{k_2}\right)\left((n^2-1)\frac{k_4}{k_2}+\frac{k_{22}}{k_2}\right),$$
$$b_n=\left((2n^2-1)\frac{k_4}{k_2}+4\frac{k_{22}}{k_2}\right)-\frac{4\left(n^2-\frac{3}{2}\right)^2}{(n^2-1)}\left(\frac{k_3}{k_2}\right)^2. \tag{10}$$

Inequality (9) is the instability condition for the circular configurations. If $f_n(\kappa^2)>0$ for any $n\geqslant 2$, the circle will be stable; however, if the circle happens to satisfy $f_n(\kappa^2)<0$ for at least one value of n, then it will no longer be stable and will change to other configurations. We found that this kind of instability is indeed induced by the chiral property of the chain, i.e., by nonzero k_3. This is demonstrated more clearly in Fig. 1. In Fig. 1 we see that f_n is always positive for any n if $k_3=0$ (curves a and e), while if $|k_3|$ becomes high enough to some threshold with environmental change, f_n may become negative (curves c and d), and the circle will deform into the nth deformation mode. We feel this is just the causation for the kink instability observed in the experiment of Ref. [15]. Figure 2 shows the top views of some deformation modes predicted theoretically; they are quite similar with the top views of actual DNA chains observed by atomic force microscopy [15] where the original circle kinked into polygonal shapes.

Inequality (9) shows that the nth deformation mode occurs whenever $\Delta=b_n^2-4a_n\geqslant 0$ (see curves c and d of Fig. 1). When $\Delta>0$, circles with curvature $\kappa\in(\kappa_1,\kappa_2)$ will be unstable, where κ_1 and κ_2 are the two solutions of $f_n(\kappa^2)=0$ (see curve d of Fig. 1). We should emphasize that the two roots, κ_1 and κ_2, are functions of n. Thus, at any specific environment condition where the elastic moduli k_2, k_3, k_{22}, and k_4 are all fixed, to determine whether a circle of certain length, i.e., of determined κ is stable or not, we need to know how κ_1 and κ_2 change with n. In Fig. 3 we have shown schematically the relation of κ_2^2 with n (the upper curve) and that of κ_1^2 with n (the lower curve), for some fixed elastic moduli. Circles with κ^2 lying in the range between these two curves will be unstable. It is obvious from Fig. 3 that when the elastic moduli is fixed, there exists a threshold radius of $R_c=1/\kappa_2$ for DNA to be unstable; DNA circles with radii less than this value will not deform, while those with radii higher than this value will be unstable. The prediction of a threshold stability radius is in good agreement with the experimental work of Han *et al.* [15], where kink deformations were observed for DNA circles with 168 base pairs but not for DNA circles 126 base pairs long.

It is useful to estimate the intensity of the elastic moduli. It is already known that the bending persistence length $A\simeq 50$ nm for DNA [9], thus $k_2=k_BTA\simeq 2\times 10^{-19}$ J nm at

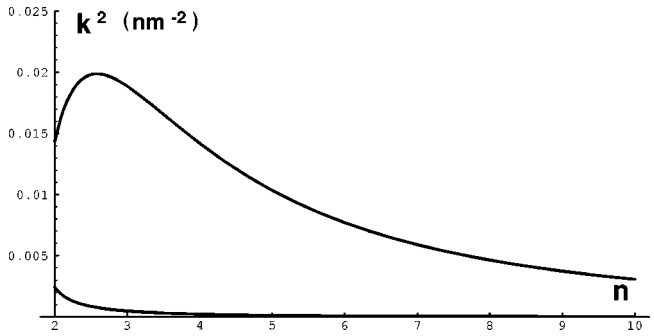

FIG. 3. Instability range of DNA circles. κ_1^2 and κ_2^2 [the two roots of $f_n(\kappa^2)$] as functions of n are calculated with Eq. (9) and shown by the lower curve and the upper curve, respectively. Between these two lines is the instability range for DNA circles. Here we use $k_3/k_2=10$ nm and $k_4/k_2=k_{22}/k_2=32$ nm^2 (see text for a detailed reason).

room temperature. For a rough estimate of the higher-order elastic moduli, we may set $k_4 \sim k_{22}$. Further, based on the usual data given by molecular biology, it is easy to evaluate that the 126 base pair DNA circle has a curvature $\kappa = 0.15$ nm^{-1}, and the 168 base pair DNA circle has $\kappa = 0.11$ nm^{-1}. Since the kink instability occurred for 168 base pair DNA and not for 126 base pair DNA, we may reasonably speculate that the threshold stability curvature lies between 0.11 nm^{-1} and 0.15 nm^{-1}. Based on the value of k_2 mentioned above, we have estimated that $k_3 \sim 2 \times 10^{-18}$ J nm^2 and $k_4 \sim k_{22} \sim 7 \times 10^{-18}$ J nm^3 at the experimental environment.

Finally, we should note that although the kink transition is attributed to the change of k_3 caused by changing divalent ions around DNA, the real case in biochemistry may be described as follows. According to [15], the difference between the ions used is chemical: zinc actually binds the DNA bases while Mg interacts electrostatically with the backbone phosphates. Therefore, the free energy of the two forms in solution may be comparable in the strained circles, but zinc binding stabilizes the kinked form. Another point is that the DNA used in these experiments was made from ligating specific ''A-tract'' sequences, therefore the present estimates of the elastic moduli k_i, k_{ij} and their relations shown in Fig. 1 may apply to only one kind of DNA. The specific sequences of DNA chosen in the experiment, ''A-tract'' sequences, which determine the elastic moduli and their variations with environment, are expected to be important for the occurrence of kinks. We also point out that based on this model even open DNA molecules consisting of ''A-tract'' sequences should become helical as they undergo the transition. This prediction should be examined by future experiment.

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