Elastic stability of DNA configurations. I. General theory

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Results are presented in the theory of the elastic rod model for DNA, among which are criteria enabling one to determine whether a calculated equilibrium configuration of a DNA segment is stable in the sense that it gives a local minimum to the sum of the segment's elastic energy and the potential of forces acting on it. The derived stability criteria are applicable to plasmids and to linear segments subject to strong anchoring end conditions. Their utility is illustrated with an example from the theory of configurations of the extranucleosomal loop of a DNA miniplasmid in a mononucleosome, with emphasis placed on the influence that nicking and ligation on one hand, and changes in the ratio of elastic coefficients on the other, have on the stability of equilibrium configurations. In that example, the configurations studied are calculated using an extension of the method of explicit solutions to cases in which the elastic rod modeling a DNA segment is considered impenetrable, and hence excluded volume effects and forces arising from self-contact are taken into account.

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I. INTRODUCTION

In this paper we derive various necessary conditions and sufficient conditions for the elastic stability of equilibrium configurations of DNA segments subject to the constraints that can arise from the presence of bound proteins and the topology of the segment. The results obtained hold in the theory of the commonly employed elastic rod model which treats a DNA segment as an inextensible rod with elastic properties characterized by two elastic constants, the flexural rigidity A and the torsional rigidity C. In that theory, the configuration of a DNA segment is determined once one knows the curve $\mathcal{C}$ representing the duplex axis and the density $\Delta\Omega$ of the excess twist (or ''overtwisting'') about $\mathcal{C}$. The total energy Φ of the segment is taken to be the sum of two terms. One term, Γ, is determined by $\mathcal{C}$ and accounts for the elastic bending energy and the possible presence of long range forces having a potential depending on $\mathcal{C}$. The other, Ψ_T, is the twisting energy:

$$\Phi=\Gamma+\Psi_T. \tag{1}$$

In conventional units,

$$\Psi_T=\frac{C}{2}\int_0^L \Delta\Omega(s)^2 ds, \tag{2}$$

with L the length of the segment and s the arc-length parameter along $\mathcal{C}$. When long range forces are absent, Γ reduces to the bending energy, Ψ_B, which is determined by the curvature κ of $\mathcal{C}$:

$$\Psi_B=\frac{A}{2}\int_0^L \kappa(s)^2 ds. \tag{3}$$

Excluded volume effects and the possibility that the segment makes contact with itself are taken into account by treating a DNA segment as an impenetrable rod with circular cross section.

So as to give the reader an idea of the nature of our theory of stability, we now state several definitions and give a summary of our principal results. The precise meaning of some of the terms employed in this Introduction will be clarified later in the paper.

An equilibrium configuration, i.e., a configuration for which the first variation of Φ vanishes for variations in configuration compatible with the imposed constraints, is here called stable if it gives a strict local minimum to Φ in the class of configurations compatible with the constraints. (The meaning of the term ''strict local minimum'' is discussed in Sec. II.) The imposed constraints include those that follow from the assumption that the DNA segment, which we treat as an impenetrable rod, either is a plasmid (i.e., a segment that is closed in the sense that each of its two DNA strands, and hence $\mathcal{C}$, forms a closed curve) or is subject to strong anchoring end conditions.

There is a topological invariant $\Delta\mathcal{L}$, called *excess link*, which is meaningful for plasmids but can be defined also for open segments (also called linear segments) that are subject to strong anchoring end conditions. In both cases, $\Delta\mathcal{L}$ is related to the total excess twist $\Delta\mathcal{T}$ of the segment and an appropriately defined writhe $\mathcal{W}$ by the relation

$$\Delta\mathcal{L}=\mathcal{W}+\Delta\mathcal{T}, \tag{4}$$

which for plasmids is equivalent to a well-known result of Calugareanu [1] and White [2]. Of course, $\Delta\mathcal{T}$ (in turns) is related as follows to $\Delta\Omega$ (in radians per unit length):

$$\Delta\mathcal{T}=\frac{1}{2\pi}\int_0^L \Delta\Omega\, ds. \tag{5}$$

The writhe $\mathcal{W}$, on the other hand, like Γ in Eq. (1), is determined by the curve $\mathcal{C}$. The fact that Φ, which is minimized

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by a stable configuration, and $\Delta\mathcal{L}$, which is preserved in variations, each can be expressed as a sum of a term depending only on $\mathcal{C}$, and a term depending only on $\Delta\Omega$, plays an important role in the derivation of stability criteria.

In our treatment of necessary conditions for stability, we follow up on ideas given in a paper of Le Bret [3] and consider cases in which the configuration whose stability is being investigated is a member of a one-parameter family E of equilibrium configurations of segments that differ only in their values of $\Delta\mathcal{L}$, a parameter that can be taken to vary continuously. (See the discussion at the beginning of Sec. III.) We show that if the configuration is stable, then the slope of the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ for E is not negative. Thus, the relation $d\Delta\mathcal{L}^E/d\mathcal{W}\geqslant 0$, which we call the E condition, is a necessary condition for stability. (This condition was obtained in a different form by Le Bret [3] for the important special case of a protein-free plasmid without long range forces affecting Γ.)

An equilibrium configuration in E for which the excess twist density $\Delta\Omega$ vanishes, remains an equilibrium configuration when the segment is nicked, i.e., when one of the two DNA strands is severed, an operation that eliminates the segment's ability to support a torsional moment. We show that if a configuration with $\Delta\Omega\equiv 0$, when considered a configuration of an intact segment, is stable, with $d\Delta\mathcal{L}^E/d\mathcal{W}<1$, it does not remain stable after nicking. In other words, the relation $d\Delta\mathcal{L}^E/d\mathcal{W}\geqslant 1$, called here the n condition, is a necessary condition for an equilibrium configuration in E to be stable both before and after nicking. In Sec. IV (in the discussion of Fig. 4), we give an example of a case in which there are three values of $\Delta\mathcal{L}$ that give rise to equilibrium configurations with $\Delta\Omega\equiv 0$ with all three stable (in fact, globally stable) while the segment remains intact, but such that one becomes unstable and two remain stable when the segment is nicked.

Since an equilibrium configuration of a segment is stable only if the corresponding configuration of each of the subsegments is stable when subject to appropriate constraints, one can obtain a strengthened form of the E condition, which we call the θ condition: In order for an equilibrium configuration of a segment of length L to be stable, it is necessary that, for each ξ between 0 and L, there holds $\theta(\xi)\geqslant 0$, where $\theta(\xi)$ is, by definition, the slope of the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ for the family of equilibrium configurations of the subsegment for which $0\leqslant s\leqslant\xi$, when the subsegment is subject to the strong anchoring end conditions that would be imposed on it if its complementary subsegment of length $L-\xi$ were held rigid.

The importance of the θ condition lies in the fact that families of equilibrium configurations can contain configurations that obey the E condition but not the θ condition, and hence are unstable. (Examples in which such is the case are given in the accompanying paper on supercoiled configurations of plasmids [4], here referred to as ''paper II.'')

Another necessary condition for the stability of an equilibrium configuration is that the curve $\mathcal{C}$, representing the duplex axis, give a local minimum to Γ in the class of curves that obey the same geometric and topological constraints, and have the same writhe as $\mathcal{C}$. We call this condition the $\mathcal{W}$ condition.

We show further that strengthened forms of the E condition and the $\mathcal{W}$ condition, taken together, yield a condition sufficient for stability, called the S condition, which we use in the discussion of the example treated in Sec. IV.

Three of the necessary conditions for stability that we present, i.e., the E, n, and θ conditions, relate the stability of an equilibrium configuration to graphs of $\Delta\mathcal{L}$ versus $\mathcal{W}$. Hence, application of these conditions requires an efficient method of finding equilibrium configurations of specified $\Delta\mathcal{L}$ and calculating their writhe.

When $\Gamma=\Psi_B$, exact closed form solutions of the equilibrium equations can be derived, and adjustment of integration constants yields explicit representations for equilibrium configurations compatible with the imposed constraints [5–7]. In previous applications of such a procedure to DNA segments, attention was restricted to configurations free from self-contact. We recently derived a generalization of the procedure to cases, such as the present, in which the cross sections are circular and impenetrable; that ''generalized method of explicit solutions'' is used here to calculate the configurations of DNA segments of a type called ''extranucleosomal loops'' (see, e.g., Sec. IV, Fig. 1, and Refs. [7] and [8]).

Adjustment of integration constants to obtain configurations with prescribed $\Delta\mathcal{L}$ requires, by Eq. (4), repeated calculations of $\mathcal{W}$, which can be a delicate and time-consuming matter. However, once the explicit expression for $\mathcal{C}$ is available, a closed form relation can be obtained for the integral along $\mathcal{C}$ of the geometric torsion [7], and it follows from observations of Calugareanu [1] and Pohl [9] that $\mathcal{W}$ differs from the torsion integral by an integer that we have found not difficult to evaluate. Thus, writhe calculations were not a major difficulty in the present research. In addition, there is now available an easily evaluated algebraic formula relating the elastic energy of an equilibrium configuration directly to the integration constants [6]. Without the new explicit representations of solutions and computational methods for $\mathcal{W}$ and Ψ_B based on these representations, precise calculations of configurations and detailed analysis of their stability would be far more difficult to perform.

In earlier work on closed form solutions of the equilibrium equations [6], we have given examples of cases in which a nicked segment of DNA subject to strong anchoring end conditions can have two equilibrium configurations with one stable and the other not (according to the present definition of stability). The conclusions about the stability of nicked configurations made in Ref. [6] were reached using criteria derived in this paper.

II. DEFINITIONS, ASSUMPTIONS, AND PRELIMINARY RESULTS

As we remarked in the Introduction, in the present theory the configuration $\mathcal{Z}$ of a segment of DNA of length L is specified by giving (i) the space curve $\mathcal{C}$ traced out by the duplex axis and (ii) the excess twist density $\Delta\Omega$ as a function of the distance s along $\mathcal{C}$; $\Delta\Omega$ is the difference between the twist density in the present state and in the torsionally relaxed, stress-free state.

For a plasmid, $\mathcal{C}$ is a closed curve. It has been known for some time that Eq. (4) holds for a plasmid, i.e., that the sum

of the excess twist $\Delta\mathcal{T}$ and the writhe $\mathcal{W}$ is a constant $\Delta\mathcal{L}$ that is topological in the sense that it is the same for all configurations that the plasmid can attain, at fixed temperature and chemical composition of the medium, without cutting one or both of its two strands. This conclusion follows from the now familiar theorem that, for a plasmid, $\mathcal{L}$, the Gauss linking number for a DNA strand and the duplex axis $\mathcal{C}$, obeys the relation [1,2] $\mathcal{L}=\mathcal{W}+\mathcal{T}$, in which $\mathcal{T}$ is the total twist of the strand about $\mathcal{C}$ in the present configuration, and $\mathcal{W}$ is the writhe of $\mathcal{C}$. If we write $\mathcal{T}_0$ for the value of $\mathcal{T}$ when the DNA segment is relaxed (i.e., stress-free), put $\Delta\mathcal{L}=\mathcal{L}-\mathcal{T}_0$, and note that $\Delta\mathcal{T}=\mathcal{T}-\mathcal{T}_0$, then Eq. (4) is the same as the relation $\mathcal{L}=\mathcal{W}+\mathcal{T}$. Although $\mathcal{L}$ is an integer and is independent of the temperature and chemical composition of the medium, $\mathcal{T}_0$ and $\Delta\mathcal{L}$ are not.

There are several equivalent ways of defining the *writhe* of a closed curve (see, e.g., the exposition of White [10]). One way [11] is to average, over all orientations of a plane, the sum of the (signed) self-crossings in the projection of the curve on the plane and set $\mathcal{W}$ equal to that average. A circle, or more generally, a plane curve that does not cross itself, has zero writhe, and a flat figure eight has a writhe of magnitude one.

There are important examples of open segments of DNA, for which one can define a writhe $\mathcal{W}$ and excess link $\Delta\mathcal{L}$, in such a way that Eq. (4) holds again with $\Delta\mathcal{L}$ constant for an appropriate class of deformations of the segment. This can be done when the location and the orientation of the base pairs at each end of the (open) segment are specified. Such a segment is said to be subject to *strong anchoring end conditions*; for it the endpoints of both the duplex axis $\mathcal{C}$ and the two DNA strands, and also the tangents to $\mathcal{C}$ at its ends, remain fixed during variations in configuration. One can show that if one joins the ends of $\mathcal{C}$ with a *fixed* curve $\mathcal{C}^*$, identifies $\mathcal{W}$ in Eq. (4) with the writhe of the closed curve formed by $\mathcal{C}$ and $\mathcal{C}^*$, and again takes $\Delta\mathcal{T}$ to be the total excess twist of the segment, then $\mathcal{W}+\Delta\mathcal{T}$ will remain constant as the configuration of the segment is varied, provided, of course, that the deformation is not such that $\mathcal{C}$ passes through $\mathcal{C}^*$. The constant $\Delta\mathcal{L}$ will then depend on how the curve $\mathcal{C}^*$ is chosen.

The extranucleosomal loops of mononucleosomes (treated in Ref. [7] and here in Sec. IV) are examples of open segments for which there is precisely one natural choice for $\mathcal{C}^*$, and it is clear that, when that choice is made, the class of admissible variations is such that $\mathcal{C}$ does not cross $\mathcal{C}^*$ [12].

Since we are treating DNA segments as inextensible, homogeneous, kinematically symmetric, intrinsically straight, elastic rods obeying the classical theory of Kirchhoff, the elastic energy Ψ of a segment is a sum,

$$\Psi=\Psi_B+\Psi_T, \tag{6}$$

in which, by Eqs. (2) and (3), the bending energy Ψ_B and the twisting energy Ψ_T in units of A/L are

$$\Psi_B=\frac{L}{2}\int_0^L \kappa(s)^2 ds, \quad \Psi_T=\frac{\omega L}{2}\int_0^L \Delta\Omega(s)^2 ds, \tag{7}$$

with

$$\omega=C/A. \tag{8}$$

Our treatment of equilibrium states and their stability is confined to DNA segments that either are closed or are open and subject to strong anchoring end conditions. The theory we develop is sufficiently general to allow for the possibility that a segment is subject to conservative forces that act along its length, such as the long-range electrostatic forces that can arise from its interaction with itself or with stationary objects. We assume, however, that the potential P of such forces is a function of $\mathcal{C}$ alone and hence is independent of $\Delta\Omega$. We write Φ for the sum of the potential P and the elastic energy Ψ:

$$\Phi=\Phi(\mathcal{Z})=\Gamma(\mathcal{C})+\Psi_T(\Delta\Omega), \quad \Gamma(\mathcal{C})=P(\mathcal{C})+\Psi_B(\mathcal{C}). \tag{9}$$

Our theory does not require the assumption that the segment be free from self-contact or from contact with stationary rigid bodies, but we do suppose that: (i) when such contact occurs, changes in configuration do no work against the forces and moments exerted at a contact point, (ii) the contact forces are normal to the surface of the segment, and (iii) the moment exerted at a contact point has no component along the tangent to $\mathcal{C}$ and hence does not affect $\Delta\Omega(s)$.

We say that a configuration $\mathcal{Z}^\#$ characterizes a state of mechanical equilibrium, or, for short, is an *equilibrium configuration*, if $\delta\Phi$, the first variation of Φ, vanishes for every variation $\delta\mathcal{Z}$ in configuration that is *admissible* in the sense that it is compatible with the imposed constraints, including (in the case of an open segment of DNA) the strong anchoring end conditions and (for both closed segments and open segments) the constraint that the topological properties of the segment (among which are the knot type of $\mathcal{C}$ and the value of $\Delta\mathcal{L}$) be preserved.

The relation

$$\delta\Phi=0, \tag{10}$$

or, equivalently,

$$\delta\Gamma=-\delta\Psi_T, \tag{11}$$

holds for each admissible variation $\delta\mathcal{Z}$ from an equilibrium configuration $\mathcal{Z}^\#$. Any variation from $\mathcal{Z}^\#$ that does not alter $\mathcal{C}$ and preserves $\Delta\mathcal{T}$ is admissible, and for it Eq. (11) reduces to $\delta\Psi_T=0$. Hence, a familiar argument tells us that, for an equilibrium configuration, $\Delta\Omega$ is constant along the segment and the second of Eqs. (7) reduces to

$$\Psi_T=2\pi^2\omega\Delta\mathcal{T}^2. \tag{12}$$

Because it is easy to show that of all twist density functions $\Delta\Omega(s)$ with a given value of $\Delta\mathcal{T}$, that for which $\Delta\Omega(s)=2\pi\Delta\mathcal{T}/L$ minimizes Ψ_T, from this point on we shall confine our attention to variations in configuration that keep $\Delta\Omega$ spatially uniform and therefore are such that

$$\delta\Psi_T=4\pi^2\omega\Delta\mathcal{T}\delta(\Delta\mathcal{T}). \tag{13}$$

This restriction will have no effect on the theory of the stability of equilibrium.

The requirement that each variation in configuration preserve $\Delta\mathcal{L}$ will be of particular importance in the treatment of the stability of equilibrium states given in the next section of the paper. For example, the variation in $\Gamma(\mathcal{C})$ due to a variation in $\mathcal{C}$, which at first sight appears difficult to calculate directly, can be evaluated for an equilibrium state by noting that Eqs. (11) and (13) and the relation

$$\delta\mathcal{W}+\delta(\Delta\mathcal{T})=0 \tag{14}$$

yield

$$\delta\Gamma=-4\pi^2\omega\Delta\mathcal{T}\delta(\Delta\mathcal{T})=4\pi^2\omega\Delta\mathcal{T}\delta\mathcal{W}. \tag{15}$$

This last relation implies that, for an equilibrium configuration,

$$\text{if}\quad \delta\mathcal{W}=0,\quad \text{then}\quad \delta\Psi_B=-\delta P. \tag{16}$$

We consider two configurations to be *equivalent* if the corresponding excess twist density functions $\Delta\Omega$ are the same and the corresponding duplex axes $\mathcal{C}$ are congruent. For example, all the configurations obtained by rotating a configuration of a closed segment in which $\mathcal{C}$ is a true circle and $\Delta\Omega$ is constant along $\mathcal{C}$ are equivalent (see also Ref. [13] §IIIB). Moreover, hypothetical traveling wave motions in which a closed segment attains equivalent equilibrium configurations of a ''figure-eight'' type are not unfamiliar in rod theory (see, e.g., Ref. [14], Fig. 4).

We call an equilibrium configuration $\mathcal{Z}^\#$ *stable* if Φ has a *strict local minimum* at $\mathcal{Z}^\#$ in the sense that $\mathcal{Z}^\#$ has a neighborhood [15] $\mathcal{N}$ such that $\Phi(\mathcal{Z})>\Phi(\mathcal{Z}^\#)$ for each $\mathcal{Z}$ in $\mathcal{N}$ that is not equivalent to $\mathcal{Z}^\#$, and can be reached from $\mathcal{Z}^\#$ by a homotopy (i.e., an appropriately regular one-parameter family) of configurations that are compatible with the imposed constraints.

This definition of stability, which requires that Φ have a strict *local* minimum, differs from an often used definition requiring Φ to have a *global* minimum. (See, e.g., the paper of Jülicher on supercoiled configurations of plasmids [16].) When global minimization is used to define stability, a configuration that is stable according to our definition may be only ''metastable.'' Global minimizers of Φ are of importance in many subjects, among which are the theory of topoisomer distributions in miniplasmids with bound proteins (cf. [7]). Our experience has indicated that conditions for local stability of the type we present here can facilitate the search for global minimizers of Φ. For an open segment, the answer to the question of whether a given local minimizer of Φ is a global minimizer depends in general upon the choice of the curve $\mathcal{C}^*$ employed to define $\mathcal{W}$ and $\Delta\mathcal{L}$.

For an equilibrium configuration $\mathcal{Z}^\#$ to be stable it is necessary, but not sufficient, that the second variation in Φ be nonnegative for each small admissible variation from $\mathcal{Z}^\#$:

$$\delta^2\Phi=\delta^2(\Gamma+\Psi_T)\geqslant 0. \tag{17}$$

In the next section we shall use the apparatus assembled here to obtain useful conditions for the stability of equilibrium states.

III. CONDITIONS FOR STABILITY

The topological invariant $\Delta\mathcal{L}$ can be altered. In the case of a plasmid, the cutting and subsequent ligation of a single DNA strand can result in a change in $\Delta\mathcal{L}$ by an integral value that depends on the relative number of full rotations made in the plane of the cut before ligation. The corresponding process for an open DNA segment is rotation about the tangents at the ends and can result in nonintegral changes in $\Delta\mathcal{L}$. Nonintegral changes in $\Delta\mathcal{L}$ can result also from changes in the twist associated with the torsionally relaxed DNA due, for example, to changes in temperature or solvent composition. Criteria to be derived here for stability of calculated configurations refer to virtual processes in which $\Delta\mathcal{L}$ varies continuously with the elastic coefficients A and C and the potential energy function P held fixed.

Let E be a smooth one-parameter family of equilibrium configurations corresponding to values of $\Delta\mathcal{L}$ in an open interval I which is sufficiently small that for each $\Delta\mathcal{L}$ in I there is a unique $\mathcal{Z}$, and hence a unique value of $\mathcal{W}$. Let J be the interval of values of $\mathcal{W}$ so obtained. The present discussion is confined to equilibrium families E for which one can take $\Delta\mathcal{L}$, $\Delta\mathcal{T}$, Γ, and Φ to be given by functions, $\Delta\mathcal{L}^E$, $\Delta\mathcal{T}^E$, Γ^E, Φ^E, of $\mathcal{W}$. With the exception of families that we shall specify below, this can be done for all $\mathcal{W}$ in J other than those that correspond to places where the graph of $\mathcal{W}$ versus $\Delta\mathcal{L}$ for E has a turning point with $d\mathcal{W}/d\Delta\mathcal{L}=0$. (A value of $\mathcal{W}$ that is singular in this sense need not have a neighborhood in which $\Delta\mathcal{L}$ is a single-valued function of $\mathcal{W}$.)

That there are exceptional families of equilibrium configurations for which $\Delta\mathcal{L}$ is not determined by $\mathcal{W}$ is a consequence of the fact that a segment of DNA that is in equilibrium and is such that $\mathcal{C}$ is a piece of a helix, an arc of a circle, or a straight line remains in equilibrium whenever $\Delta\mathcal{T}$ (and hence, $\Delta\mathcal{L}$) is changed with $\mathcal{C}$ (and hence $\mathcal{W}$) kept constant. The most important of the exceptional families of equilibrium configurations, namely the circular configurations of a protein-free plasmid, will be discussed in paper II, which deals with bifurcation diagrams for such plasmids.

Let $\mathcal{Z}^\#$ be the equilibrium configuration in the family E which gives a nonsingular value $\mathcal{W}^\#$ to the writhe. For each admissible variation $\delta\mathcal{Z}$ taking $\mathcal{Z}^\#$ into a configuration for which the curve $\mathcal{C}$ equals the duplex axis of a configuration that is both in E and near to $\mathcal{Z}^\#$, there holds

$$\delta\Gamma=\left[\frac{d\Gamma^E}{d\mathcal{W}}\right]_{\mathcal{W}=\mathcal{W}^\#}\delta\mathcal{W}, \tag{18}$$

$$\delta^2\Gamma=\left[\frac{d^2\Gamma^E}{d\mathcal{W}^2}\right]_{\mathcal{W}=\mathcal{W}^\#}(\delta\mathcal{W})^2. \tag{19}$$

It follows from Eqs. (15) and (18) that, along the family E, the derivative of Γ with respect to the writhe of $\mathcal{C}$ is, to within the (constant) factor $4\pi^2\omega$, the total excess twist [17]:

$$\frac{d\Gamma^E}{d\mathcal{W}}=4\pi^2\omega\Delta\mathcal{T}^E. \tag{20}$$

Equations (19), (20), and (4) yield

$$\delta^2\Gamma = 4\pi^2\omega\left(\frac{d\Delta\mathcal{L}^E}{d\mathcal{W}} - 1\right)(\delta\mathcal{W})^2. \tag{21}$$

Since we are considering variations that keep the excess twist density uniform along $\mathcal{C}$, we have, by Eq. (13),

$$\delta^2\Psi_T = 4\pi^2\omega\,\delta(\Delta\mathcal{T})^2, \tag{22}$$

and hence, by Eq. (21),

$$\delta^2\Phi = 4\pi^2\omega\,\frac{d\Delta\mathcal{L}^E}{d\mathcal{W}}(\delta\mathcal{W})^2. \tag{23}$$

If the equilibrium configuration $\mathcal{Z}^\#$ is stable, the second variation of Φ is nonnegative for each admissible variation from that configuration. Thus, a *necessary condition* for $\mathcal{Z}^\#$ to be stable is that at the point in E where $\mathcal{W}=\mathcal{W}^\#$,

$$\frac{d\Delta\mathcal{L}^E}{d\mathcal{W}} \geqslant 0. \tag{24}$$

This condition on a derivative along E, called the *E condition*, is far from sufficient for stability. In paper II we present examples of configurations of plasmids that obey the condition, but for which there are admissible variations that violate the following, also necessary, condition for an equilibrium configuration $\mathcal{Z}^\#$ to be stable:

$$\text{If } \delta\mathcal{Z} \text{ is such that } \delta\mathcal{W}=0, \quad \text{then} \quad \delta^2\Gamma \geqslant 0. \tag{25}$$

That this, the $\mathcal{W}$ *condition*, is necessary for the stability of $\mathcal{Z}^\#$ may be verified by using the relation (17) and noting that $\delta^2\Psi_T=0$ for those admissible variations $\delta\mathcal{Z}$ that leave $\Delta\Omega$ the same and change $\mathcal{C}$ without changing $\mathcal{W}$.

The $\mathcal{W}$ condition, like the E condition, is not, by itself, sufficient for stability. However, strengthened forms of the two conditions can be combined in the following way to obtain a sufficient condition which we call the *S condition*:

Let $\mathcal{Z}^\#$ be in a family E of equilibrium configurations such that (i) the curve $\mathcal{C}(\mathcal{Z}^\#)$ corresponding to $\mathcal{Z}^\#$ has (in an appropriate space of curves) a neighborhood $\mathcal{N}$ with the property that for each $\mathcal{Z}^*$ in E with writhe $\mathcal{W}^*$ close to the (nonsingular) writhe $\mathcal{W}^\#$ of $\mathcal{Z}^\#$, there holds $\Gamma(\mathcal{C}) > \Gamma(\mathcal{C}^E(\mathcal{Z}^*))$ for every $\mathcal{C}$ in $\mathcal{N}$ that has writhe $\mathcal{W}^*$, is compatible with the boundary or closure conditions imposed on the segment, and is not congruent to $\mathcal{C}(\mathcal{Z}^*)$, *and* (ii) the relation (24) holds with $\geqslant$ replaced by $>$.

When such is the case, $\Phi(\mathcal{Z})>\Phi(\mathcal{Z}^\#)$ for each configuration $\mathcal{Z}$ that is close but not equivalent to $\mathcal{Z}^\#$, and hence $\mathcal{Z}^\#$ is stable.

To prove the last assertion we let $\delta\mathcal{Z}$ be an admissible variation taking $\mathcal{Z}^\#$ into a configuration $\mathcal{Z}$ which is not equivalent to $\mathcal{Z}^\#$ but is such that $\mathcal{C}(\mathcal{Z})$ is in $\mathcal{N}$. We can consider $\delta\mathcal{Z}$ to be the result of the successive application of two admissible variations, $(\delta\mathcal{Z})_1$ and $(\delta\mathcal{Z})_2$, where $(\delta\mathcal{Z})_1$ is a variation of the type employed in the discussion containing Eqs. (18)–(23), and $(\delta\mathcal{Z})_2$ is a variation in which $\Delta\mathcal{T}$ (and hence $\mathcal{W}$) is held fixed while $\mathcal{C}$ changes in such a way that the configuration $\mathcal{Z}$ is attained. We then employ arguments similar to others in this section to show that neither $(\delta\mathcal{Z})_1$ nor $(\delta\mathcal{Z})_2$ decreases the energy Φ.

To be specific: the variation $(\delta\mathcal{Z})_1$ takes $\mathcal{Z}^\#$ into a configuration $\mathcal{Z}_1$ for which the duplex axis, $\mathcal{C}_1$, equals that of a configuration in E near to $\mathcal{Z}^\#$ with writhe equal to the writhe of $\mathcal{C}(\mathcal{Z})$. In view of Eq. (23), the hypothesis (ii) tells us that, when $\mathcal{Z}_1$ is not equivalent to $\mathcal{Z}^\#$, the second variation in Φ corresponding to $(\delta\mathcal{Z})_1$ is positive, and hence

$$\Phi(\mathcal{Z}_1) > \Phi(\mathcal{Z}^\#). \tag{26}$$

Since the variation $(\delta\mathcal{Z})_2$ does not change $\mathcal{W}$ or $\Delta\mathcal{T}$, the change it induces in Φ equals the change in Γ. In view of the hypothesis (i), $\mathcal{C}_1$ gives to Γ a strict minimum in the class of all curves in $\mathcal{N}$ with writhe equal to that of $\mathcal{C}(\mathcal{Z}_1)$, and hence, when $\mathcal{Z}$ is not equivalent to $\mathcal{Z}_1$,

$$\Phi(\mathcal{Z}) > \Phi(\mathcal{Z}_1). \tag{27}$$

As $\mathcal{Z}$ and $\mathcal{Z}^\#$ are not equivalent to each other, they cannot both be equivalent to $\mathcal{Z}_1$. Thus, in view of Eqs. (26) and (27), $\Phi(\mathcal{Z})>\Phi(\mathcal{Z}^\#)$ and the equilibrium configuration $\mathcal{Z}^\#$ must be stable.

The utility of the θ condition, which we now render precise, is based on the fact that in order for an equilibrium configuration of a segment to be stable, the corresponding configuration of each subsegment must be stable when the subsegment is subject to appropriate constraints.

We continue to take it for granted that the DNA segment under consideration has the following properties: (i) the curve $\mathcal{C}$ is smooth in the sense that the spatial positions of points on $\mathcal{C}$ and the tangent vectors to $\mathcal{C}$ are continuous functions of the arc-length distance s, and (ii) unless we state otherwise (as in a discussion of nicked DNA), the two DNA strands are continuous structures. For a DNA segment $\mathcal{D}$ that is in a given equilibrium configuration $\mathcal{Z}^\#$ and is either closed or subject to strong anchoring end conditions, we may consider, for each ξ with $0<\xi\leqslant L$, the subsegment $\mathcal{D}_\xi$ of $\mathcal{D}$ that corresponds to values of s between 0 and ξ, and we may imagine cases in which $\mathcal{D}_\xi$ is subject to the geometric constraints (including end conditions) and topological restrictions that would be imposed on it if its complementary subsegment (for which $\xi\leqslant s\leqslant L$) were held rigid. When the complement of $\mathcal{D}_\xi$ is held rigid, the writhe $\mathcal{W}(\mathcal{Z}_\xi)$ and the excess link $\Delta\mathcal{L}(\mathcal{Z}_\xi)$ for a configuration $\mathcal{Z}_\xi$ of the subsegment $\mathcal{D}_\xi$ can be defined by setting $\mathcal{W}(\mathcal{Z}_\xi)$ equal to $\mathcal{W}(\mathcal{Z})$, and $\Delta\mathcal{L}(\mathcal{Z}_\xi)$ equal to $\Delta\mathcal{L}(\mathcal{Z})$, where $\mathcal{Z}$ is the configuration of $\mathcal{D}$. Moreover, if $\mathcal{Z}$ is the equilibrium configuration $\mathcal{Z}^\#$, then $\mathcal{Z}^\#_\xi$, the corresponding configuration of $\mathcal{D}_\xi$, is an equilibrium configuration for the geometric constraints imposed on $\mathcal{D}_\xi$, and each admissible variation $\delta\mathcal{Z}_\xi$ in the configuration of $\mathcal{D}_\xi$ then corresponds to a unique admissible variation $\delta\mathcal{Z}$ in the configuration of $\mathcal{D}$. [The constraints, including end conditions, imposed on $\mathcal{D}_\xi$ are a consequence of the smoothness assumptions (i) and (ii) the fact that the configuration of the (rigid) complement of $\mathcal{D}_\xi$ is specified once $\mathcal{Z}^\#$ is given.] If $\mathcal{Z}^\#$ is stable, then, for each ξ between 0 and L, $\mathcal{Z}^\#_\xi$ is stable and hence obeys the E condition, which implies that the following assertion is true: For an equilibrium configuration $\mathcal{Z}^\#$ of $\mathcal{D}$ to be stable it is necessary that the θ condition hold, i.e., that for each ξ with $0<\xi\leqslant L$,

$$\theta(\xi) \geqslant 0, \tag{28}$$

where

$$\theta(\xi)=\left[\frac{d\Delta\mathcal{L}^{E_\xi}}{d\mathcal{W}}\right]_{\mathcal{W}=\mathcal{W}^{\#}}, \tag{29}$$

for the one-parameter family E_ξ of equilibrium configurations of $\mathcal{D}_\xi$ that contains $\mathcal{Z}^{\#}_\xi$ as a nonsingular point. [In the exceptional cases in which ξ is such that $\mathcal{Z}^{\#}_\xi$ corresponds to a bifurcation point for $\mathcal{D}_\xi$, and hence the family E_ξ is not unique, $\theta(\xi)$ should be taken to be the minimum, over all E_ξ, of the value of the right-hand side of Eq. (29).]

It is clear that a configuration that obeys the θ condition also obeys the E condition. Although, for the examples of families E that we present for miniplasmids in mononucleosomes in the final section of this paper, the configurations that obey the E condition also obey the θ condition, such is not always the case. In a subsequent paper we shall show that there are branches of the bifurcation diagram for protein-free miniplasmids that contain configurations at which the inequality, $d\Delta\mathcal{L}^E/d\mathcal{W}>0$, holds, but which are nonetheless unstable because they do not obey the θ condition.

We say that a segment of DNA is *nicked* if one of its two DNA strands has been severed. Once this has happened, $\mathcal{C}$ and $\Delta\Omega$ can be varied independently, and the concepts of linking number and excess link are no longer applicable. Thus, we say that a variation $\delta\mathcal{Z}$ in the configuration of a nicked segment is *admissible* if it is compatible with all the constraints that would be imposed if the segment were not nicked, with the single exception of the constraint that $\delta\mathcal{Z}$ preserve the sum of $\Delta\mathcal{T}$ and $\mathcal{W}$.

As in the case of a segment that is not nicked, a configuration $\mathcal{Z}^{\#}$ of a nicked segment is called an *equilibrium configuration* if $\delta\Phi=0$ for each admissible variation $\delta\mathcal{Z}$ from $\mathcal{Z}^{\#}$. Because variations in $\mathcal{C}$ and $\Delta\Omega$ for a nicked segment are independent, for an equilibrium configuration $\mathcal{Z}^{\#}$ of such a segment,

$$\Delta\Omega(s)=0 \quad \text{for all } s. \tag{30}$$

It follows from this last relation and Eqs. (11) and (7) that in order for $\mathcal{Z}^{\#}$ to be in equilibrium, it is not only necessary but also sufficient that

$$\delta\Gamma=0 \tag{31}$$

for each admissible variation $\delta\mathcal{Z}$.

In general, in order for $\mathcal{Z}^{\#}$ to be stable, it is necessary that $\delta^2\Phi\geqslant 0$ for each admissible variation. For a nicked segment, in view of Eqs. (7) and (30), it is clear that the stability of an equilibrium configuration requires that

$$\delta^2\Gamma\geqslant 0. \tag{32}$$

In fact, as Ψ_T attains its minimum value when $\Delta\Omega\equiv 0$, an equilibrium state $\mathcal{Z}^{\#}$ of a nicked segment is stable if and only if $\Gamma(\mathcal{C})$ has a strict local minimum at $\mathcal{C}^{\#}$, in the sense that $\Gamma(\mathcal{C})>\Gamma(\mathcal{C}^{\#})$ for all $\mathcal{C}$ corresponding to configurations that are in a neighborhood of $\mathcal{Z}^{\#}$ and are not equivalent to $\mathcal{Z}^{\#}$.

Let us now consider cases in which a family E of equilibrium configurations of an intact (i.e., not nicked) DNA segment $\mathcal{D}$ contains a configuration $\mathcal{Z}^{\#}$ with $\Delta\mathcal{T}=0$ and hence $\Delta\Omega(s)=0$. Equation (15) with $\Delta\mathcal{T}=0$ then tells us that Eq. (31) holds not only for all variations from $\mathcal{Z}^{\#}$ that are admissible for $\mathcal{D}$, but also for all variations admissible for a segment $\mathcal{D}^n$ obtained by nicking $\mathcal{D}$ (without changing end conditions or closure properties). (This follows from the fact that, because the curve $\mathcal{C}$ and the twist density $\Delta\Omega$ in $\mathcal{D}^n$ can be varied independently, for each admissible variation in the configuration of $\mathcal{D}^n$ there is another that gives rise to the same change in $\mathcal{C}$, but for which the change in $\Delta\mathcal{T}$ equals the negative of the change in $\mathcal{W}$, and which is admissible not only for $\mathcal{D}^n$ but also for $\mathcal{D}$.) Thus, a configuration with $\Delta\Omega(s)=0$ would remain in equilibrium if $\mathcal{D}$ were nicked.

If $\mathcal{Z}^{\#}$ is stable when considered a configuration of $\mathcal{D}$, it is not necessarily true that $\mathcal{Z}^{\#}$ is also stable as a configuration of $\mathcal{D}^n$. For although, by Eqs. (17) and (22), stability of $\mathcal{Z}^{\#}$ as a configuration of $\mathcal{D}$ requires that, for all admissible variations,

$$\delta^2\Gamma\geqslant -4\pi^2\omega(\delta\mathcal{W})^2, \tag{33}$$

stability of $\mathcal{Z}^{\#}$ as a configuration of $\mathcal{D}^n$ requires that $\delta^2\Gamma\geqslant 0$ for all such variations.

Now, Eqs. (18)–(21) hold for each variation $\delta\mathcal{Z}$ that is admissible for $\mathcal{D}$ and takes $\mathcal{Z}^{\#}$ into a configuration that has an axial curve equal to that of a nearby configuration in E. It follows from Eq. (19) that when $\delta\mathcal{Z}$ is such that $\delta\mathcal{W}\neq 0$ (i.e., when, as we assume, $\mathcal{W}^{\#}$ is not a singular value of $\mathcal{W}$ for the family E), the relation $\delta^2\Gamma\geqslant 0$ can hold for $\delta\mathcal{Z}$ only if

$$\frac{d^2\Gamma^E}{d\mathcal{W}^2}\geqslant 0, \tag{34}$$

and, by Eq. (20) [or Eq. (21)], we may conclude that in order for $\mathcal{Z}^{\#}$ to be a stable configuration of $\mathcal{D}^n$ it is necessary that the relation,

$$\frac{d\Delta\mathcal{L}^E}{d\mathcal{W}}\geqslant 1, \tag{35}$$

or, equivalently,

$$\frac{d\Delta\mathcal{L}^E}{d\Delta\mathcal{T}}\geqslant 0, \tag{36}$$

hold at the point in E where $\mathcal{W}=\mathcal{W}^{\#}$, i.e., where $\Delta\mathcal{T}=\Delta\mathcal{T}^E(\mathcal{W}^{\#})$. This condition, called the *n* condition, tells us that when the intact DNA segment $\mathcal{D}$ is nicked, a stable equilibrium configuration $\mathcal{Z}^{\#}$ in the family E with $\Delta\mathcal{T}^{\#}=0$ will remain in equilibrium, but will lose its stability, if

$$0\leqslant\frac{d\Delta\mathcal{L}^E}{d\mathcal{W}}<1. \tag{37}$$

Using an argument analogous to the one that gave us the *S* condition for the stability of an intact segment, one can prove the following assertion: If a family E of equilibrium configurations of an intact segment contains a configuration $\mathcal{Z}^{\#}$ with $\Delta\mathcal{T}^{\#}=0$ and with $\mathcal{W}^{\#}$ nonsingular, and is such that (i) the curve $\mathcal{C}(\mathcal{Z}^{\#})$ has a neighborhood $\mathcal{N}$ with the property that, for each writhe $\mathcal{W}^*$ close to $\mathcal{W}^{\#}$, $\mathcal{C}^E(\mathcal{W}^*)$ gives to Γ a strict minimum in the class of all curves $\mathcal{C}$ that are in $\mathcal{N}$, have writhe $\mathcal{W}^*$, and are compatible with the boundary or closure

conditions imposed on $\mathcal{D}$, and (ii) the inequality (35) is strict, then $\Gamma(\mathcal{C})>\Gamma(\mathcal{C}^{\#})$ for each such curve $\mathcal{C}$ not congruent to $\mathcal{C}^{\#}$, and hence $\mathcal{Z}^{\#}$ is stable and would remain stable if the segment $\mathcal{D}$ were nicked.

It follows from Eqs. (13) and (20) that for a family E of equilibrium configurations of an intact segment there holds the relation,

$$\frac{d\Phi^E}{d\Delta\mathcal{L}^E}=4\pi^2\omega\Delta\mathcal{T}^E=4\pi^2\omega(\Delta\mathcal{L}^E-\mathcal{W}), \tag{38}$$

which tells us how the graphs of Φ^E versus $\Delta\mathcal{L}^E$ and $\Delta\mathcal{L}^E$ versus $\mathcal{W}$ determine each other.

If a point on the graph of Φ^E versus $\Delta\mathcal{L}^E$ happens to correspond to a configuration $\mathcal{Z}^{\#}$ with $\Delta\mathcal{T}^{\#}=0$, then that point is a local minimum, a local maximum, or a point of horizontal inflexion. A necessary, but not sufficient, condition for the configuration $\mathcal{Z}^{\#}$ with $\Delta\mathcal{T}^{\#}=0$ to be stable when the segment is nicked is that the corresponding point of the graph of Φ^E versus $\Delta\mathcal{L}^E$ be a local minimum.

A relation between free energy and average writhe analogous to Eq. (38) holds even for segments with length such that fluctuations in configuration cannot be neglected. (For pertinent discussions of the statistical mechanics of protein-free DNA plasmids free from long range interactions, see Refs. [18] and [19].)

We close our discussion of the general theory with a rule for constructing a graph of $\Delta\mathcal{L}^E$ versus $\mathcal{W}$ for a family $E_{(\omega)}$ of equilibrium configurations of a DNA segment when one has such a graph for the corresponding family $E_{(\omega^*)}$ for a segment that is subject to the same constraints, has the same value of A, but has a different value of C and hence of ω.

The first of Eq. (7) and the second of the Eq. (9) imply that both sides of Eq. (20) are independent of ω, and hence

$$\Delta\mathcal{T}^E(\mathcal{W},\omega,A)=\frac{\omega^*}{\omega}\Delta\mathcal{T}^E(\mathcal{W},\omega^*,A), \tag{39}$$

i.e.,

$$\Delta\mathcal{L}^E(\mathcal{W},\omega,A)=\mathcal{W}+\frac{\omega^*}{\omega}(\Delta\mathcal{L}^E(\mathcal{W},\omega^*,A)-\mathcal{W}). \tag{40}$$

(As our notation indicates, $\Delta\mathcal{T}^E$ and $\Delta\mathcal{L}^E$ can be considered functions of $\mathcal{W}$, ω, and A. If long range interactions are absent, i.e., if $P=0$, then $\Delta\mathcal{T}^E$ and $\Delta\mathcal{L}^E$ are independent of A.) Equation (40) tells us that under a change in ω, the vertical distance of a point on the graph of $\Delta\mathcal{L}^E$ versus $\mathcal{W}$, from the line $\Delta\mathcal{L}^E=\mathcal{W}$, scales as ω^{-1}. Of course, the location of the points that correspond to configurations with $\Delta\mathcal{T}^E=0$, i.e., the points where that graph crosses the line $\Delta\mathcal{L}^E=\mathcal{W}$, is independent of the assumed value of ω.

IV. MINIPLASMIDS IN MONONUCLEOSOMES

A. Method for calculating configurations

This section contains examples of calculated equilibrium configurations to which results presented above are applicable. The configurations are calculated using explicit solutions obtained under the assumption that no external forces act at a cross section, other than those that may arise from contact of the cross section with others or with stationary objects. Hence the potential P is set equal to zero, we have $\Gamma=\Psi_B$, $\Phi=\Psi$, and the variational equation characterizing an equilibrium configuration, i.e.,

$$\delta\Psi_B+\delta\Psi_T=0, \tag{41}$$

is equivalent to the field equations of Kirchhoff's theory of (inextensible and symmetric) rods:

$$\frac{d\mathbf{F}}{ds}=\mathbf{0},\quad \frac{d\mathbf{M}}{ds}=\mathbf{F}\times\mathbf{t}. \tag{42}$$

Here, $\mathbf{t}(s)$ is the unit tangent vector to $\mathcal{C}$; $\mathbf{F}(s)$, the result of the internal forces acting on the cross section under consideration, is a reactive force not given by a constitutive relation; and $\mathbf{M}(s)$, the result of moments of the internal forces, is given by a relation that, in the units employed for Eqs. (2) and (3), can be written as

$$\mathbf{M}=A\mathbf{t}\times\frac{d\mathbf{t}}{ds}+C\Delta\Omega\mathbf{t}. \tag{43}$$

A DNA molecule is here approximated by a tube with circular cross sections of diameter D. The number $d=D/L$, like $\omega=A/C$, is an important dimensionless parameter in our theory. We assume that the contact force $\mathbf{f}^*$ (i.e., the force exerted on the cross section with $s=s^*$ by a cross section with $s=s^{**}\neq s^*$) is a reactive force concentrated at the point of contact that is normal to the surface of the tube at $s=s^*$. Geometrical considerations yield

$$|\mathbf{x}(s^*)-\mathbf{x}(s^{**})|=D,\quad \mathbf{t}(s^*)\cdot(\mathbf{x}(s^*)-\mathbf{x}(s^{**}))=0, \tag{44}$$

where $\mathbf{x}(s)$ is the position in space of the point on $\mathcal{C}$ with arc-length parameter s. Because contact forces are assumed to be normal to the surface of the tube,

$$\mathbf{f}^*=f^*\frac{\mathbf{x}(s^*)-\mathbf{x}(s^{**})}{D}, \tag{45a}$$

$$\mathbf{F}(s^*+0)-\mathbf{F}(s^*-0)+\mathbf{f}^*=\mathbf{0},$$

$$\mathbf{M}(s^*+0)-\mathbf{M}(s^*-0)=\mathbf{0}. \tag{45b}$$

The relations (42) and (43) can be considered differential equations for $\mathbf{x}$ and $\Delta\Omega$. In our previous studies of the elastic rod model [5–7], explicit solutions of these equations were employed to calculate equilibrium configurations free from points of self-contact. In the present research we use a generalization of the explicit solution method to cases in which self-contact occurs at isolated points. We make use of the fact that the configuration of each subsegment lying between two consecutive points of contact is given by an explicit solution that depends on six parameters which are related to integration constants for the differential equations (42) and (43). These parameters determine, among other things, the excess twist density $\Delta\Omega$ (which turns out to be constant in s) and the moduli of elliptic functions and integrals occurring in expressions for the coordinates of $\mathbf{x}(s)$. A segment with n points of contact has $2n+1$ contact-free subsegments if it is open, or $2n$ if it is closed, and hence its configuration is

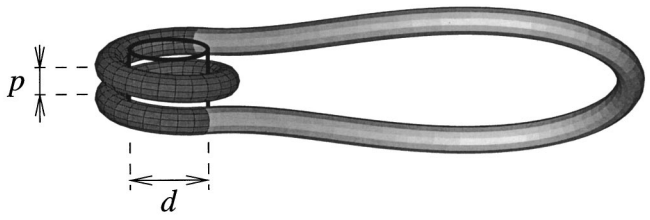

FIG. 1. Drawing of a DNA miniplasmid in a mononucleosome, with the core particle shown schematically as a cylinder. The relative lengths of the bound and free segments, $\mathcal{D}^b$ and $\mathcal{D}^f$, correspond to a miniplasmid of 359 base pairs that is wrapped about the core particle for 1.7 turns. Here, as elsewhere in the paper, DNA is depicted as a tube of diameter 20 Å.

determined when $12n+6$ parameters are specified if it is open, or $12n$ if it is closed. The boundary conditions (or, in the case of a protein-free plasmid, the closure conditions) imposed on the segment, the geometric relations (44), the balance equations (45) for the forces and moments at the points of self-contact, and the condition of preassigned $\Delta\mathcal{L}$ yield $12n+6$ (or $12n$) equations which are solved to calculate the $12n+6$ (or $12n$) parameters. Once the parameters are fixed, the reactive force vectors $\mathbf{F}(s)$ and $\mathbf{f}(s^*)$ are determined, and, in addition, the calculation of the elastic energy and the writhe is facilitated by use of expressions given in earlier work ([6], Eqs. (17)–(19), and [7], Appendix B).

For the present work on examples intended to be illustrative of possible applications of the theory developed in Sec. II and III, we have set $D=20$ nm. The calculations we report do not account for the effects of electrostatic repulsion.

B. Mononucleosomes

In a recent paper [7], we presented calculations of configurations that minimize the elastic energy of miniplasmids in mononucleosomes with specified values of the plasmid size N, the extent w of wrapping of DNA about the histone core particle, the helical repeat h_0^b of the bound DNA, and the linking number $\mathcal{L}$ of the plasmid. There the emphasis was placed on calculations of equilibrium topoisomer distributions for miniplasmids in mononucleosomes and the results obtained were for configurations free from self-contact. Here we present calculated graphs of $\Delta\mathcal{L}$ versus $\mathcal{W}$ and Ψ versus $\Delta\mathcal{L}$ for the extranucleosomal loop, for a range of $\Delta\mathcal{L}$ in which self-contact can occur.

A plasmid of length L or of size N in base pairs (bp) in a mononucleosome is made up of (i) the nucleosomal DNA, i.e., a bound segment $\mathcal{D}^b$ with duplex axis $\mathcal{C}^b$ and length L^b which is in contact with the core particle, and (ii) a ''free'' segment $\mathcal{D}^f$ (the extranucleosomal loop) with duplex axis $\mathcal{C}^f$ and length $L^f=L-L^b$. (See Fig. 1.) The segment $\mathcal{D}^f$ can be thought of as an open segment of DNA subject to strong anchoring end conditions, that follow from the assumption that the duplex axis $\mathcal{C}$, the tangent to $\mathcal{C}$, and both DNA strands are continuous at the places where $\mathcal{D}^f$ and $\mathcal{D}^b$ meet. We suppose that $\mathcal{D}^b$ is rigid with the curve $\mathcal{C}^b$ being a left-handed helix that, in accord with the structural information recently reported [20], has a pitch p of 2.39 nm and a diameter d of 8.36 nm.

For miniplasmids in mononucleosomes, values between 1.4 and 1.8 have been observed for the wrap w, i.e., the number of turns about the core particle made by $\mathcal{D}^b$ [8]. Expressions relating w, p, d, and L to such end conditions for $\mathcal{C}^f$ as the distance between the endpoints of $\mathcal{C}^f$ and the orientation of the tangents to $\mathcal{C}^f$ at those endpoints can be found in our recent paper [7], where, in addition to calculating equilibrium configurations for specified values of N, w, and $\mathcal{L}$, we develop a multistate model in which w is allowed to fluctuate. Study of the model has suggested that, in principle, DNA-histone binding energy can be obtained from comparisons of measured and calculated topoisomer distributions.

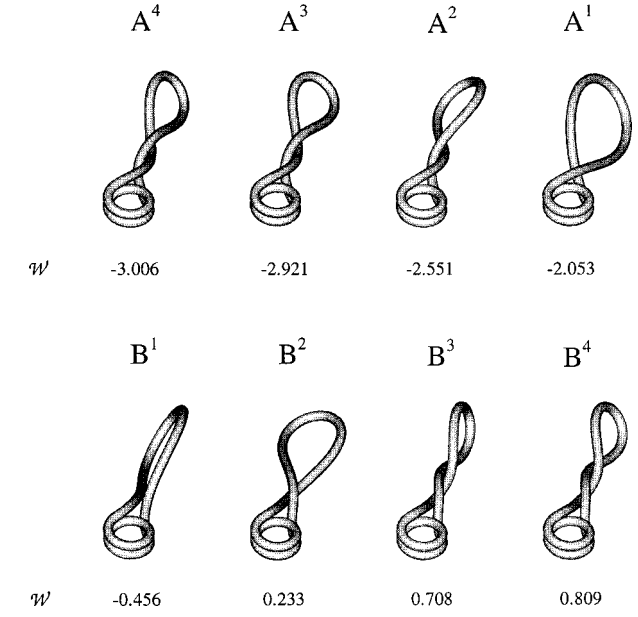

FIG. 2. Equilibrium configurations of DNA miniplasmids in mononucleosomes with $N=359$ bp, $w=1.7$ turns, and $\mathcal{W}$ as listed in the figure. In the configurations shown here, $\mathcal{D}^f$ has one or more points of self-contact. The corresponding values of $\Delta\mathcal{L}$ and Ψ are given in Table I for $\omega=1.4$ and 0.7; see Figs. 4 and 5 for the corresponding graphs of $\Delta\mathcal{L}$ versus $\mathcal{W}$ and Ψ versus $\Delta\mathcal{L}$.

So as to have specific examples of families E of equilibrium configurations with varying $\Delta\mathcal{L}$ but fixed end conditions, we here put $N=359$ and $w=1.7$. We take the excess link $\Delta\mathcal{L}$ of the free segment $\mathcal{D}^f$ to be defined by Eq. (4) with $\mathcal{W}$ the writhe of the duplex axis of the miniplasmid (i.e., of the closed curve $\mathcal{C}$) and with $\Delta\mathcal{T}$ equal to the total excess twist $\Delta\mathcal{T}^f$ of $\mathcal{D}^f$. The curve $\mathcal{C}^b$ plays the role of $\mathcal{C}^*$, the curve employed to close the open curve called $\mathcal{C}$ in the discussion of the ways of defining $\Delta\mathcal{L}$ for open segments subject to strong anchoring end conditions [which appears in the paragraph immediately preceding the one containing Eq. (6)]. Here, that open curve is $\mathcal{C}^f$. With the present (and natural) choice of $\mathcal{C}^*$ and the present definition of the excess link $\Delta\mathcal{L}$ of $\mathcal{D}^f$, the integral valued linking number of the plasmid $\mathcal{L}$, is

$$\mathcal{L}=\Delta\mathcal{L}+\mathcal{T}^b+\mathcal{T}_0^f, \tag{46}$$

where $\mathcal{T}^b$ is the total twist in the segment $\mathcal{D}^b$, and $\mathcal{T}_0^f$ is the total twist in $\mathcal{D}^f$ when $\mathcal{D}^f$ is in a torsionally relaxed state with $\Delta\Omega=0$.

In the calculations we report, A was set equal to 2.058×10^{-12} erg nm (which corresponds to a persistence length A/RT of 50.0 nm at $T=298$ K; cf. [21]. For $\omega=C/A$ we

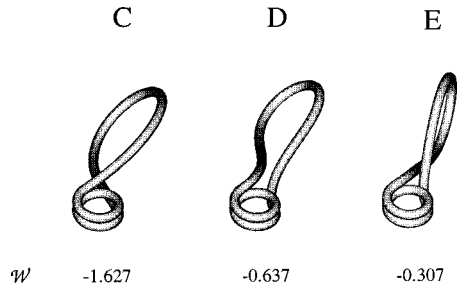

FIG. 3. Equilibrium configurations of DNA miniplasmids in mononucleosomes with $N=359$ bp, $w=1.7$ turns, and with $\Delta\mathcal{L}$ such that the configuration would remain in equilibrium if $\mathcal{D}^f$ were nicked. $\mathcal{D}^f$ has one point of self-contact in E; it has none in C and D.

employed two values: 1.4 and 0.7. The choice $\omega=1.4$ is compatible with the measurements of topoisomer distribution for miniplasmids [22], and appears appropriate for physical applications of our calculations. The value $\omega=0.7$ is compatible with measurements of fluorescence polarization anisotropy of dyes intercalated in open segments of DNA with $N\geqslant 10^4$ bp (see [23]); it is employed here to illustrate a way in which stability can be sensitive to the parameter ω. [That the value of ω can depend on the topology (and perhaps size) of the segment under investigation is a matter of current interest and concern; see, e.g., [24]. At the very least it suggests that one must be cautious in the interpretation of quantitative results of calculations based on a theory in which a DNA segment is modeled as a homogeneous rod obeying the special case of Kirchhoff's constitutive relations, in which the rod is assumed to be both inextensible and axially symmetric.]

C. Results for $C/A=1.4$

We suppose first that $\omega=1.4$. When ω has that value, each of the configurations seen in Figs. 2 and 3 gives a global minimum to the elastic energy of $\mathcal{D}^f$ for a value of $\Delta\mathcal{L}$ that is listed in Table I and hence is not only an equilibrium configuration but also a stable configuration. These configurations belong to a single one-parameter family $E_{(1.4)}$ of equilibrium configurations, whose members minimize Ψ at fixed $\Delta\mathcal{L}$, and hence minimize Ψ_B at fixed $\mathcal{W}$ (in the class of configurations of $\mathcal{D}^f$ compatible with the given N and w). The solid curve in Fig. 4(a) is the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ for that family. Because each configuration in the family is stable, each obeys the E condition, and the slope of the graph is positive. The configurations of $\mathcal{D}^f$ corresponding to points on the graph between A^1 and B^1 are free from self-contact; for $1\leqslant n\leqslant 4$, the configurations corresponding to points between A^n and A^{n+1} or between B^n and B^{n+1} have n points of self-contact. (Our calculations show that when one point of self-contact is present, the contact force varies nearly linearly with $\Delta\mathcal{L}$, increasing from 0 to 4.5 pN as $\Delta\mathcal{L}$ decreases from its value at A^1 to its value at A^2, and from 0 to 8.9 pN as $\Delta\mathcal{L}$ increases from its value at B^1 to its value at B^2.) As we see in Fig. 4(a), the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ is continuous, and at the places where the derivative $d\Delta\mathcal{L}/d\mathcal{W}$ suffers a jump (i.e., at the points A^n and B^n at which the number of self-contacts in $\mathcal{D}^f$ increases), the left-hand and right-hand derivatives agree in sign and are positive.

There is a reason why the graphs presented in Fig. 4 are confined to values of $\Delta\mathcal{L}$ greater than $\Delta\mathcal{L}$ at A^4 and less than $\Delta\mathcal{L}$ at B^4. For $\Delta\mathcal{L}$ in that range, $\mathcal{D}^f$ has less than four self-contacts. Our numerical results strongly indicate that, for $\Delta\mathcal{L}$ outside of that range, $\mathcal{D}^f$ has not only discrete points but also regions of self-contact, and as a result, precise calculation of $\mathcal{C}$ requires an extension of our present method that will be given in subsequent publications.

In Fig. 4(a), there are three points, C, D, E, at which the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ intersects a line with a slope of 45° passing through the origin. These points correspond to configurations which, because they have $\Delta\mathcal{T}=0$, would remain in equilibrium if $\mathcal{D}^f$ were nicked. These three configurations, like all other configurations in $E_{(1.4)}$, minimize Ψ_B in the class of configurations that have the same writhe. The slope

TABLE I. Calculated values of $\mathcal{W}$, Ψ_B, $\Delta\mathcal{L}$, and Ψ for the configurations shown in Figs. 2 and 3.

			$\omega=1.4$		$\omega=0.7$	
	$\mathcal{W}$	Ψ_B [kcal/mol]	$\Delta\mathcal{L}$	Ψ [kcal/mol]	$\Delta\mathcal{L}$	Ψ [kcal/mol]
A^4	−3.006	15.31	−3.443	17.33	−3.880	19.36
A^3	−2.921	14.54	−3.333	16.34	−3.745	18.14
A^2	−2.551	11.66	−2.890	12.88	−3.228	14.09
A^1	−2.053	7.89	−2.347	8.81	−2.641	9.72
B^1	−0.456	9.69	−0.560	9.81	−0.664	9.92
B^2	0.233	12.76	0.560	13.89	0.887	15.02
B^3	0.708	16.31	1.109	18.01	1.511	19.72
B^4	0.809	17.19	1.237	19.13	1.665	21.08
C	−1.627	5.94	−1.627	5.94	−1.627	5.94
D	−0.637	9.88	−0.637	9.88	−0.637	9.88
E	−0.307	9.50	−0.307	9.50	−0.307	9.50
S	−1.240	7.72			−0.664	9.47
X	−0.958	9.16			−0.578	9.96

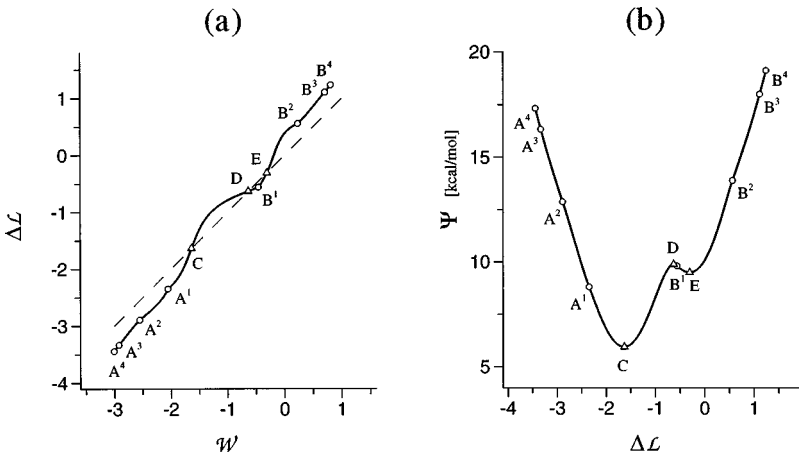

FIG. 4. Graphs of $\Delta\mathcal{L}$ versus $\mathcal{W}$, and Ψ versus $\Delta\mathcal{L}$, for equilibrium configurations with $\omega=1.4$ and end conditions corresponding to $N=359$ bp and $w=1.7$ turns. Here, as in Fig. 5 below, the symbol ○ indicates that the configuration (A^n or B^n) is such that the number of points of self-contact changes with $\Delta\mathcal{L}$, and △ indicates that the configuration (C, D, or E) is such that $\Delta\mathcal{T}=0$. The dashed line in (a) is the graph of $\Delta\mathcal{L}=\mathcal{W}$.

$d\Delta\mathcal{L}/d\mathcal{W}$ of the solid curve in Fig. 4(a) exceeds 1 at C and E and is less than 1 at D (i.e., the *n* condition holds at C and E but not at D). Hence, even though each of these three points corresponds to a stable configuration of the intact segment, if the segment $\mathcal{D}^f$ were nicked, the configuration at point D would not be stable. Since the configurations at points C and E meet conditions (i) and (ii) given in the paragraph that follows relation (37), these two configurations would be stable if $\mathcal{D}^f$ were nicked.

In Fig. 4(b), we see that the graph of Ψ versus $\Delta\mathcal{L}$ has a global minimum at C, a local minimum at E, and a local maximum at point D that corresponds to an equilibrium configuration that loses stability when nicked.

Remark: In our previous paper on mononucleosomes [7] we put $\omega=1.4$ and considered the set $\mathcal{S}(N,w)$ of (integer) values of the linking number $\mathcal{L}$ for which the configuration of $\mathcal{D}^f$ with minimum elastic energy Ψ does not show self-contact, and we remarked that preliminary calculations had strongly indicated that, for a range of N in which Ψ can be identified with the free energy G of $\mathcal{D}^f$, when $\mathcal{L}$ is not in $\mathcal{S}(N,w)$ the configurations of $\mathcal{D}^f$ compatible with $\mathcal{L}$ have elastic energies high enough to preclude the occurrence of an observable concentration of topoisomer $\mathcal{L}$ in an equilibrium distribution of topoisomers in a mononucleosome with the specified N and w. Careful calculations of the type that gave us Fig. 4(b) confirm the validity of this property of $\mathcal{S}(N,w)$ for $\omega=1.4$, when $330<N<370$ and $1.4<w<1.8$.

D. Results for $C/A=0.7$

Once we have in hand the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ shown in Fig. 4(a) for $E_{(1.4)}$, Eq. (40) yields, forthwith, corresponding graphs for families $E_{(\omega)}$ with other values of ω. Such a graph for $\omega=0.7$ is shown in Fig. 5(a). For families $E_{(\omega)}$ related in this way, the curve $\mathcal{C}$ corresponding to a given value of $\mathcal{W}$ is independent of ω, and the calculated configurations seen in Figs. 2 and 3 are appropriate to each such family, with $\Delta\mathcal{L}$ and Ψ dependent on ω in accord with Eqs. (40) and (38). As each configuration in $E_{(1.4)}$ minimizes Ψ_B at fixed $\mathcal{W}$, so also does each configuration in $E_{(\omega)}$ for arbitrary ω.

Returning now to Fig. 5(a), we note that as each configuration in $E_{(0.7)}$ is a minimizer of Ψ_B at fixed $\mathcal{W}$, we can apply the *S* condition and assert that a configuration in $E_{(0.7)}$ is stable if $d\Delta\mathcal{L}/d\mathcal{W}$ is strictly positive. The derivative $d\Delta\mathcal{L}/d\mathcal{W}$ suffers a jump at points A^n and B^n. In the present case, both the right-hand and left-hand derivatives are positive at A^1, A^2, A^3, B^2, and B^3, and hence the configurations corresponding to those points are stable. At B^1, the left-hand and right-hand derivatives do not agree in sign; at point X, shown as a solid circle in Fig. 5, the derivative $d\Delta\mathcal{L}/d\mathcal{W}$ is continuous but changes sign. As the branch traverses points X and B^1, the corresponding configurations either gain or lose stability; such points are called the points of exchange of stability. As $\Delta\mathcal{L}$ is here a (strictly) increasing function of $\mathcal{W}$ between A^4 and X and between B^1 and B^4, but is a de-

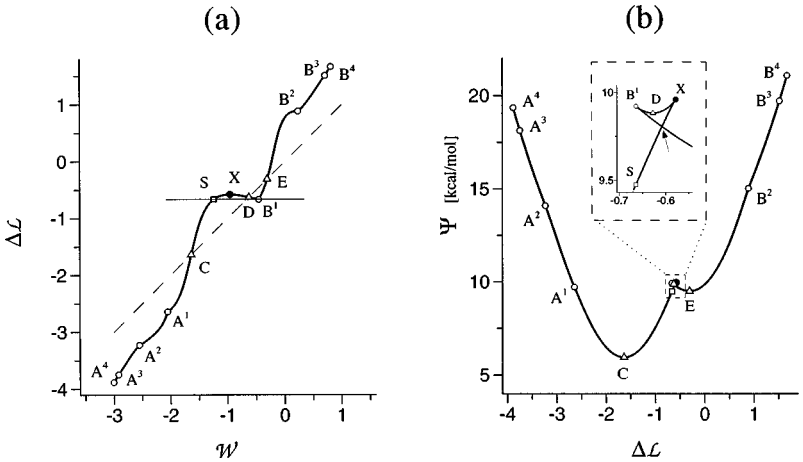

FIG. 5. Graphs of $\Delta\mathcal{L}$ versus $\mathcal{W}$, and Ψ versus $\Delta\mathcal{L}$, for equilibrium configurations with $\omega=0.7$, $N=359$ bp and $w=1.7$ turns. The symbol ● indicates a point of exchange of stability with $d\Delta\mathcal{L}/d\mathcal{W}$ continuous and equal to 0. The point B^1 is a point of exchange of stability with $d\Delta\mathcal{L}/d\mathcal{W}$ discontinuous. In (a), the horizontal lightface solid line with $\Delta\mathcal{L}=-0.664$ passes through the point B^1 and a point S corresponding to a stable configuration with lower elastic energy than B^1.

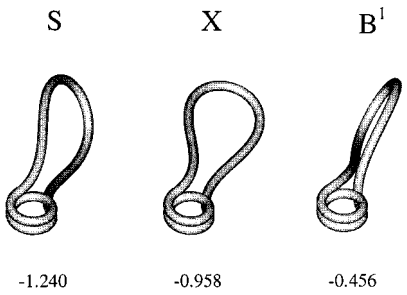

FIG. 6. Equilibrium configurations corresponding to points S, X, and B^1 of Fig. 5.

creasing function between X and B^1, the configurations in $E_{(0.7)}$ with $\mathcal{W}$ either between its values at A^4 and X or between its values at B^1 and B^4 are stable, and those with $\mathcal{W}$ between its values at X and B^1 are unstable.

Our calculations show that there is a critical value ω^c of ω such that $d\Delta\mathcal{L}/d\mathcal{W}>0$ for all values of $\mathcal{W}$ between -3.006 ($\mathcal{W}$ for A^4) and 0.809 ($\mathcal{W}$ for B^4) when $\omega>\omega^c$. For $\omega=\omega^c$, $d\Delta\mathcal{L}/d\mathcal{W}\geqslant 0$, and there is a (single) value of $\mathcal{W}$ (namely -0.606) at which $d\Delta\mathcal{L}/d\mathcal{W}=0$ (the corresponding configuration is one for which $\mathcal{D}^f$ is without self-contact). For $\omega<\omega^c$, there is at least one interval of values of $\mathcal{W}$ in which the E condition does not hold, and hence the corresponding configurations are unstable. The number ω^c and the values of $\mathcal{W}$ that we have mentioned depend on the assumed values of N and w. In the present case, in which $N=359$ bp and $w=1.7$ turns, $\omega^c=0.860$.

The configuration corresponding to each labeled point, A^n,B^n,C,D,E,X,S, of the graph in Fig. 5(a) is shown in Fig. 2, 3, or 6. The corresponding values of $\Delta\mathcal{L}$, Ψ, and Ψ_B are listed in Table I. (Of course, Ψ and Ψ_B are the total elastic energy and the total bending energy of the segment $\mathcal{D}^f$ which, in the present case in which $N=359$, has length $L^f=77.23$ nm and therefore contains approximately 227 bp.)

We now give a detailed analysis of some properties of the graphs shown in Fig. 5, not because they are immediately relevant to experiment (at the present time the value of 0.7 for ω is not expected to be appropriate for miniplasmids), but because they illustrate well certain possible consequences of a lack of monotonicity in a graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ for a segment subject to strong anchoring end conditions. The graph in Fig. 5(a) has a local maximum at point X (where $\mathcal{W}=-0.958$ and $\Delta\mathcal{L}=-0.578$) and a local minimum at B^1 (where $\mathcal{W}=-0.456$ and $\Delta\mathcal{L}=-0.664$). For each $\Delta\mathcal{L}$ with $-0.664<\Delta\mathcal{L}<-0.578$ there are three configurations in $E_{(0.7)}$, two of which are stable with $\mathcal{W}$ either less than its value at X or greater than its value at B^1. For $\Delta\mathcal{L}=-0.664$, there are two configurations in $E_{(0.7)}$: the unstable configuration B^1 and the stable configuration S. As Fig. 5(b) and the last column in Table I show, for the configuration S, which gives not only a local but also a global minimum to Ψ at fixed $\Delta\mathcal{L}$, Ψ at S has a value approximately 0.45 kcal/mole less than its value at B^1. The configurations S, X, and B^1 are shown in Fig. 6. In the inset to Fig. 5(b), an arrow points to the place where the graph of Ψ versus $\Delta\mathcal{L}$ crosses itself. At that point, $\Delta\mathcal{L}$ has a value (namely -0.608) for which there are two configurations in $E_{(0.7)}$ with equal values of Ψ but distinct values of $\mathcal{W}$ (i.e., $\mathcal{W}^\dagger=-1.139$ and $\mathcal{W}^\ddagger=-0.425$). To each value of $\mathcal{W}$ there corresponds a unique configuration in $E_{(0.7)}$, and if $\mathcal{W}<\mathcal{W}^\dagger$ or $\mathcal{W}>\mathcal{W}^\ddagger$, then that configuration not only is stable but gives a global minimum to Ψ in the class of all configurations with equal $\Delta\mathcal{L}$.

The rule that we employed to generate, from the graph of $\Delta\mathcal{L}$ versus $\mathcal{W}$ for $E_{(1.4)}$, corresponding graphs for families $E_{(\omega)}$ with different ω is such that the value of $\mathcal{W}$ for which $\Delta\mathcal{L}=\mathcal{W}$ is independent of ω. Although the stability of configurations of the intact segment corresponding to such values of $\mathcal{W}$ can depend on ω (e.g., for the intact segment, the configuration D is stable when $\omega=1.4$ but unstable when $\omega=0.7$), the stability of corresponding configurations of nicked segments (as it is governed by the dependence of Ψ_B on $\mathcal{C}$) is independent of ω.

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