Statistical mechanics of supercoiling-induced *B*-to-*Z* transitions in a closed circular DNA: One-dimensional model system with a double quadratic displacement potential and long range interactions

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(Received 4 January 1985; accepted 11 February 1985)

A configurational partition function for transitions between the right-handed *B* helix and the left-handed *Z* helix in a closed circular double stranded DNA is formulated in an idealized model. The twisting potential of base pairs is assumed to be a double quadratic potential with two minima at the *B* and *Z* forms and nearest neighbor coupling between base pair twists to be harmonic. In addition, long range interactions attributable to the conservation of linking number between two closed DNA strands are approximated by a harmonic potential of the writhing number that is equal to the linking number minus the total twist. Interactions between phonons and *B*-*Z* junctions are neglected as well as interactions between junctions. The configuration partition function is formulated for two cases, one that all base pairs can take the *Z* form as well as the *B* form, and another that only part of DNA can take the *Z* form. Then it is applied to analyzing *B*-to-*Z* transitions induced by changing the linking number of a closed circular DNA. The characteristics of *B*-to-*Z* transitions are examined in detail as well as experimental data analyses.

I. INTRODUCTION

Structural transitions in one-dimensional systems are often found in biological systems; of course, they are not phase transitions. Helix-coil transitions of polypeptides, and double helix-random coil transitions of linear DNAs[1] are one of typical examples that have been extensively studied so far.[2] Although there are many similiarities, the most distinctive feature from such helix-coil transitions in *B*-to-*Z* transitions of a closed circular double stranded DNA is that interactions which are essentially long range ones are involved in the system.

DNA is well known to take the Watson–Crick right-handed double helix[3] called the *B* form under physiological conditions of high humidity, although another type of right handed conformation, the *A* form, is observed when humidity is lower. Single-crystal x-ray analyses of double-stranded oligodeoxyribonucleotides revealed a novel structure of DNA which is the left-handed helix termed the *Z* form[4,5]; see Refs. 6 and 7 for a review of structural differences among the *A*, *B*, and *Z* forms. Although all kinds of base sequences cannot take the *Z* form, specific base sequences of alternating pyrimidine–purine such as $d(pCpG)_n \cdot d(pCpG)_n$[4,5] and $d(pTpG)_n \cdot d(pCpA)_n$[8,9] have been confirmed to take the *Z* form; the *Z* conformation is usually less stable under physiological ionic conditions than the *B* form. *B*-to-*Z* transitions of a linear DNA, which would be induced by changing environmental conditions such as salt concentration, might be analyzed by a simple version of Ising model in which each base pair is assumed to take either the *B* or *Z* form with a certain statistical weight and nearest neighbor interactions depending on base pair conformations are assumed. This simple model, however, cannot be employed to analyze *B*-to-*Z* transitions of a closed circular DNA, because long range interactions must be taken into account in the case of a circular DNA.

In a closed circular double stranded DNA, a topological property of two closed strands, which is termed the linking number, is invariant as long as neither one is broken, no matter how they are deformed. White[10] proved that the linking number and the total twist, which is termed the twisting number, of a closed ribbon or two closed DNA strands differ by a quantity which depends exclusively on the curve of the axis of a closed ribbon or the helical axis of closed DNA double strands and which was later termed the writhing number by Fuller[11]; see also Refs. 12–14. In other words, the conservation of linking number in a closed circular DNA imposes the condition that a change of the total helical twist must be compensated by the change of the writhing number that is associated with those of the average total bending energy and conformational entropy. This condition specific to a closed DNA is equivalent to introducing interactions among base pair twists, which are essentially long range interactions and depend on the writhing number that is equal to the linking number minus the twisting number. In the result, such long range interactions must be taken into account to analyze *B*-to-*Z* transitions of a closed circular double stranded DNA. Thus in the case of closed circular DNAs, changing the linking number causes twisting stress. This specific feature of closed circular DNAs has been utilized to induce the structural transition from the right-handed *B* form to the left-handed *Z* form; *B*-to-*Z* transitions of a circular DNA induced by reducing the linking number have been confirmed to occur even under the physiological conditions.[15–17] On the other hand, changing the linking number writhes a DNA double helix and causes its supercoiling[18,19] in the left- or right-handed way as well as the deformation of base pair twists, increasing the free energy of the molecule; thus *B*-to-*Z* transitions induced by changing the linking number is called supercoiling-induced *B*-to-*Z* transitions. *B*-to-*Z* transitions induced by changing the linking number are

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structural transitions induced by changing long range interactions rather than short range interactions. The dependence of the free energy of a B-form circular DNA on its linking number has been experimentally evaluated[20–24] in a quadratic function of the linking number, i.e., a harmonic potential.

In models[25–27] that have been employed so far to analyze experimental data of B-to-Z transitions, an empirical formula[20–24] of the free energy of a B-form circular DNA as a function of the linking number was explicitly employed with the assumption that the change of the total twist due to B-to-Z conformational changes would be effectively equivalent to the change of the linking number, in order to estimate the free energy of conformations with Z forms. In these analyses, the contribution of the configurational entropy, which originates from a number of possible arrangements of B and Z forms in a circular DNA, was not completely[25] or not taken into account at all.[26,27] In other theoretical models,[27–31] B-to-Z transitions induced by torsional stress were discussed; however, these models cannot be applied to structural transitions of closed circular DNAs induced by changing the linking number, because the long range interactions due to the conservation of linking number are not taken into account and therefore the free energy of the supercoil formation is completely neglected.

Here we will rather rigorously formulate the configurational partition function of a closed circular DNA in an idealized model. A twisting potential of base pairs will be explicitly assumed to be a double quadratic potential with two minima at the B and Z forms. Also, nearest neighbor coupling between twists is assumed to be hamonic as a simple model of interactions that favor a consecutive stretch of the B or Z form. Long range interactions among twists due to the conservation of linking number will be approximated by taking a first significant term, a harmonic term, in the Taylor expansion with the writhing number. Although these assumptions, specifically for the twisting potential, are certainly idealizations, this simple system is worth analyzing as a one-dimensional model system in which a displacement potential is strongly anharmonic and also long range interactions are included, and which undergoes structural transitions by the change of long range interactions.

It is important to distinguish two different regimes according to the relative strength of the twisting potential and interactions between neighbors; the Ising limit in which the twisting potential is so strong that each base pair fluctuates almost independently, and the opposite limit termed the displacive limit in which interactions between neighbors are strong enough to make the variation of base pair twists smooth. In the displacive limit, Krumhansl and Schrieffer[32] and Currie *et al.*[33] have extensively studied dynamics and statistical mechanics of one-dimensional systems whose scalar field Hamiltonian includes an anharmonic potential and whose Euler–Lagrange equation of motion is a nonlinear Klein–Gordon equation that has solitary-wave (called soliton, kink or antikink, or domain wall) solutions; anharmonic potentials are specifically ϕ^4, sine-Gordon and double-quadratic potentials with two degenerate minima. They[33] have proved that the ideal-gas phenomenology in which kink-phonon interactions are exactly taken into account but kink-kink interactions are neglected gives exact results for the various low-temperature thermodynamic functions and correlation lengths. They discussed dynamics and thermodynamics of solitons excited by temperature changes. In the present system, B-Z junctions, which correspond[27] to kinks or domain walls, are induced by changing long range interactions rather than temperature or other environmental parameters that affect the relative energy of the Z form to the B form. In the continuous limit of base pair position, the analytical expression of a B-Z junction can be obtained for double quadratic potentials, and then it can be used to calculate the potential energy stored at a junction (the rest energy of a kink) and the twist associated with the formation of a junction. However, the presence of long range interactions makes it difficult to obtain an exact result of the partition function and even the estimate of junction–phonon interactions. Thus junction–phonon interactions are neglected here as well as junction–junction interactions, and the configurational partition function are phenomenologically formulated. As a result, this approximation is appropriate for the case that interactions between neighbors are comparable with or weaker than the twisting potential. Also the twisting potential is simplified as a dobule quadratic function whose second derivative takes the same value at the B and Z forms, so that phonons will be equally distributed over B and Z conformational regions rather than trapped in either B or Z regions. These simplifications lead to the approximation, which is similar to the Ising model, that librational contributions around each of conformational states, which are the overall B conformation, the alternating B–Z conformation and if possible, the overall Z conformation, in the configurational partition function do not depend on those conformational states. In the Ising limit, however, the partition function can be formulated even for the general case in which the twisting force constants for the Z and B forms are different from each other; the formulation of the configurational partition function in the Ising limit will be presented in the Appendix B.

In Sec. II, we describe in detail the formulation of the configurational partition function; it is represented as the sum of conformational states such as overall B or Z conformations and alternating B-Z conformations. The configurational partition function formulated is consistent with the experimental fact[22,23] that the equilibrium ensemble of closed circular DNAs over the linking number obeys the Gaussian distribution, indicating that the second order approximation for long range interactions, i.e., the assumption of a harmonic potential of the writhing number, would be adequate. In Sec. III, the combinatory factor that is defined as the number of ways to choose a given number of Z forms with a given number of Z-conformational regions in a circular DNA is evaluated for two cases; case (a) in which all base pairs can take both the B and Z forms, and case (b) in which only a part of a closed circular DNA can take both the B and Z forms but other base pairs take only the B form. The case (b) is considered because most experiments have been performed under such circumstances. Section IV deals with the statistical characteristics of each of overall B and Z conformations and alternating B-Z conformations; the statistical

average and variance of the twisting number will be discussed among others. An important fact is that the variance of the total twist in a closed circular DNA is not the same as that expected for a linear DNA but reduced by the long range interactions due to the conservation of linking number, as if the twisting force constant increased. In Sec. V, the characteristics of B-to-Z transitions induced by changing the linking number, specifically the dependencies of the transition linking number on external variables, will be examined. On the contrary to the fact, it was claimed[26] that in the limit of a long DNA, a segment of the DNA that could take the Z form would wholly change from the B to the Z form at the transition point. It will be proved that such a transition will occur only if the segment that can take the Z form is short. In Sec. VI, the experimental data of B-to-Z transitions reported by Peck and Wang[25] are analyzed and the structural parameters, such as the relative energy of the Z form to the B form and the energy of the formation of a B-Z junction among others, are estimated. Also, the dependencies of the transition point on external variables are discussed with examples of numerical results for some cases.

II. FORMULATION OF THE CONFIGURATIONAL PARTITION FUNCTION

A principal parameter that discriminates the B and Z forms is of course the twisting angle of a base pair along the helical axis of DNA. Thus in the present simple model of B-to-Z transitions, only base pair twists are explicitly taken into account. Let us suppose that the partition function is represented in an orthogonal coordinates system. By integrating the Boltzmann factor over a whole momentum space, the partition function may be divided into two factors, the momentum factor Z_P and the configurational partition function Z_C that includes coordinates variables.

$$Z = Z_P Z_C. \tag{1}$$

This configurational partition function could be represented as follows in terms of an effective potential U_E for base pair twists $\{\tau_i\}$.

$$Z_C \equiv \int \prod_{i=1}^{N} d\tau_i \exp[-\beta U_E(\{\tau_i\})], \tag{2}$$

where

$$\exp[-\beta U_E(\{\tau_i\})] \equiv \text{const} \int \prod_k dq_k \exp[-\beta U(\{q_k\})] \times \prod_i \delta[\tau_i(\{q_k\}) - \tau_i].$$

$\{q_k\}$ and U are a set of orthogonal coordinates and a potential energy function to describe a whole system. τ_i is defined as the twist of the ith base pair around the helical axis. β is $1/(kT)$, where k is the Boltzmann's constant and T is absolute temperature. δ is the Dirac's distribution. Equation (2) represents the configurational partition function for a linear DNA consisting of $N+1$ base pairs or a closed circular DNA consisting of N base pairs. An essential difference between linear and closed circular DNA resides in the effective potential U_E, which will be discussed in the following.

The potential U_E is divided here into three terms depending on the range of interactions; a twisting potential $V_{1i}(\tau_i)$ that may reflect interactions between nearest neighbor base pairs and depends only on the base pair twist, $V_{2i}(\tau_i - \tau_{i-1})$ that is a nearest neighbor interaction potential between base pair twists, and a remaining part V_L that represents longer range interactions.

$$U_E(\{\tau_i\}) = \left[\sum_{i=1}^{N} V_{1i}(\tau_i) + V_{2i}(\tau_i - \tau_{i-1})\right] + V_L(\{\tau_i\}). \tag{3}$$

Simple functions are assumed here for those short range interaction potentials. Because experiments showed that the stability of the Z form strongly depends on the short range order of a base sequence, two type of functions are employed for the twisting potential V_{1i}; specific base sequences of alternating pyrimidine–purine such as $d(pCpG)_n \cdot d(pCpG)_n$[4,5] and $d(pTpG)_n \cdot d(pCpA)_n$[8,9] have been confirmed to take the Z form. For simplicity, we assume a double quadratic potential with two minima at the B and Z forms for base pairs that can take the Z form, and a harmonic potential with a minimum at the B form for base pairs that take only the B form. We also assume that the twisting force constants for the B and Z forms are assumed to be the same;

$$V_{1i}(\tau) \simeq \begin{cases} e_B + (\omega_0^2/2)(\tau - \tau_B)^2 & \text{for } \tau \geqslant \tau_J \\ e_Z + (\omega_0^2/2)(\tau - \tau_Z)^2 & \text{for } \tau \leqslant \tau_J \end{cases} \tag{4a}$$

or

$$V_{1i}(\tau) \simeq e_B + (\omega_0^2/2)(\tau - \tau_B)^2, \tag{4b}$$

where

$$e_Z - e_B = -\omega_0^2(\tau_Z - \tau_B)(\tau_B + \tau_Z - 2\tau_J)/2. \tag{5}$$

In the following, Eq. (4a) will be implicitly referred to unless Eq. (4b) is explicitly cited. The left and right figures in Fig. 1 illustrate the double quadratic potential of Eq. (4a) and the harmonic potential of Eq. (4b), respectively. In the left-handed Z helix of $d(pCpG)_n \cdot d(pCpG)_n$,[4,5] C–N glycosyl bond between sugar and base takes the anti conformation, which appears in all A and B DNAs, at every pyrimidine (cytosine) and the syn conformation at every purine (guanine), making the twist of each base pair significantly different from each other. However, such a base dependence of twist in the Z helix is neglected in the present idealized model; the fact that the repeating unit of the helix is not a single base pair, as it is in A and B DNAs, but rather two successive base pairs (pyrimidine–purine pair) will be taken into account later. The average twists of the standard B and Z forms, τ_B and τ_Z, are approximately equal to[6,7]

$$\tau_B \simeq 1/10, \quad \tau_Z \simeq -1/12.$$

A twist τ is defined here as a twisting angle divided by 2π, and the right-handed twist is taken as the positive direction of twist. These values are based on the results of single-crystal x-ray analyses; $\tau_B = 1/10.4$[34] or $1/10.5$[25] and $\tau_Z = -1/11.6$[25] were obtained in solution measurements. Variation in individual twist values in B-form DNAs is not insignificant and appears to depend on base sequences.[7,35] Also, the twisting force constant was reported to depend on the base composition of base sequences.[36] However, such base sequence dependencies of the twisting potential are completely neglected in the present model.

The nearest neighbor interactions between the ith and $(i-1)$th base pair twists are approximated by a harmonic

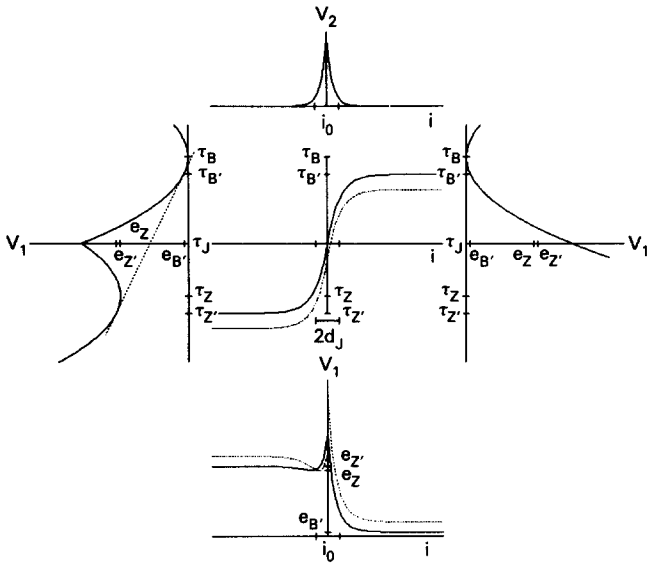

FIG. 1. Schematic plot of a Z–B junction or a kink. A kink of Eq. (25), which is a solution of Eq. (14) with the double quadratic function Eq. (4a) shown in the left figure as the twisting potential V_1, is drawn by a solid line in the middle figure; the dotted line in the left figure shows a cotangent line whose slope gives a constraining force λ needed to make the kink. In the middle figure, the dotted curve is simply a shift of the solid curve and shows a resting kink of Eq. (31) that can be formed at the position i_0 at the right-hand side of which the twisting potential is equal to the harmonic potential Eq. (4b) shown in the right figure. The bottom figure shows the twisting potentials V_1 for both kinks as a function of position i. In the top figure, the nearest neighbor interaction potential $V_2 = (C_0^2/2)(d\tau/di)^2$ is plotted against position i; it is the same for both kinks. See the text for details.

potential that favors the regular repetition of the same twisting angle.

$$V_{2i}(\tau_i - \tau_{i-1}) \simeq \frac{C_0^2}{2}(\tau_i - \tau_{i-1})^2. \tag{6}$$

For a closed circular DNA, the periodic boundary condition must be satisfied.

$$\tau_{i \pm N} \equiv \tau_i. \tag{7}$$

For the case of a linear DNA, V_{21} is defined as zero; alternatively τ_0 is regarded as $\tau_0 \equiv \tau_1$. For simplicity, the force constant C_0^2 is assumed here to be the same for any base pair.

Taking account of only short range interactions might be sufficient unless long range correlations among base pair twists are an object of consideration. This might be proper for a linear DNA, but in the case of a closed circular DNA, long range interactions play significant roles on its conformation. One essential property of a closed circular DNA is a topological one of two closed strands that is called the linking number; the linking number is deformation invariant as long as neither one of the closed strands is broken. White[10] proved that the linking number L_k of a closed ribbon or two closed DNA strands and its total twist T_w differ by the quantity W_r which depends exclusively on the curve of the axis of a closed ribbon or the helical axis of closed DNA double strands and which was later termed the writhing number by Fuller[11]; refer to Refs. 10–14 for details.

$$L_k = T_w + W_r, \tag{8}$$

where

$$T_w = \sum_{i=1}^{N} \tau_i.$$

Although the twisting number T_w is, of course, a property of a ribbon or two strands of DNA as well as the linking number, their difference, the writhing number, is a property of a closed curve such as the ribbon's axis and the DNA helical axis. The writhing number is a geometrical property as well as the twisting number. The writhing and twisting numbers are both invariant under rigid motions and dilatations. Mirror reflection changes signs of these three quantities. Thus the writing number of any curve which is its own mirror image such as a circle is necessarily zero. Also, the writhing number will be zero if the axis of ribbon or the helical axis is on a flat plane or entirely on the surface of a sphere.[14] Both the twisting and writhing numbers can take any real number but the linking number must be integral. The relationship [Eq. (8)] between the topological and the geometrical quantities imposes an additional restriction on the conformations of a circular DNA, causing long range interactions among base pair twists; in a closed circular DNA, a change of the total twist must be compensated by the change of the writhing number that is associated with those of the average total bending energy and conformational entropy.

The long range interaction potential for a closed circular DNA of a linking number L_k is approximated here by taking the first significant term, a harmonic term, in the Taylor expansion with the writhing number.

$$V_L \simeq \frac{B_0^2}{2N}(W_r)^2 = \frac{B_0^2}{2N}(L_k - T_w)^2. \tag{9}$$

B_0^2 is termed here the writhing force constant; for a linear chain, B_0^2 is, of course, regarded to be zero. It should be noted that the long range energy defined by Eq. (9) is free energy rather than energy, because there may be multiple conformations whose bending energies are different from each other but whose writhing numbers are the same.

Then the configurational partition function of Eq. (2) is approximated as follows:

$$Z_C \simeq \int dT_w \exp(-\beta V_L) \int \prod_i d\tau_i \times \exp\left[-\beta\left(\sum_i V_{1i} + V_{2i}\right)\right] \delta\left(\sum_i \tau_i - T_w\right) \tag{10}$$

$$\simeq \int dT_w \exp(-\beta V_L) \sum_m Z_{m,T_w} \times \exp\left[-\beta\left(\sum_i V_{1i} + V_{2i}\right)_{\{\tau_i = \tau_i^{m,Tw}\}}\right], \tag{11}$$

where $\{\tau_i^{m,Tw}\}$ is defined to be the set of twists that corresponds to a local minimum m of the short range interaction potential $(\Sigma_i V_{1i} + V_{2i})$ for a given value $\{\tau_i^{m,Tw}\}$ of the total twist, and $Z_{m,Tw}$ represents librational contribution around such a local minimum. In other words, $\{\tau_i^{m,Tw}\}$ corresponds to one of the most probable configurations at a given total twist T_w, which may contribute significantly to the partition function. $\{\tau_i = \tau_i^{m,Tw}\}$ must satisfy

$$\frac{\partial}{\partial \tau_i}\left[V_{1i}(\tau_i)+V_{2,i+1}(\tau_{i+1}-\tau_i)+V_{2i}(\tau_i-\tau_{i-1})\right]-\lambda=0, \tag{12}$$

$$\sum_i \tau_i = T_w,$$

where λ is a Lagrange's undetermined multiplier.

$$\lambda=\frac{1}{N}\left[\sum_{i=1}^{N}\frac{d}{d\tau_i}V_{1i}\right]_{\{\tau_i=\tau_i^{m,Tw}\}}. \tag{13}$$

In a simple case in which the twisting potentials V_{1i} are the same for all i, a trivial solution of Eq.(12) is one representing a uniform twist; $\tau_i = T_w/N$. In this case, the constraining force λ is nonzero unless V_{1i} has an extremum at T_w/N; $\lambda=\omega_0^2(T_w/N-\tau_\sigma)$ with $\tau_\sigma=\tau_B$ or τ_Z for the double quadratic potential of Eq. (4a). If the twisting potential V_{1i} were a harmonic potential, Eq. (12) would have no other solution except the uniform displacement. However, in general V_{1i} has at least two minima at the B and Z forms. For such anharmonic potentials, there may be other solutions such as alternating B–Z conformations in which deformation is localized at junctions between B and Z regions.

In the limit of $C_0/\omega_0\to 0$, it is obvious that each base pair can take the B or Z form independently. Therefore, let us consider the opposite limit, i.e., $C_0/\omega_0 \gg 1$. In this displacive limit, the position i may be approximated as a continuous variable. Here in the continuous approximation of Eq. (12), we show one example of such a solution of nonuniform deformation that consists of a Z–B or B–Z junction; since there is only one junction, this conformation is feasible only in a linear DNA but not in a closed circular DNA; the periodic condition of Eq. (7) is not applied to this case. The continuous approximaion for Eq. (12) is represented by

$$\frac{d}{d\tau}V_{1i}[\tau(i)]-C_0^2\frac{d^2}{di^2}\tau(i)-\lambda=0,$$

$$\int di\,\tau(i)=T_w. \tag{14}$$

The variable i is regarded here as a continuous one. Let us consider the simple case that the twisting potential V_{1i} does not depend on the base pair position i, i.e., $V_{1i}(\tau)=V_1(\tau)$. The first integral of Eq. (14) is

$$\frac{C_0^2}{2}\left(\frac{d\tau}{di}\right)^2 = V_1(\tau)-V_1(\tau_{B'})-\lambda(\tau-\tau_{B'})$$
$$= V_1(\tau)-V_1(\tau_{Z'})-\lambda(\tau-\tau_{Z'}) \quad \text{for } V_{1i}=V_1 \tag{15}$$

with the following boundary conditions for solutions of a Z–B or B–Z junction.

$$\lim_{i\to\pm\infty}\tau(i)=\tau_{B'} \text{ or } \tau_{Z'},$$

$$\lim_{i\to\pm\infty}\frac{d\tau}{di}=0. \tag{16}$$

The constraining force λ must satisfy the following equations:

$$\left[\frac{dV_1(\tau)}{d\tau}\right]_{\tau=\tau_{Z'}}=\left[\frac{dV_1(\tau)}{d\tau}\right]_{\tau=\tau_{B'}}=\lambda,$$

$$\frac{V_1(\tau_{Z'})-V_1(\tau_{B'})}{\tau_{Z'}-\tau_{B'}}=\lambda. \tag{17}$$

The first equation above would be obvious from the definition of the constraining force, and the second equation is obtained from Eq. (15). Equation (17) indicates that $\tau_{B'}$ and $\tau_{Z'}$ are points where the potential V_1 has a cotangent line whose slope is equal to the constraining force λ; the constraining force λ for this case is a constant depending only on the twisting potential V_1, unlike that depending on the amount of uniform deformation in the case of uniform deformations. It should be noted here that the twists $\tau_{Z'}$ and $\tau_{B'}$ of base pairs far from a junction are different from the standard twists τ_Z and τ_B of the Z and B forms where the twisting potential V_1 takes minimal values; if and only if the constraining force λ is zero, they will be equal to the standard twists. From Eq. (15), a solution of Eq. (14) is obtained.

$$i-i_0=\pm\int_{\tau(i_0)}^{\tau(i)}d\tau\left[\frac{2}{C_0^2}\left\{V_1(\tau)-V_1(\tau_{B'})-\left[\frac{dV_1}{d\tau}\right]_{\tau=\tau_{B'}}(\tau-\tau_{B'})\right\}\right]^{-1/2} \tag{18a}$$

$$=\pm\int_{\tau(i_0)}^{\tau(i)}d\tau\left[\frac{2}{C_0^2}\left\{V_1(\tau)-V_1(\tau_{Z'})-\left[\frac{dV_1}{d\tau}\right]_{\tau=\tau_{Z'}}(\tau-\tau_{Z'})\right\}\right]^{-1/2}. \tag{18b}$$

Each solution with plus or minus in the above equation corresponds to the Z–B or B–Z junction. The position i_0 where the junction is located is an arbitrary constant; the translational invariance of Eq. (14) is restored. This solution is an extension of the solution[33] of kink or anti-kink for potential functions with two degenerate minima to general double-well potentials. The twist $\tau_{J'}$ and the energy $e_{J'}$ associated with the formation of a junction are defined as follows:

$$\tau_{J'}\equiv\int_{-\infty}^{i_0}di[\tau(i)-\tau(-\infty)]+\int_{i_0}^{\infty}di[\tau(i)-\tau(\infty)], \tag{19}$$

$$e_{J'}\equiv\int_{-\infty}^{i_0}di\left\{V_1[\tau(i)]+\frac{C_0^2}{2}\left(\frac{d\tau(i)}{di}\right)^2-V_1[\tau(-\infty)]\right\}$$
$$+\int_{i_0}^{\infty}di\left\{V_1[\tau(i)]+\frac{C_0^2}{2}\left(\frac{d\tau(i)}{di}\right)^2-V_1[\tau(\infty)]\right\}. \tag{20}$$

The energies $e_{Z'}$ and $e_{B'}$ of the Z and B forms in conformations with a junction are defined as potential energies per base pair in the regular conformation of the twist $\tau_{Z'}$ or $\tau_{B'}$, respectively.

$$e_{B'}\equiv V_1(\tau_{B'}),\quad e_{Z'}\equiv V_1(\tau_{Z'}). \tag{21}$$

If V_1 is the double quadratic function of Eq. (4a), Eqs. (18a) or (18b) will be represented by

$$\tau(i)=\begin{cases}\tau_J+(\tau_{B'}-\tau_J)\left[1-\exp\left(-\dfrac{\omega_0}{C_0}|i-i_0|\right)\right] \\ \quad \text{for } \pm(i-i_0)\geqslant 0 \\ \tau_J+(\tau_{Z'}-\tau_J)\left[1-\exp\left(-\dfrac{\omega_0}{C_0}|i-i_0|\right)\right] \\ \quad \text{for } \pm(i-i_0)\leqslant 0,\end{cases} \tag{22}$$

where

$$\tau_{B'} = \tau_B + \lambda/\omega_0^2, \quad \tau_{Z'} = \tau_Z + \lambda/\omega_0^2. \tag{23}$$

This equation indicates that $\tau(i)$ will become asymmetric at $\tau(i_0) = \tau_J$ if the twisting force constant is different in the regions of $\tau \leqslant \tau_J$ and $\tau \geqslant \tau_J$. In case of Eq. (4a) in which the twisting force constant is assumed to be the same ω_0^2 in both the Z and B conformational regions, the constraining force is

$$\lambda = (e_Z - e_B)/(\tau_Z - \tau_B) = -\omega_0^2(\tau_B + \tau_Z - 2\tau_J)/2 \tag{24}$$

and then Eq. (22) is alternatively represented by

$$\tau(i) = \tau_J \pm \mathrm{sgn}(i - i_0)\frac{\tau_B - \tau_Z}{2}[1 - \exp(-|i - i_0|/d_J)], \tag{25}$$

where

$$d_J \equiv C_0/\omega_0. \tag{26}$$

The solution of Eq. (25) for a kink or Z–B junction is plotted by a solid line at the middle in Fig. 1. The bottom and top figures in Fig. 1 also show the twisting potential V_1, and the nearest neighbor interaction potential $V_2 = (C_0^2/2)(d\tau/di)^2$ as functions of position i. From Eq. (25), the energy $e_{J'}$ associated with the formation of a junction, which is defined by Eq. (20), has been calculated and is listed in Table I; in this table, the width of a junction is regarded to be equal to $2d_J$. $\tau_{J'}$ is zero, because τ_i must be central symmetric at the position i_0 of a junction in case of the twisting potential Eq. (4a).

Next, let us consider the case that the twisting potential differs at each side of the position i_0.

$$V_{1i}(\tau) \equiv V_{1-}(\tau)[1 - H(i - i_0)] + V_{1+}(\tau)H(i - i_0), \tag{27}$$

where H is the Heaviside's function. In this case, the first integral of Eq. (14) is

$$\frac{C_0^2}{2}\left(\frac{d\tau}{di}\right)^2 = V_{1i}(\tau) - V_{1+}[\tau(\infty)] - \lambda[\tau - \tau(\infty)] - \int_\infty^i di[V_{1+}(\tau) - V_{1-}(\tau)]\delta(i - i_0) \tag{28a}$$

$$= V_{1i}(\tau) - V_{1-}[\tau(-\infty)] - \lambda[\tau - \tau(-\infty)] - \int_{-\infty}^i di[V_{1+}(\tau) - V_{1-}(\tau)]\delta(i - i_0), \tag{28b}$$

$$= \begin{cases} V_{1+}(\tau) - V_{1+}[\tau(\infty)] - \lambda[\tau - \tau(\infty)] & \text{for } i \geqslant i_0 \\ V_{1-}(\tau) - V_{1-}[\tau(-\infty)] - \lambda[\tau - \tau(-\infty)] & \text{for } i \leqslant i_0 \end{cases} \tag{28c}$$

The constraining force λ, $\tau(-\infty)$, and $\tau(\infty)$ are obtained from

$$\left[\frac{dV_{1-}(\tau)}{d\tau}\right]_{\tau=\tau(-\infty)} = \left[\frac{dV_{1+}(\tau)}{d\tau}\right]_{\tau=\tau(\infty)} = \lambda, \tag{29}$$

$$\frac{\{V_{1-}[\tau(-\infty)] - V_{1-}[\tau(i_0)]\} - \{V_{1+}[\tau(\infty)] - V_{1+}[\tau(i_0)]\}}{\tau(-\infty) - \tau(\infty)} = \lambda.$$

The second equation above is obtained from Eqs. (28a) and (28b). $\tau(-\infty)$ and $\tau(\infty)$ are points where the functions $\{V_{1-}(\tau) - V_{1-}[\tau(i_0)]\}$ and $\{V_{1+}(\tau) - V_{1+}[\tau(i_0)]\}$ have a cotangent line whose slope is equal to the constraining force λ. The formal solution $\tau(i)$ would be obvious from Eq. (28c).

As a specific example, it is worthwhile considering a solution that describes a Z–B or B–Z junction located at a specific site i_0 of a composite DNA whose twisting potential at each side of i_0 is equal to Eq. (4a) or Eq. (4b); the left-hand side or right-hand side of the specific site i_0 takes only the B form. In othe words, it is supposed that the twisting potential is

$$V_{1i} \equiv \begin{cases} \text{Eq. (4a)} & \text{for } \pm(i - i_0) \leqslant 0 \\ \text{Eq. (4b)} & \text{for } \pm(i - i_0) \geqslant 0 \end{cases} \tag{30}$$

For the twisting potential of Eq. (30), it is obvious from Eq. (12) that solutions of Eq. (14), which describe a Z–B or B–Z junction located at i_0, correspond to uniform negative twists of the conformation of Eq. (25).

$$\tau(i) = \tau_J \pm \mathrm{sgn}(i - i_0)\frac{\tau_B - \tau_Z}{2}[1 - \exp(-|i - i_0|/d_J)] + \Delta\tau, \tag{31}$$

where

$$\Delta\tau \leqslant 0.$$

In this case the constraining force λ is equal to

$$\lambda = (e_Z - e_B)/(\tau_Z - \tau_B) + \omega_0^2\Delta\tau = -\omega_0^2(\tau_B + \tau_Z - 2\tau_J - 2\Delta\tau)/2. \tag{32}$$

The solution Eq. (31) for a kink or Z–B junction is shown by a dotted line at the middle in Fig. 1. The twisting potential V_{1i} for this case is also shown by a dotted line at the bottom in Fig. 1. The nearest neighbor interaction potential $V_2 = (C_0^2/2)(d\tau/di)^2$, as a function of position i, does not depend on $\Delta\tau$, because of uniform change of twists. The conformational characteristics of this solution are described by each entry in Table I with Eq. (32) as the definition of λ. The idealization that the twisting force constant is the same for both the B and Z forms makes τ_i central symmetric at the position i_0 of a junction for both twisting potentials of Eqs. (4a) and (30), leading to simple pictures that $\tau_{J'}$ is zero and $e_{J'}$ is constant.

In case of a closed circular DNA, alternating B–Z conformations must have at least two junctions, a Z–B and a B–Z junction; note the periodic boundary conditions [Eq. (7)]. Equation (12) or (14) may have solutions that correspond to alternating B–Z conformations with multiple junctions. It is

TABLE I. Characteristics of alternating B–Z conformations.[a]

	Z form	Z–B or B–Z junction in alternating B–Z conformations	B form
Width		$2d_J \equiv 2C_0/\omega_0$	
Twist	$\tau_{Z'} = \tau_Z + \lambda/\omega_0^2$	$d_J\tau_{Z'} + \tau_{J'} + d_J\tau_{B'}$ $\tau_{J'} = 0$	$\tau_{B'} = \tau_B + \lambda/\omega_0^2$
Short range interaction energy	$e_{Z'} = e_Z + \lambda^2/(2\omega_0^2)$	$d_J e_{Z'} + e_{J'} + d_J e_{B'}$ $\{e_{J'} = C_0\omega_0[(\tau_B - \tau_Z)/2]^2\}$[b]	$e_{B'} = e_B + \lambda^2/(2\omega_0^2)$

[a] For Eq. (4a), $\lambda = (e_Z - e_B)/(\tau_Z - \tau_B) = -\omega_0^2(\tau_B + \tau_Z - 2\tau_J)/2$ or for Eq. (30), $\lambda = (e_Z - e_B)/(\tau_Z - \tau_B) + \omega_0^2\Delta\tau = -\omega_0^2(\tau_B + \tau_Z - 2\tau_J - 2\Delta\tau)/2$ with $\Delta\tau \leqslant 0$.

[b] This formula of $e_{J'}$ is appropriate for the regime $d_J \gg 1$ because Eq. (25) or (31) based on the continuous approximation is used.

hard to obtain such solutions of Eq. (12) or (14), because of a nonlinear equation. However, if the density of B–Z and $Z-B$ junctions in alternating B–Z conformations is so low that junctions are well separated from one another, then interactions between junctions could be neglected, and each junction in such conformations will be well approximated by solutions with only one junction like Eqs. (25) and (31). The continuous approximation has been used to obtain explicit functional forms of B–Z junctions. It should be noted here that all derived in the continuous approximation except the functional form of a junction and the estimate of $e_{J'}$ based on it do not depend on the approximation; $\tau_{J'} = 0$ for the twisting potential Eq. (4a) can be proved without the explicit functional form of τ_i.

In the following, the configurational partition function of Eq. (11) will be formulated phenomenologically for two cases; one in which the twisting potentials for all base pairs are represented by Eq. (4a), and the other in which DNA consists of two types of segments whose twisting potential is assumed to be Eq. (4a) or (4b). In either case, it is assumed that all possible conformations that consist of alternating Z and B regions separated by the Z–B or B–Z junction contribute significantly to the configurational partition function Z_C. Also, the average conformational characteristics of the Z and B regions and junctions are assumed to be well approximated by those in conformations that have only one junction.

A. Case (a) in which any base pair can take both the B and Z forms

The configurational partition function of Eq. (11) can be approximated by the sum of terms each of which represents the ensemble of uniform deformations over an entire DNA from the standard twist τ_B of the B form, or that from τ_Z of the Z form, or the ensemble of alternating B–Z conformations.

$$Z_C \simeq \sum_{\sigma\in\{B,Z\}} Z_{c,\sigma} + Z_{C,BZ}, \tag{33}$$

where the first and second terms are represented by

$$Z_{C,\sigma(\in\{B,Z\})} \simeq \int_{T_w/N > \tau_J \text{ or } T_w/N < \tau_J} dT_w\, Z_{\{\tau_i = T_w/N\}} \times \exp[-\beta V_L(T_w)] \times \exp\left[-\beta \sum_i V_{1i}(T_w/N)\right], \tag{34}$$

$$Z_{C,BZ} \simeq \sum_{m\in\{\text{alternating } B\text{–}Z \text{ conformations}\}} Z_{m,T_w(m)} \times \exp\{-\beta V_L[T_w(m)]\} \times \exp\left(-\beta\left[\sum_i V_{1i} + V_{2i}\right]_{\{\tau_i = \tau_i^m\}}\right). \tag{35}$$

First let us discuss Eq. (34). The librational factor in Eq. (34) is defined as

$$Z_{\{\tau_i = T_w/N\}} \equiv \int \prod_i d\Delta\tau_i \exp\left\{-\beta\left(\lambda\sum_i \Delta\tau_i + \sum_i\sum_j \frac{\Delta\tau_i\Delta\tau_j}{2} \times [V_{\tau_i\tau_j}]_{\{\tau_i = T_w/N\}}\right)\right\}\delta\left(\sum_i \Delta\tau_i\right) = \int \prod_i d\Delta\tau_i \exp\left\{-\beta\left(\sum_i\sum_j \frac{\Delta\tau_i\Delta\tau_j}{2} \times [V_{\tau_i\tau_j}]_{\{\tau_i = T_w/N\}}\right)\right\}\delta\left(\sum_i \Delta\tau_i\right), \tag{36}$$

where λ is the constraining force that is defined by Eq. (13). The second derivative matrix of the short range interaction potential is defined as

$$V_{\tau_i\tau_j} \equiv \left[\delta_{ij}\frac{d^2}{d\tau_i^2} V_{1i} - C_0^2(\delta_{i+1,j} - 2\delta_{ij} + \delta_{i-1,j})\right], \tag{37}$$

where δ_{ij} is Kronecker's δ. If V_{1i} is equal to Eq. (4a),

$$\frac{d^2}{d\tau_i^2} V_{1i} = \omega_0^2\left[1 - \delta\left(\frac{\tau_i - \tau_J}{\tau_B - \tau_Z}\right)\right] \tag{38}$$

and then the second derivative matrix of Eq. (37) will be equal to

$$V^0_{\tau_i\tau_j} \equiv [\omega_0^2\delta_{ij} - C_0^2(\delta_{i+1,j} - 2\delta_{ij} + \delta_{i-1,j})], \tag{39}$$

except $V_{\tau_i\tau_i}$ at $\tau_i = \tau_J$; the uniform deformation of $\tau_i = \tau_J$ corresponds to an unstable point in the short range interaction potential. The domain of integration of T_w in Eq. (34) is the region of the B or Z form, i.e., $T_w/N > \tau_J$ or $T_w/N < \tau_J$; however, significant contributions may come only from the small range of T_w/N near τ_B or τ_Z. In the result, the second

derivative matrix $V_{\tau_i\tau_j}$ in Eq. (36) can be replaced by $V^0_{\tau_i\tau_j}$ and then Eq. (34) is approximated by changing the domain of integration of T_w from a limited range to a whole domain.

$$Z_{C,\sigma(\in\{B,Z\})} \simeq \int dT_w \, Z^0_{\Sigma\Delta\tau_i=0} \exp[-\beta V_L(T_w)] \times \exp\left\{-\beta N\left[e_\sigma + \frac{\omega_0^2}{2}\left(\frac{T_w}{N} - \tau_\sigma\right)^2\right]\right\}, \quad (40)$$

where

$$Z^0_{\Sigma\Delta\tau_i=0} \equiv \int \prod_i d\Delta\tau_i \exp\left\{-\beta\left(\sum_i \sum_j \frac{\Delta\tau_i\Delta\tau_j}{2} V^0_{\tau_i\tau_j}\right)\right\} \times \delta\left(\sum_i \Delta\tau_i\right). \quad (41)$$

The librational factor [Eq. [41]] can easily be calculated from the fact that if $V_L = 0$ and $e_\sigma = 0$, Eq. (40) will be equal to the configurational partition function Z^0_{CP} for harmonic lattice vibrations with the force constant $V^0_{\tau_i\tau_i}$.

$$Z^0_{\Sigma\Delta\tau_i=0} = Z^0_{CP}\left(\frac{\beta\omega_0^2}{2\pi N}\right)^{1/2}, \quad (42)$$

where the configurational partition function Z^0_{CP} for harmonic lattice vibrations or phonons is

$$Z^0_{CP} \equiv \int \prod_i d\Delta\tau_i \exp\left\{-\beta\left(\sum_i \sum_j \frac{\Delta\tau_i\Delta\tau_j}{2} V^0_{\tau_i\tau_j}\right)\right\} = \left[\left(\frac{2\pi}{\beta\omega_0^2}\right)^{1/2} \frac{2}{\{1 + [1 + 4(C_0^2/\omega_0^2)]^{1/2}\}}\right]^N. \quad (43)$$

The derivation of Eq. (43) will be given in Appendix A. In the result, the ensemble of uniform deformations is represented by

$$Z_{C,\sigma(\in\{B,Z\})} \simeq Z^0_{CP}\left(\frac{\beta\omega_0^2}{2\pi N}\right)^{1/2}\left[\int dT_w \exp\left\{-\beta\frac{\omega_0^2 + B_0^2}{2N}\left(T_w - \frac{\omega_0^2 N\tau_\sigma + B_0^2 L_k}{\omega_0^2 + B_0^2}\right)^2\right\}\right] \quad (44a)$$

$$\times \exp(-\beta N e_\sigma)\exp\left[-\beta\frac{\omega_0^2 B_0^2}{2(\omega_0^2 + B_0^2)N}(L_k - N\tau_\sigma)^2\right]$$

$$= Z^0_{CP}\left(\frac{\omega_0^2}{\omega_0^2 + B_0^2}\right)^{1/2} \exp(-\beta N e_\sigma)\exp\left[-\beta\frac{\omega_0^2 B_0^2}{2(\omega_0^2 + B_0^2)N}(L_k - N\tau_\sigma)^2\right]. \quad (44b)$$

Next let us discuss Eq. (35) that represents the ensemble of alternating B–Z conformations. On the basis of the discrete system, the integral of T_w and the sum over energy-minimal conformations in Eq. (11) are replaced in Eq. (35) by the sum of $T_w(m)$ over alternating B–Z conformations; in a discrete system, the total twist $T_w(m)$ of an alternating B–Z conformation that corresponds to a local minimum of the short range interaction potential is quantized. If it is assumed that the average conformational characteristics of Z and B regions and junctions in alternating B–Z conformations with multiple junctions are well approximated by those in conformations consisting of one junction, the total twist $T_w(m)$ and the total short range interaction energy will be approximated by $(N_B\tau_{B'} + N_Z\tau_{Z'} + j\tau_{J'})$ and $(N_B e_{B'} + N_Z e_{Z'} + j e_{J'})$, respectively; N_B, N_Z, and j are the numbers of the B forms, Z forms, and junctions, respectively. The twists $\tau_{B'}$ and $\tau_{Z'}$ and the energies $e_{B'}$ and $e_{Z'}$ of the B and Z forms are defined by Eqs. (17) and (21), and the twist $\tau_{J'}$ and the energy $e_{J'}$ associated with the formation of a junction are defined by equivalent equations in the discrete system to Eqs. (19) and (20). Let us consider libration around such alternating B–Z conformations. If the twisting potential V_{1i} is the double quadratic potential of Eq. (4a), the short range interaction potential for small fluctuation of such energy-minimal conformations will be approximated as follows:

$$\left[\sum_i V_{1i} + V_{2i}\right]_{\{\tau_i = \tau_i^m + \Delta T_w/N + \Delta\tau_i \,|\, \sum_i \Delta\tau_i = 0\}}$$

$$= \left[\sum_i V_{1i} + V_{2i}\right]_{\{\tau_i=\tau_i^m\}} + \lambda\Delta T_w + \sum_i\sum_j \frac{1}{2}\left(\frac{\Delta T_w}{N} + \Delta\tau_i\right)\left(\frac{\Delta T_w}{N} + \Delta\tau_j\right)\left[V_{\tau_i\tau_j}\right]_{\{\tau_i=\tau_i^m\}} + \cdots$$

$$\simeq N_B e_{B'} + N_Z e_{Z'} + j e_{J'} + \lambda\Delta T_w + \frac{N\omega_0^2}{2}\left(\frac{\Delta T_w}{N}\right)^2 + \sum_i\sum_j \frac{\Delta\tau_i\Delta\tau_j}{2} V^0_{\tau_i\tau_j}$$

$$= N_B e_B + N_Z e_Z + j e_{J'} + \frac{N\omega_0^2}{2}\left(\frac{N_B(\tau_{B'} - \tau_B) + N_Z(\tau_{Z'} - \tau_Z) + \Delta T_w}{N}\right)^2 + \sum_i\sum_j \frac{\Delta\tau_i\Delta\tau_j}{2} V^0_{\tau_i\tau_j}, \quad (45)$$

where λ is the constraining force that is defined by Eq. (13). The second derivative matrix $V_{\tau_i\tau_j}$ at an alternating B–Z conformation is approximated as $V^0_{\tau_i\tau_j}$ with neglecting the singular point of $\tau_i = \tau_J$; refer to Eqs. (37)–(39). This approximation would be appropriate for small fluctuations in the discrete system; fluctuations must be too small to change positions of B–Z junctions. Equations (21), (23), and (24), which describe relationships among $\tau_{B'}$, $\tau_{Z'}$, $e_{B'}$, $e_{Z'}$, and λ for the twisting potential Eq. (4a), are used to transform the second to the third equation above. Then Eq. (35) would be approximated as follows; $\tau_{J'}$ is equal to zero in case of Eq. (4a).

$$Z_{C,BZ}\simeq Z^0_{\Sigma\Delta\tau_i=0}\sum_{\{N_B,N_Z,j|N=N_B+N_Z,j>1\}}\sum C(N_B,N_Z,j)\exp[-\beta(N_Be_B+N_Ze_Z+je_{J'})]$$
$$\times\int_{\Delta T_w\sim 0}d\Delta T_w\exp\left\{-\frac{\beta N\omega_0^2}{2}\left(\frac{N_B\tau_{B'}+N_Z\tau_{Z'}+j\tau_{J'}+\Delta T_w-N_B\tau_B-N_Z\tau_Z}{N}\right)^2\right\}$$
$$\times\exp\left\{-\frac{\beta B_0^2}{2N}(L_k-N_B\tau_{B'}-N_Z\tau_{Z'}-j\tau_{J'}-\Delta T_w)^2\right\}. \tag{46}$$

$C(N_B,N_Z,j)$ is the number of configurations in which the number of base pairs in the B form, the number of Z forms, and the total number of B–Z and Z–B junctions are equal to N_B, N_Z, and j, respectively; base pairs at a junction are regarded as either the Z form or B form and therefore $N=N_B+N_Z$. The sums in Eq. (46) are performed over all possible combinations of (N_B,N_Z,j) with at least a junction, i.e., $j\geqslant 1$. Then, with the approximation of extending the domain of integration of ΔT_w from a limited range to a whole domain, Eq. (46) is transformed to

$$Z_{C,BZ}\simeq Z^0_{CP}\left(\frac{\beta\omega_0^2}{2\pi N}\right)^{1/2}\sum_{\{N_B,N_Z,j|N=N_B+N_Z,j>1\}}\sum C(N_B,N_Z,j)\exp[-\beta(N_Be_B+N_Ze_Z+je_{J'})]$$
$$\times\int d\Delta T_w\exp\left\{-\frac{\beta(\omega_0^2+B_0^2)}{2N}\left(T_w+N_B\tau_{B'}+N_Z\tau_{Z'}+j\tau_{J'}-\frac{\omega_0^2(N_B\tau_B+N_Z\tau_Z+j\tau_{J'})+B_0^2L_k}{\omega_0^2+B_0^2}\right)^2\right\}$$
$$\times\exp\left\{-\beta\frac{\omega_0^2B_0^2}{2(\omega_0^2+B_0^2)N}(L_K-N_B\tau_B-N_Z\tau_Z-j\tau_{J'})^2\right\} \tag{47a}$$

$$=Z^0_{CP}\left(\frac{\omega_0^2}{\omega_0^2+B_0^2}\right)^{1/2}\sum_{\{N_B,N_Z,j|N=N_B+N_Z,j>1\}}\sum C(N_B,N_Z,j)\exp[-\beta(N_Be_B+N_Ze_Z+je_{J'})]$$
$$\times\exp\left\{-\beta\frac{\omega_0^2B_0^2}{2(\omega_0^2+B_0^2)N}(L_k-N_B\tau_B-N_Z\tau_Z-j\tau_{J'})^2\right\}. \tag{47b}$$

The assumptions and approximations used to derive Eqs. (47a) and (47b) are equivalent to neglecting interactions between phonons and junctions, and also neglecting the possibility that phonons are localized at either Z or B conformational regions; phonons will be equally distributed over Z and B regions in case of Eq. (4a), because the twisting force constant is the same for the Z and B forms. The approximation of neglecting phonon–junction interactions is more valid for smaller values of d_J; the limit of $d_J\to 0$ corresponds to the approximation of Ising model.

B. Case (b) in which only part of DNA can take both the B and Z forms

DNA is assumed to consist of two types of segments. Multiple segments with the total length N_2 that can take the Z form with the twisting potential of Eq. (4a) are supposed to be inserted into a host DNA with the length $N_1(=N-N_2)$ that can take only the B form with the potential of Eq. (4b); the number of junctions between those two types of segments is supposed to be equal to J. In this case, the limiting conformations induced by reducing the linking number cannot be uniformly, negatively twisted ones from the Z conformation but from the conformation in which the specific segements of DNA that can take the Z form are completely in the Z form and the other segments are in the B form; each segment is assumed to be longer than the width of a Z–B or B–Z junction $2d_J$. The presence of such conformations as energy-minimal ones is already proved; see Eq. (31). The second derivative of the twisting potential

$$\frac{d^2}{d\tau_i^2}V_{1i}=\begin{cases}\omega_0^2\left[1-\delta\left(\dfrac{\tau_i-\tau_J}{\tau_B-\tau_Z}\right)\right] & \text{if } V_{1i} \text{ is equal to Eq. (4a)}\\ \omega_0^2 & \text{if } V_{1i} \text{ is equal to Eq. (4b)}\end{cases} \tag{48}$$

is equal to ω_0^2 at every position i in the uniformly, negatively twisted conformations from the alternating B–Z conformation. Therefore, the second derivative matrix $V_{\tau_i\tau_j}$ at such conformations is equal to $V^0_{\tau_i\tau_j}$. The short range interaction potential for small fluctuation of such energy-minimal conformations is represented by

$$\left[\sum_i V_{1i}+V_{2i}\right]_{\{\tau_i=\tau_i^m+\Delta T_w/N+\Delta\tau_i|\ \Delta T_w<0,\sum_i\Delta\tau_i=0\}}$$
$$=\left[\sum_i V_{1i}+V_{2i}\right]_{\{\tau_i=\tau_i^m+\Delta T_w/N\}}+\left(\lambda+\frac{\omega_0^2T_w}{N}\right)\sum_i\Delta\tau_i+\sum_i\sum_j\frac{\Delta\tau_i\Delta\tau_j}{2}[V_{\tau_i\tau_j}]_{\{\tau_i=\tau_i^m+\Delta T_w/N\}}+\cdots$$
$$=N_1e_B+N_2e_Z+Je_{J'}+\frac{N\omega_0^2}{2}\left(\frac{N_1(\tau_{B'}-\tau_B)+N_2(\tau_{Z'}-\tau_Z)+\Delta T_w}{N}\right)^2+\sum_i\sum_j\frac{\Delta\tau_i\Delta\tau_j}{2}V^0_{\tau_i\tau_j}. \tag{49}$$

$(\lambda + \omega_0^2 \Delta T_w/N)$ with the definition Eq.(24) for λ is the constraining force for this specific conformation; see Eq. (32). Here it should be noted that Eq. (49) is, of course, the same as Eq. (45) with $N_B = N_1$, $N_Z = N_2$, and $j = J$. Thus the term of $N_B = N_1$, $N_Z = N_2$, and $j = J$ in Eq. (46) must be modified for the present case by extending the domain of integration of ΔT_w to include the range of 0 to $-\infty$. However, this modification does not change the final expressions of Eqs. (47a) and (47b). In the result, the partition function for case (b) is represented by

$$Z_C \simeq Z_{C,B} + Z_{C,BZ}, \tag{50}$$

where the first and second terms are given by Eqs. (44) and (47), respectively.

It should be noted here that the configurational partition functions [Eqs. (44) and (47)] includes the case of linear DNAs as a trivial case of $B_0^2 = 0$; the long range interaction potential V_L is assumed here to originate only from the conservation of linking number for a closed circular DNA. Of course, the combinatory factor $C(N_B,N_Z,j)$ in Eq. (47) depends upon whether a given system is a linear DNA or a closed circular DNA. In the next section, $C(N_B,N_Z,j)$ will be calculated specifically for a closed circular DNA, because we are interested in B-to-Z transitions of a closed circular DNA.

Before going further, it would be of interest to point out here that the librational factor $Z_{m,Tw}$ in Eq.(11) is just equal to the configurational partition function for lattice vibrations with a constraint described by the equation of motion shown in the following; however, a zero-frequency translation mode that generates another energy-minimal conformation must be excluded for $Z_{m,Tw}$. The continuous approximation for a position variable i is employed here to consider interactions between phonons and junctions; the displacive limit $d_J \equiv C_0/\omega_0 \gg 1$ is assumed.

$$\frac{d^2}{dt^2}\Delta\tau(i,t) + \left[\frac{d^2}{d\tau(i)^2} V_{1i}\right]_{\tau(i)=\tau^{m,Tw}(i)} \Delta\tau(i,t) - C_0^2 \frac{d^2}{di^2}\Delta\tau(i,t) - \mu(t) = 0, \tag{51}$$

$$\int di\, \Delta\tau(i,t) = 0.$$

The constraining force $\mu(t)$ is

$$\mu(t) = \frac{1}{N}\int_0^N di \left[\frac{d^2}{d\tau(i)^2} V_{1i}\right]_{\tau(i)=\tau^{m,Tw}(i)} \Delta\tau(i,t). \tag{52}$$

If $d^2V_{1i}/d\tau(i)^2$ with $\tau(i) = \tau^{m,Tw}(i)$ takes the same value irrespective of position i, the constraining force will be zero. Then libration around such conformations is represented by a simple form, Eq.(51) with $\mu = 0$, which does not explicitly include the long range constraining force. This is the case for libration around uniform deformations from the B and Z conformations and from the alternating B–Z conformation in case (b); the dispersion relation for this system is simply represented by Eq. (A3) with nonzero wave vectors $k \neq 0$ in Appendix A. Libration around alternating B–Z conformations is also described by Eq. (51). $d^2V_{1i}/d\tau^2$ for the twisting potential Eq. (4a) takes $d^2V_{1i}/d\tau_i^2 = \omega_0^2\{1 - \delta\{[\tau(i) - \tau_J]/(\tau_B - \tau_Z)\}) = \omega_0^2\{1 - \delta[(i - i_0)/(2d_J)]\}$ near $i \simeq i_0$ where a junction is located; Eq. (25) is used for $\tau(i)$. Thus the interactions between phonons and junctions must be taken into account. Currie *et al.*[33] discussed such interactions by solving Eq. (51) without a constraint of $\int di \Delta\tau(i,t) = 0$ and therefore with $\mu = 0$. However, in the present case, a constraining force originated from the presence of junctions is introduced as long range interactions into the system; see Eq. (52). Therefore even for a simple potential like double quadratic functions, if $d_J \gg 1$, Eq. (51) that includes long range interactions must be dealt with to estimate librational contributions. In general, the twisting potential V_{1i} may not be represented by simple functions like the double quadratic function Eq. (4a), and therefore $d^2V_{1i}/d\tau_i^2$ with $\{\tau_i = \tau_i^{m,Tw}\}$ may vary significantly at junctions and also take different values at the Z form and B form. Then phonons may be bound to or localized at junctions and Z or B regions. As a result, it becomes difficult to determine each vibrational mode and then to calculate the configuration partition function for such systems.

In the present formulation of librational contributions, interactions between phonons and junctions are neglected; see Eq. (45). Thus, strictly speaking, it is appropriate for the case of $d_J \equiv C_0/\omega_0 \lesssim 0(1)$, in other words, the case that the nearest neighbor interaction energy between twists is comparable with or smaller than the twisting potential. Also, phonons are treated to equally distribute over B and Z regions by assuming that the twisting force constants for the Z and B forms are the same. The case that the Z and B forms have different twisting force constants will be easily dealt with, if an Ising model is employed, i.e., if the limit of $d_J \equiv C_0/\omega_0 \to 0$ is assumed; in the Ising model, each twist is assumed to fluctuate independently. The formulation of the configurational partition function based on the Ising Model is discussed in Appendix B.

III. COMBINATORY FACTOR $C(N_B,N_Z,2n_J)$ FOR A CLOSED CIRCULAR DNA

In this section, the number $C(N_B,N_Z,2n_J)$ of ways to choose N_B B forms and N_Z Z forms with n_J regions in a closed circular DNA will be calculated; in a circular DNA, the number of B–Z or Z–B junctions must be equal to the number of B or Z regions and is represented here by n_J. The combinatory factor is formulated for two cases; (a) a simple case in which any base pair can take both the B and Z forms, and (b) another case that only a part of DNA can take the Z form as well as the B form. In the latter case, it is supposed that a DNA segment consisting of N_2 base pairs that can take the Z form is inserted into a host DNA with the length $N_1 (= N - N_2)$ that can take only the B form; for simplicity, the number of such inserted segments is assumed to be only one. The case (b) is considered because most experiments have been performed under such circumstances.

As stated in Sec. II, the repeating unit of the Z helix is not a single base pair, as it is in A and B DNA, but rather two successive base pairs (pyrimidine–purine pair).[4–7] Although the structure of a B–Z junction is not known, it might be better to assume that each Z conformational region in alternating B–Z conformations consists of even number of base pairs. In this section, the combinatory factor will be calculat-

ed for each case in which a Z region consisting of odd number of base pairs is either accepted or not accepted.

Let us define a generating function for $C(i,j,k)$ as follows:

$$g \equiv \sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\sum_{k=0}^{\infty} C(i,j,k) X_B^i X_Z^j X_J^k. \tag{53}$$

This generating function can be represented as the sum of all possible configurations consisting of alternating Z and B regions with various lengths separated by Z–B and B–Z junctions with a fixed length. First let us deal with the case that any base pair of DNA can take the Z form.

A. Case (a) in which any base pair can take both the B and Z forms

If Z helices consisting of odd numbers of base pairs are permitted, Eq. (53) will be represented by

$$\begin{aligned} g = {} & g_B(1) + g_Z(1) \\ & + \sum_{n_J=1}^{\infty}\Bigg[\sum_{\substack{i,j>0 \\ i+j>m_B}}\sum X_B^i [X_J g_Z(m_Z) X_J g_B(m_B)]^{n_J-1} \\ & \times X_J g_Z(m_Z) X_J X_B^j \\ & + \sum_{\substack{i,j>0 \\ i+j>m_Z}}\sum X_Z^i [X_J g_B(m_B) X_J g_Z(m_Z)]^{n_J-1} \\ & \times X_J g_B(m_B) X_J X_Z^j \Bigg], \end{aligned} \tag{54}$$

where

$$g_\sigma(m) \equiv \sum_{i=m}^{\infty} X_\sigma^i = \frac{X_\sigma^m}{1-X_\sigma} \quad \text{with } \sigma \in \{B,Z\}. \tag{55}$$

If it is assumed that each Z helix must consist of even number of base pairs, the summation of i and j in the fourth term of Eq. (54) will be restricted in such a way that $(i+j)$ must be even. Also, Eq. (55) will be modified as follows:

$$g_Z(m_Z) \equiv \sum_{i=m_Z/2}^{\infty} X_Z^{2i} = \frac{X_Z^{m_Z}}{1-X_Z^2}. \tag{56}$$

m_Z and m_B are the minimum lengths for Z and B regions; because each junction is regarded to consist of the d_J Z forms and d_J B forms, $m_Z \geqslant 2d_J$ and $m_B \geqslant 2d_J$. The first and second terms in Eq. (54) are generating functions for configurations consisting of B forms only or Z forms only. The third and fourth terms that represent the sum over distinguishable alternating B–Z conformations are formulated by assuming that the origin of the base sequence in this closed circular DNA can be identified. By expanding Eq. (54) in a series of X_B, X_Z, and X_J, the combinatory factor can be calculated.

Nonzero elements of the combinatory factor $C_{a_1}(N_B,N_Z,2n_J)$ for the case that Z helices consisting of odd numbers of base pairs are permitted are

$$\begin{aligned} & C_{a_1}(n_J m_B + i, n_J m_Z + j, 2n_J) \\ & = \frac{(n_J-1+i)!\,(n_J-1+j)!}{i!\,(n_J-1)!\,j!\,(n_J-1)!}\binom{n_J m_B + i + n_J m_Z + j}{n_J}, \end{aligned} \tag{57}$$

$$C_{a_1}(N,0,0) = C_{a_1}(0,N,0) = 1,$$

where

$$n_J m_B + i + n_J m_Z + j = N, \quad m_B \geqslant 2d_J, \quad m_Z \geqslant 2d_J, \quad n_J \geqslant 1, \quad i,j \geqslant 0.$$

If each Z helix is assumed to consist of even number of base pairs, Eq. (57) will be modified as follows; m_Z must be even in this case.

$$C_{a_2}(n_J m_B + i, n_J m_Z + j, 2n_J) = \begin{cases} \dfrac{(n_J-1+i)!\,(n_J-1+j/2)!}{i!\,(n_J-1)!\,(j/2)!\,(n_J-1)!}\binom{n_J m_B + i + n_J m_Z + j}{n_J} & \text{for even } j \\ 0 & \text{for odd } j \end{cases}, \tag{58}$$

$$C_{a_2}(N,0,0) = 1, \quad C_{a_2}(0,N,0) = \begin{cases} 1 & \text{for even } N \\ 0 & \text{for odd } N \end{cases}.$$

B. Case (b) in which only a part of DNA can take both the B and Z forms

Next, let us consider the case in which only a specific segment with the length N_2 in a closed circular DNA with the total length $N = N_1 + N_2$ can take the Z form; for simplicity, the number of such segments is assumed to be only one. The generating function of Eq. (53) for this case is represented by

$$\begin{aligned} g = X_B^{N_1}\Bigg\{ & g_B(0) + \sum_{n_J=1}^{\infty} g_B(0)[X_J g_Z(m_Z) X_J g_B(m_B)]^{n_J-1} \\ & \times X_J g_Z(m_Z) X_J g_B(0)\Bigg\}, \end{aligned} \tag{59}$$

where $N_1 \geqslant m_B$ is assumed; g_B and g_Z are given by Eq. (55) or (56). The first term in Eq. (59) represents a generating function for configurations consisting of B forms only and the second term for configurations of alternating B–Z conformations that contain a fixed B-form region with the length N_1. Then, if Z helices consisting of odd numbers of base pairs are permitted, nonzero elements of C_{b_1} will be

$$\begin{aligned} & C_{b_1}[N_1 + (n_J-1)m_B + i, n_J m_Z + j, 2n_J] \\ & = \frac{(n_J+i)!\,(n_J-1+j)!}{i!\,n_J!\,j!\,(n_J-1)!}, \end{aligned} \tag{60}$$

$$C_{b_1}(N,0,0) = 1,$$

where

$$(n_J-1)m_B+i+n_Jm_Z+j=N_2,\quad N_1+N_2=N,$$
$$m_B\geqslant 2d_J,\quad m_Z\geqslant 2d_J,\quad n_J\geqslant 1,\ i,j\geqslant 0.$$

If each Z helix is assumed to consist of even number of base pairs, Eq. (60) will be modified as follows; m_Z must be even in this case.

$$C_{b_2}[N_1+(n_J-1)m_B+i,\,n_Jm_Z+j,\,2n_J]=\begin{cases}\dfrac{(n_J+i)!\,(n_J-1+j/2)!}{i!\,n_J!\,(j/2)!\,(n_J-1)!} & \text{for even } j\\ 0 & \text{for odd } j\end{cases}, \tag{61}$$

$$C_{b_2}(N,0,0)=1.$$

In the result, from Eqs.(33), (44), (47), and (50), the configurational partition function is represented as follows:

For case (a),

$$Z_C(L_k)\simeq\left\{W_a(N,0,0)+W_a(0,N,0)+\sum_{n_J=1}^{\max(n_J)}\sum_{j=0}^{N-(m_B+m_Z)n_J}W_a[n_Jm_B+(N-n_Jm_B-n_Jm_Z-j),\,n_Jm_Z+j,\,2n_J]\right\}, \tag{62}$$

where

$$\max(n_J)=\lfloor N/(m_B+m_Z)\rfloor$$

and for case (b),

$$Z_C(L_k)\simeq\left(W_b(N,0,0)+\sum_{n_J=1}^{\max(n_J)}\sum_{j=0}^{N_2+m_B-(m_B+m_Z)n_J}W_b\{N_1+(n_J-1)m_B+[N_2-(n_J-1)m_B-n_Jm_Z-j],n_Jm_Z+j,2n_J\}\right), \tag{63}$$

where

$$\max(n_J)=\lfloor (N_2+m_B)/(m_B+m_Z)\rfloor.$$

The statistical weight of each configuration, W_I for case $I\in\{a,b\}$, is given by

$$\begin{aligned}W_I(N_B,N_Z,2n_J)\equiv&Z^0_{CP}\left(\frac{\omega_0^2}{\omega_0^2+B_0^2}\right)^{1/2}C_I(N_B,N_Z,2n_J)\ \exp[-\beta(N_Be_B+N_Ze_Z+2n_Je_{J'})]\\&\times\left(\frac{\beta(\omega_0^2+B_0^2)}{2\pi N}\right)^{1/2}\int dT_w\exp\left[-\frac{\beta(\omega_0^2+B_0^2)}{2N}\left(T_w-\frac{\omega_0^2(N_B\tau_B+N_Z\tau_Z+2n_J\tau_{J'})+B_0^2L_k}{\omega_0^2+B_0^2}\right)^2\right]\\&\times\exp\left[-\beta\frac{\omega_0^2B_0^2}{2(\omega_0^2+B_0^2)N}(L_k-N_B\tau_B-N_Z\tau_Z-2n_J\tau_{J'})^2\right]\end{aligned} \tag{64a}$$

$$\begin{aligned}=&Z^0_{CP}\left(\frac{\omega_0^2}{\omega_0^2+B_0^2}\right)^{1/2}C_I(N_B,N_Z,2n_J)\exp[-\beta(N_Be_B+N_Ze_Z+2n_Je_{J'})]\\&\times\exp\left[-\beta\frac{\omega_0^2B_0^2}{2(\omega_0^2+B_0^2)N}(L_k-N_B\tau_B-N_Z\tau_Z-2n_J\tau_{J'})^2\right].\end{aligned} \tag{64b}$$

The combinatory factors C_I are given by Eqs.(57), (58), (60), or (61). In the present model, $\tau_{J'}$ is equal to zero, because the twisting potential is assumed to be Eq. (4a). Z^0_{CP} is the configurational partition function for harmonic lattice vibrations or phonons and given by Eq. (43). It should be noted here that although Eq. (64b) is similar to formulas[25–27] in which the free energy of a closed circular DNA with a given linking number is evaluated by the empirical function,[20–24] it is rather derived here by explicitly taking account of twisting and writhing potentials. For the case in which the B and Z forms have different twisting force constants, the statistical weight W_I has been calculated in the Ising limit $d_J\equiv C_0/w_0\to 0$; the result is given in Appendix B.

IV. STATISTICAL CHARACTERISTICS OF B, Z, AND ALTERNATING B–Z CONFORMATIONS

Before discussing B-to-Z transitions in a closed circular DNA, let us consider conformational characteristics of each of B, Z, and alternating B–Z conformations. From Eq. (44a) or (64a), the statistical average of the total twist over the ensemble of B-form DNA or Z-form DNA is equal to

$$\langle T_w\rangle_\sigma-N\tau_\sigma=\frac{B_0^2(L_k-N\tau_\sigma)}{\omega_0^2+B_0^2}\quad\text{with }\sigma\in\{B,Z\}. \tag{65}$$

Angle brackets with the subscript σ represent the statistical average over the ensemble of the σ-type of conformations; those without a subscript simply represent the statistical average over all conformations. The right-hand side of the above equation represents the shift of twist from the standard twist of the B or Z form due to the linking difference $(L_k-N\tau_\sigma)$. Also the linking difference causes the writhe of DNA that leads to the supercoil formation. The statistical average of the writhing number would be obvious from Eq. (8), i.e.,

$$\langle T_w\rangle_\sigma+\langle W_r\rangle_\sigma=L_k\quad\text{with }\sigma\in\{B,Z,BZ\}. \tag{66}$$

If the twisting force constant ω_0^2 is much larger than the writhing force constant B_0^2, most of the linking difference will be transformed into the writhing number. These equa-

tions describe how the linking number is distributed between the writhing and twisting numbers. Likewise, the statistical average of the total twist in alternating B–Z conformations can be calculated from Eq. (64a). A general form including Eq. (65) as a special case is

$$\langle T_w\rangle_\sigma = \frac{\omega_0^2\langle(N_B\tau_B + N_Z\tau_Z + 2n_J\tau_{J'})\rangle_\sigma + B_0^2 L_k}{\omega_0^2 + B_0^2}$$
with $\sigma\in\{B,Z,BZ\}$. (67)

Here it should be noted that Eqs. (66) and (67) also represent the statistical averages of the twisting number and the writhing number over all conformations because both equations can be applied to conformations of all types, i.e., B, Z, and B–Z.

The statistical average of N_Z over alternating B–Z conformations would be approximated by the most probable value of N_Z.

$$[-\ln W_I(N-\langle N_Z\rangle_{BZ},\langle N_Z\rangle_{BZ},2\langle n_J\rangle_{BZ})]$$
$$\simeq \min_{N_Z}[-\ln W_I(N-N_Z,N_Z,2\langle n_J\rangle_{BZ})]. \quad (68)$$

If $m_Z < \langle N_Z\rangle_{BZ} < N_2$, then $\langle N_Z\rangle_{BZ}$ will satisfy

$$\left\{\frac{\partial}{\partial N_Z}[-1/\beta\ln W_I(N-N_Z,N_Z,2n_J)]\right\}_{n_J=\langle n_J\rangle_{BZ}}$$
$$= \left\{\frac{\partial}{\partial N_Z}[-1/\beta\ln C_I(N-N_Z,N_Z,2n_J)]\right\}_{n_J=\langle n_J\rangle_{BZ}}$$
$$+ (e_Z-e_B) - \frac{\omega_0^2 B_0^2}{(\omega_0^2+B_0^2)N}(\tau_Z-\tau_B)$$
$$\times[L_k - N\tau_B - N_Z(\tau_Z-\tau_B) - 2\langle n_J\rangle_{BZ}\tau_{J'}]$$
$$\simeq 0. \quad (69)$$

Likewise, the statistical average of n_J would be approximated by the most probable value of n_J.

$$[-\ln W_I(N-\langle N_Z\rangle_{BZ},\langle N_Z\rangle_{BZ},2\langle n_J\rangle_{BZ})]$$
$$\simeq \min_{n_J}[-\ln W_I(N-\langle N_Z\rangle_{BZ},\langle N_Z\rangle_{BZ},2n_J)]. \quad (70)$$

If $1 < \langle n_J\rangle_{BZ} < \max(n_J),\langle n_J\rangle_{BZ}$ will satisfy the following equation:

$$\left\{\frac{\partial}{\partial n_J}[-1/\beta\ln W_I(N-N_Z,N_Z,2n_J)]\right\}_{N_Z=\langle N_Z\rangle_{BZ}} \simeq 0. \quad (71)$$

Therefore, in case of $1 < \langle N_Z\rangle_{BZ} < N_2$, Eq. (67) for alternating B–Z conformations can be approximated as follows by using Eq. (69).

$$\langle T_w\rangle_{BZ} - \langle(N_B\tau_B + N_Z\tau_Z + 2n_J\tau_{J'})\rangle_{BZ} - \frac{N(e_B-e_Z)}{\omega_0^2(\tau_B-\tau_Z)} \left[= \langle T_w\rangle_{BZ} - \langle(N_B\tau_{B'} + N_Z\tau_{Z'} + 2n_J\tau_{J'})\rangle_{BZ}\right]$$
$$\simeq \frac{N}{\beta\omega_0^2(\tau_B-\tau_Z)}\left\{\frac{\partial}{\partial N_Z}[\ln C_I(N-N_Z,N_Z,2n_J)]\right\}_{N_Z=\langle N_Z\rangle_{BZ},n_J=\langle n_J\rangle_{BZ}} \quad (72a)$$
$$\langle T_w\rangle_{BZ} \simeq L_k - \frac{N(e_B-e_Z)}{B_0^2(\tau_B-\tau_Z)} - \frac{N}{\beta B_0^2(\tau_B-\tau_Z)}\left\{\frac{\partial}{\partial N_Z}[\ln C_I(N-N_Z,N_Z,2n_J)]\right\}_{N_Z=\langle N_Z\rangle_{BZ},n_J=\langle n_J\rangle_{BZ}}. \quad (72b)$$

Equation (72a) indicates that the statistical average of the total twist over alternating B–Z conformations deviates from its mechanically stable point $\langle N_B\tau_{B'} + N_Z\tau_{Z'} + 2n_J\tau_{J'}\rangle_{BZ}$ due to the contribution of entropy; the twists $\tau_{B'}$ and $\tau_{Z'}$ of B and Z forms at the mechanically stable point differ from the standard twists τ_B and τ_Z of the B and Z forms, respectively, unless e_Z is equal to e_B.

Another interesting feature is that the variance of the total twist in a closed circular DNA is not the same as that expected for a linear DNA. From Eq. (64a), the variance of the total twist is the same irrespectively of conformational types.

$$\langle(T_w-\langle T_w\rangle_\sigma)^2\rangle_\sigma = \langle(W_r-\langle W_r\rangle_\sigma)^2\rangle_\sigma = \frac{N}{\beta(\omega_0^2+B_0^2)}$$
with $\sigma\in\{B,Z,BZ\}$. (73)

Long range interactions due to the conservation of linking number that is specific to closed circular DNAs reduce the variance of the total twist as if the twisting force constant increased from ω_0^2 to $(\omega_0^2 + B_0^2)$. This is consistent with the experimental fact[37] that a supercoiled DNA is torsionally more rigid than a linear DNA. It should be noted here that Eq. (73) is applied to the ensemble of closed circular DNAs with the same linking number. If the ensemble of closed DNAs is in equilibrium with regard to the linking number, the variance of the twisting number must be larger than Eq. (73) because of the variation of the linking number, and must be equal to that for a linear DNA.

Let us suppose that the ensemble of closed circular DNAs is in equilibrium with regard to the linking number. The partition function for this case is equal to the sum of $Z_C(L_k)$ over the linking number L_k. Equation (64b) indicates that the equilibrium ensemble of closed circular DNAs over the linking number obeys a Gaussian distribution:

$$\overline{L_k} = \overline{\langle N_B\tau_B + N_Z\tau_Z + 2n_J\tau_{J'}\rangle},$$
$$\overline{(L_k-\overline{L_k})^2} = \frac{N}{\beta\kappa}, \quad (74)$$

where

$$\kappa \equiv \frac{\omega_0^2 B_0^2}{(\omega_0^2+B_0^2)}. \quad (75)$$

Here the bar on variables represents their statistical averages with regard to the linking number. This is consistent with the experimental fact[22,23] that the equilibrium ensemble of topoisomers with different linking numbers, which is generated by using nicking-closing enzymes, obeys a Gaussian distribution of the linking number whose variance is almost

proportional to the total length of DNA, indicating that the second order approximation [Eq. (9)] for the writhing potential is adequate.

Then, the statistical average and the variance of the twisting number in the equilibrium ensemble of a closed DNA with regard to the linking number are, of course, equal to those for a linear DNA.

$$\overline{\langle T_w \rangle} = \frac{\overline{\omega_0^2 \langle (N_B \tau_B^2 + N_Z \tau_Z + 2 n_J \tau_{J'}) \rangle} + B_0^2 L_k}{\omega_0^2 + B_0^2}$$

$$= \overline{\langle (N_B \tau_B + N_Z \tau_Z + 2 n_J \tau_{J'}) \rangle} = \overline{L_k}, \tag{76}$$

$$\overline{\langle (T_w - \overline{\langle T_w \rangle})^2 \rangle}$$

$$= \overline{\langle (T_w - \langle T_w \rangle)^2 \rangle} + \overline{\langle (\langle T_w \rangle - \overline{\langle T_w \rangle})^2 \rangle}$$

$$= \frac{N}{\beta(\omega_0^2 + B_0^2)} + \overline{\left(\frac{B_0^2 (L_k - \overline{L_k})}{\omega_0^2 + B_0^2} \right)^2} = \frac{N}{\beta \omega_0^2}.$$

Likewise, the statistical average and the variance of the writhing number are

$$\overline{\langle W_r \rangle} = 0, \quad \overline{\langle (W_r - \overline{\langle W_r \rangle})^2 \rangle} = \frac{N}{\beta B_0^2}. \tag{77}$$

Thus the sum of the variances of the twisting number and the writhing number is equal to the variance of the liking number; in this case, the twisting number and writhing number are independent variables.

V. B-TO-Z TRANSITIONS BY CHANGING THE LINKING NUMBER

Transitions between B and Z conformations in a closed circular DNA may be defined as the structural transition between overall B or Z conformations and alternating B–Z conformations with a certain number of Z or B forms; changing an external variable beyond the transition point causes a gradual increase of Z or B forms. Thus, the order parameter for this kind of transition is not the proportion of the number of base pairs in the Z form $\langle N_Z \rangle / N_2$, but that of alternating B–Z conformations $\langle N_Z \rangle / \langle N_Z \rangle_{BZ}$; the former may be the order parameter, if transitions are induced in a linear DNA by changing environmental parameters such as the salt condition that would correspond to changing the relative energy $(e_Z - e_B)$ of the Z form to the B form. Although such transitions in a closed circular DNA may be induced by changing one of the external variables, we will here deal with those induced by changing the linking number; specifically B-to-Z transitions from overall B conformations to alternating B–Z conformations.

The transition point for the B-to-Z transition may be defined to be where the free energy of overall B conformations is equal to that of alternating B–Z conformations, which is then approximated by the free energy of the conformations with N_Z and n_J equal to their most probable values. In other words, the following equation would approximately be satisfied at the transition point:

$$\beta \Delta F(\langle N_Z \rangle_{BZ}(\{x_i\}), \langle n_J \rangle_{BZ}(\{x_i\}), \{x_i\})$$
$$\equiv [-\ln W_I(\langle N_B \rangle_{BZ}, \langle N_Z \rangle_{BZ}, 2\langle n_J \rangle_{BZ})]$$
$$- [-\ln W_I(N, 0, 0)] \simeq 0. \tag{78}$$

The statistical weight W_I is given by Eq. (64b). In this section, $\langle N_Z \rangle_{BZ}$ and $\langle n_J \rangle_{BZ}$ should be regarded as the most probable values of N_Z and n_J rather than their statistical averages. The free energy difference ΔF between overall B conformations and alternating B–Z conformations is a function of external variables $\{x_i\}$; $\langle N_Z \rangle_{BZ}$ and $\langle n_J \rangle_{BZ}$ are internal variables that are functions of $\{x_i\}$. The external variables $\{x_i\}$ may be chosen as

$$\{x_i\} \equiv \{\Delta L_k, N/(\beta\kappa), N_2, \beta(e_Z - e_B), \beta e_{J'}\}, \tag{79}$$

where the linking difference ΔL_k is defined as

$$\Delta L_k \equiv L_k - N\tau_B. \tag{80}$$

κ is already defined by Eq. (75). The variables N_2 is the number of base pairs that can take the Z form; in case (a), N_2 might be regarded as N. The linking number at the transition point is termed the transition linking number and represented by $L_{k,t}$ in the following.

The explicit expression of Eq. (78), obtained by substituting W_I by Eq. (64b), is

$$(L_{k,t} - N\tau_B) \simeq \frac{1}{2}(\langle N_Z \rangle_{BZ}(\tau_Z - \tau_B) + 2\langle n_J \rangle_{BZ} \tau_{J'})$$
$$+ \frac{N}{\beta\kappa} \frac{[\langle N_Z \rangle_{BZ} \beta(e_Z - e_B) + 2\langle n_J \rangle_{BZ} \beta e_{J'} - \ln C_I(\langle N_B \rangle_{BZ}, \langle N_Z \rangle_{BZ}, 2\langle n_J \rangle_{BZ})]}{\langle N_Z \rangle_{BZ}(\tau_Z - \tau_B) + 2\langle n_J \rangle_{BZ} \tau_{J'}}. \tag{81}$$

The transition linking number $L_{k,t}$ might be calculated by solving the simultaneous equations, (68), (70), and (81); however, it will be difficult to obtain $L_{k,t}$ for general cases, because of complicated forms of the combinatory factor. In the following, we will consider the dependencies of the transition point on the external variables given by Eq. (79).

First let us consider the dependencies of the transition linking number $L_{k,t}$ on the other external variables x_j. From Eq. (78),

$$\left(\frac{\partial \Delta L_{k,t}}{\partial x_j} \right)_{\{x_i | x_i \neq \Delta L_{k,t}, x_j\}} \simeq -\left(\frac{\partial \beta \Delta F}{\partial x_j} \right)_{\{x_i | x_i \neq x_j\}} \Big/ \left(\frac{\partial \beta \Delta F}{\partial \Delta L_{k,t}} \right)_{\{x_i | x_i \neq \Delta L_{k,t}\}}. \tag{82}$$

Unless x_k is N_2, the partial derivative of $\beta\Delta F$ by an external variable x_k will be

$$\left(\frac{\partial \beta \Delta F}{\partial x_k} \right)_{\{x_i | x_i \neq x_k\}} = \left(\frac{\partial \beta \Delta F}{\partial x_k} \right)_{\langle N_Z \rangle_{BZ}, \langle n_J \rangle_{BZ}, \{x_i | x_i \neq x_k\}} \tag{83}$$

because

$$\left(\frac{\partial\beta\Delta F}{\partial\langle N_Z\rangle_{Bz}}\right)_{\langle n_J\rangle_{BZ},\,\{x_i\}}\left(\frac{\partial\langle N_Z\rangle_{BZ}}{\partial x_k}\right)_{\{x_i|x_i\neq x_k\}}=0, \tag{84}$$

$$\left(\frac{\partial\beta\Delta F}{\partial\langle n_J\rangle_{Bz}}\right)_{\langle N_Z\rangle_{BZ},\,\{x_i\}}\left(\frac{\partial\langle n_J\rangle_{BZ}}{\partial x_k}\right)_{\{x_i|x_i\neq x_k\}}=0. \tag{85}$$

Equations (84) and (85) are satisfied because either the first factor or second factor is zero; because $\langle N_Z\rangle_{BZ}$ and $\langle n_J\rangle_{BZ}$ are defined here as the most probable values of N_Z and n_J, the first factor in Eq. (84) will be zero if $m_Z<\langle N_Z\rangle_{BZ}<N_2$, and that in Eq. (85) will be zero if $1<\langle n_J\rangle_{BZ}<\max(n_J)$, otherwise the second factor in their equations will be zero. Refer to Eqs. (68)–(71). In the result, the left-hand side of Eq. (82) becomes

$$\left(\frac{\partial\Delta L_{k,t}}{\partial x_k}\right)_{\{x_i|x_i\neq\Delta L_{k,t},\,x_k\}}\simeq\left(\frac{\partial\Delta L_{k,t}}{\partial x_k}\right)_{\langle N_Z\rangle_{BZ},\,\langle n_J\rangle_{BZ},\,\{x_i|x_i\neq\Delta L_{k,t},\,x_k\}} \tag{86}$$

In case of $x_k=N_2$, if $m_Z\leqslant\langle N_Z\rangle_{BZ}<N_2$ and $1\leqslant\langle n_J\rangle_{BZ}<\max(n_J)$, Equations (84) and (85) and then Eqs. (83) and (86) will be satisfied; otherwise the following equation will be satisfied instead of Eq. (83).

$$\left(\frac{\partial\beta\Delta F}{\partial N_2}\right)_{\{x_i|x_i\neq N_2\}}\leqslant\left(\frac{\partial\beta\Delta F}{\partial N_2}\right)_{\langle N_Z\rangle_{BZ},\,\langle n_J\rangle_{BZ},\,\{x_i|x_i\neq N_2\}}. \tag{87}$$

The inequality in Eq. (87) comes from the fact that $\langle N_Z\rangle_{BZ}$ and $\langle n_J\rangle_{BZ}$ are the most probable values of N_Z and n_J. Then, from Eqs. (82), (83), and (87), the following equation is obtained for the case of $\langle N_Z\rangle_{BZ}=N_2$ or $\langle n_J\rangle_{BZ}=\max(n_J)$:

$$\left(\frac{\partial\Delta L_{k,t}}{\partial N_2}\right)_{\{x_i|x_i\neq\Delta L_{k,t},\,N_2\}}\gtrsim\left(\frac{\partial\Delta L_{k,t}}{\partial N_2}\right)_{\langle N_Z\rangle_{BZ},\,\langle n_J\rangle_{BZ},\,\{x_i|x_i\neq\Delta L_{k,t},\,N_2\}} \tag{88}$$

because the denominator in the right-hand side of Eq. (82) is supposed to be positive;

$$\left(\frac{\partial\beta\Delta F}{\partial\Delta L_k}\right)_{\{x_i|x_i\neq\Delta L_{k,t}\}}=-\frac{\beta\kappa}{N}\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'}>0. \tag{89}$$

Equation (89) simply represents that reducing the linking number is favorable to more negatively twisted conformations with Z forms than overall B conformations. Equations (86) and (88) indicate that the transition linking number $L_{k,t}$ as a function of $\langle N_Z\rangle_{BZ}$ and $\langle n_J\rangle_{BZ}$ takes a maximal value at $\langle N_Z\rangle_{BZ}(\Delta L_{k,t})$ and $\langle n_J\rangle_{BZ}(\Delta L_{k,t})$.

Now let us evaluate the dependencies of the transition linking number on the external variables from Eqs. (81) and (86) or (88).

$$\left(\frac{\partial(L_{k,t}-N\tau_B)}{\partial N/(\beta\kappa)}\right)_{N_2,\,\beta(e_Z-e_B),\,\beta e_{J'}}\simeq\frac{[\langle N_Z\rangle_{BZ}\beta(e_Z-e_B)+2\langle n_J\rangle_{BZ}\beta e_{J'}-\ln C_I(\langle N_B\rangle_{BZ},\langle N_Z\rangle_{BZ},2\langle n_J\rangle_{BZ})]}{\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'}}. \tag{90}$$

The right-hand side of Eq. (90) will be negative unless N_2 is so large that the combinatory entropy (the third term of the numerator) overcomes the short range interaction energies (the first and second terms); the total short range energy is supposed to be positive. On the other hand, the dependency of the transition linking number density on the DNA length is simple.

$$\left(\frac{\partial(L_{k,t}-N\tau_B)/N}{\partial N}\right)_{N_2,\,\beta(e_Z-e_B),\,\beta e_{J'},\,\beta\kappa}\simeq-\frac{1}{2N^2}(\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'})>0. \tag{91}$$

The above equation indicates that as DNA becomes long, the density of the linking difference at the transition point always increases. The second term in Eq. (81) is the asymptotic form of $\Delta L_{k,t}$ in the limit of large N. The dependence of $L_{k,t}$ on N_2 is

$$\left(\frac{\partial(L_{k,t}-N\tau_B)}{\partial N_2}\right)_{N/(\beta\kappa),\,\beta(e_Z-e_B),\,\beta e_{J'}}\gtrsim\frac{-N}{\beta\kappa(\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'})}\times\left[\frac{\partial}{\partial N_2}\ln C_I(\langle N_B\rangle_{BZ},\langle N_Z\rangle_{BZ},2\langle n_J\rangle_{BZ})\right]_{\langle N_Z\rangle_{BZ},\,\langle n_J\rangle_{BZ}}>0. \tag{92}$$

Here it should be noted that unless $\langle N_Z\rangle_{BZ}=N_2$ or $\langle n_J\rangle_{BZ}=\max(n_J)$, the equality will be satisfied in the equation above. The right-hand side of the above equation must be positive because the combinatory entropy always increases as N_2 increases. Thus, the transition linking number increases as N_2 increases. Likewise, the dependencies of the transition linking number on the structural parameters of DNA such as $\beta(e_Z-e_B)$ and $\beta e_{J'}$ are obtained as follows:

$$\left(\frac{\partial(L_{k,t}-N\tau_B)}{\partial\beta(e_Z-e_B)}\right)_{N/(\beta\kappa),\,N_2,\,\beta e_{J'}}\simeq\frac{N}{\beta\kappa}\frac{\langle N_Z\rangle_{BZ}}{(\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'})}<0, \tag{93}$$

$$\left(\frac{\partial(L_{k,t}-N\tau_B)}{\partial\beta e_{J'}}\right)_{N/(\beta\kappa),\,N_2,\,\beta(e_Z-e_B)}\simeq\frac{N}{\beta\kappa}\frac{2\langle n_J\rangle_{BZ}}{(\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'})}<0. \tag{94}$$

Equations (93) and (94) may be obvious; since the increase of $\beta(e_Z-e_B)$ makes the Z form unstable, and that of $\beta e_{J'}$ is unfavorable to the formation of a B–Z junction, large twisting stress is necessary to induce the B-to-Z transition.

Next, let us consider the dependencies of the internal variables, the most probable values of N_Z and n_J, on the external variables at the transition point. If $m_Z < \langle N_Z \rangle_{BZ} < N_2$, and $1 < \langle n_J \rangle_{BZ} < \max(n_J)$ at $L_k = L_{k,t}$, the partial derivatives of the right-hand side of Eq. (81) with regard to the variables $\langle N_Z \rangle_{BZ}$ and $\langle n_J \rangle_{BZ}$ must be zero at $L_k = L_{k,t}$; refer to Eqs. (86) and (88).

$$\left(\frac{\partial(L_{k,t} - N\tau_B)}{\partial\langle N_Z\rangle_{BZ}}\right)_{\langle n_J\rangle_{BZ},\,\{x_i|x_i\neq\Delta L_{k,t}\}} \simeq 0,$$
$$\left(\frac{\partial(L_{k,t} - N\tau_B)}{\partial\langle n_J\rangle_{BZ}}\right)_{\langle N_Z\rangle_{BZ},\,\{x_i|x_i\neq\Delta L_{k,t}\}} \simeq 0. \tag{95}$$

The following equation is obtained by partially differentiating these equations by an external variable $x_j \in \{N/(\beta\kappa), N_2, \beta(e_Z - e_B), \beta e_{J'}\}$.

$$\begin{pmatrix} \partial\langle N_Z\rangle_{BZ}/\partial x_j \\ \partial\langle n_J\rangle_{BZ}/\partial x_j \end{pmatrix} \simeq M^{-1} \begin{pmatrix} \partial^2\Delta L_{k,t}/\partial x_j \partial\langle N_Z\rangle_{BZ} \\ \partial^2\Delta L_{k,t}/\partial x_j \partial\langle n_J\rangle_{BZ} \end{pmatrix}, \tag{96}$$

where

$$M \equiv \begin{pmatrix} \partial^2(-\Delta L_{k,t})/\partial\langle N_Z\rangle_{BZ}^2 & \partial^2(-\Delta L_{k,t})/\partial\langle n_J\rangle_{BZ}\partial\langle N_Z\rangle_{BZ} \\ \partial^2(-\Delta L_{k,t})/\partial\langle N_Z\rangle_{BZ}\partial\langle n_J\rangle_{BZ} & \partial^2(-\Delta L_{k,t})/\partial\langle n_J\rangle_{BZ}^2 \end{pmatrix}. \tag{97}$$

The second derivative matrix M of $(-\Delta L_{k,t})$ at $L_k = L_{k,t}$ is positive definite and then the inverse matrix of M is also positive definite; Eqs. (86) and (88) indicate that the transition linking number $L_{k,t}$ as a function of $\langle N_Z \rangle_{BZ}$ and $\langle n_J \rangle_{BZ}$ takes a maximal value at $\langle N_Z \rangle_{BZ}(\Delta L_{k,t})$ and $\langle n_J \rangle_{BZ}(\Delta L_{k,t})$. In case of $x_j = \beta(e_Z - e_B)$, it is obvious from Eq. (93) that if $\tau_{J'} = 0$, the second factor in the right-hand side of Eq. (96) will be equal to zero; therefore, the left-hand side of Eq. (96) must be zero.

If $\tau_{J'} = 0$,

$$\left(\frac{\partial\langle N_Z\rangle_{BZ}(\Delta L_{k,t})}{\partial\beta(e_Z - e_B)}\right)_{N/(\beta\kappa),\,N_2,\,\beta e_{J'}} \simeq 0, \tag{98}$$

$$\left(\frac{\partial\langle n_J\rangle_{BZ}(\Delta L_{k,t})}{\partial\beta(e_Z - e_B)}\right)_{N/(\beta\kappa),\,N_2,\,\beta e_{J'}} \simeq 0. \tag{99}$$

In other words, if $\tau_{J'} = 0$, the values of $\langle N_Z \rangle_{BZ}$ and $\langle n_J \rangle_{BZ}$ at the transition point will not depend on $\beta(e_Z - e_B)$ at all; in the present model, $\tau_{J'}$ is equal to zero.

On the other hand, the second factor in Eq. (96) in case of $x_j = N/(\beta\kappa)$ is

$$\partial^2\Delta L_{k,t}/\partial(N/(\beta\kappa))\partial\langle N_Z\rangle_{BZ} \simeq \frac{-\beta\kappa}{2N}(\tau_Z - \tau_B) > 0,$$
$$\partial^2\Delta L_{k,t}/\partial(N/(\beta\kappa))\partial\langle n_J\rangle_{BZ} \simeq \frac{-\beta\kappa}{2N} 2\tau_{J'}. \tag{100}$$

Then, the following relationship may be obtained from Eqs. (96) and (100):

If $m_Z < \langle N_Z \rangle_{BZ} < N_2$ and $\partial\langle n_J \rangle_{BZ}/\partial N = 0$ or $m_Z < \langle N_Z \rangle_{BZ} < N_2$, $1 < \langle n_J \rangle_{BZ} < \max(n_J)$, and $\tau_{J'} = 0$,

$$\left(\frac{\partial\langle N_Z\rangle_{BZ}(\Delta L_{k,t})}{\partial N/(\beta\kappa)}\right)_{N_2,\,\beta(e_Z - e_B),\,\beta e_{J'}} > 0. \tag{101}$$

The above equation indicates that $\langle N_Z \rangle_{BZ}$ increases as $N/(\beta\kappa)$ increases; however it must converge to a constant, because it is bounded by N_2. If $e_{J'}$ is large positive, there will be a marginal value $N_{2,M}$ for N_2 below which the most probable number of N_Z at the transition point is equal to N_2; if $N_2 \lesssim N_{2,M}$, then $\langle N_Z \rangle_{BZ} \simeq N_2$. The marginal value $N_{2,M}$ is the value of N_2 such that the derivative of $L_{k,t}$ by $\langle N_Z \rangle_{BZ}$ takes zero at $\langle N_Z \rangle_{BZ} = N_2$, or $\langle N_Z \rangle_{BZ} = N_2$ satisfies the simultaneous equations (69) and (81). Thus, $N_{2,M}$ will be estimated by solving Eq. (95), $[\partial L_{k,t}/\partial\langle N_Z \rangle_{BZ}] = 0$, or the simultaneous equations (69) and (81) with $C_b \simeq (N_2 - \langle N_Z \rangle_{BZ} + 1)$ and $\langle n_J \rangle_{BZ} \simeq 1$, if case (b), in which only a segment in a circular DNA can take both the Z and B forms, is assumed:

$$N_{2,M} \simeq \frac{N}{\beta\kappa(\tau_Z - \tau_B)^2}\left(-1 + \left\{1 + \frac{2\beta\kappa(\tau_Z - \tau_B)^2}{N}\left[2\beta e_{J'} - \beta(e_Z - e_B)\frac{2\tau_{J'}}{\tau_Z - \tau_B}\right]\right\}^{1/2}\right) - \frac{2\tau_{J'}}{\tau_Z - \tau_B}$$
$$\underset{N/(\beta\kappa)\to\infty}{\to} \left(2\beta e_{J'} - \beta(e_Z - e_B)\frac{2\tau_{J'}}{\tau_Z - \tau_B}\right) - \frac{2\tau_{J'}}{\tau_Z - \tau_B}. \tag{102}$$

In case of $\langle N_Z\rangle_{BZ}\simeq N_2$ and $\langle n_J\rangle_{BZ}\simeq 1$, Eq. (81) is transformed to

$$(L_{k,t}-N\tau_B)\simeq\tfrac{1}{2}[N_2(\tau_Z-\tau_B)+2\tau_{J'}] + \frac{N}{\beta\kappa}\left(\frac{N_2\beta(e_Z-e_B)+2\beta e_{J'}}{N_2(\tau_Z-\tau_B)+2\tau_{J'}}\right). \quad (103)$$

In Ref. 26, the marginal value $N_{2,M}$ was estimated by completely neglecting the contributions of the combinatory entropy; Eq. (81) without the term of $\ln C_b$ was used. Therefore, $N_{2,M}$ was estimated to be proportional to the square root of N; if $\ln C_b = 0$, Eq. (102) would not have the terms, -1 in the bracket and 1 in the square root. Equation (102) indicates that $N_{2,M}$ converges to a constant in the limit of large N rather than increases with the square root of N. Therefore, on the contrary to the claim in Ref. 26, the expression equation (103) for the transition linking number is much limited to use.

It should be noted here that Eqs. (78), (81), (82), (86), (88), and (90)–(103) are approximate relationships, because the free energy of alternating B–Z conformations is approximated by that of the conformations with N_Z and n_J equal to their most probable values; this approximation might be inadequate for large N, because the free energy curve of alternating B–Z conformations becomes shallow at the minimum $\langle N_Z\rangle_{BZ}$.

In this section, the B-to-Z transition induced by changing the linking number has been defined, and the dependencies of the transition point on the external variables have been examined. Physical meanings and interpretations of these dependencies will be discussed in the next section with examples of numerical results for some cases.

VI. NUMERICAL ANALYSES OF SUPERCOILING-INDUCED B-TO-Z TRANSITIONS

In the present model, there are four parameters to describe a system; e.g. $\beta(e_Z-e_B)$, $\beta e_{J'}$, $\beta\omega_0^2$, and βB_0^2, besides basic parameters τ_B and τ_Z, and trivial ones such as m_B and m_Z; τ_f is treated to be zero. However, unless the distribution of the twisting number or the writhing number is concerned, only three parameters will be required to specify; see Eqs. (62), (63), and (64b). One of interesting parameter sets would be $\beta(e_Z-e_B)$, $\beta e_{J'}$, and $\beta\kappa\equiv\beta[\omega_0^2B_0^2/(\omega_0^2+B_0^2)]$.

The value of $\beta\kappa/2$ was estimated to be in the range of 920–1560 from the measurements of Boltzmann's distribution of topoisomers with different linking numbers, depending upon experimental condition;[22–24] much lower values 530 and 900 were obtained from ethidium binding.[16,20,21] For the twisting force constant ω_0^2, Thomas *et al.*[38] obtained $\beta\omega_0^2=3700$ [$(1.29\pm0.10)\times10^{-19}$ erg cm/rad^2] from the kinetics of DNA twisting measured by fluorescence depolarization of ethidium bromide intercalated into DNA, and Millar *et al.*[37] obtained $\beta\omega_0^2=4100$ [$(1.43\pm0.11)\times10^{-19}$ erg cm/rad^2] for the linear DNA of calf thymus and 5600 (1.95×10^{-19} erg cm/rad^2 for a closed circular DNA of the plasmid pBR322 from the same kind of measurements on the basis of the elastic model developed by Barkley and Zimm[39]; the helix rise per base pair is assumed to be 3.4 Å and $T=293$ K is used. Shore and Baldwin[40] obtained $\beta\omega_0^2=6840$ (2.4×10^{-19} erg cm/rad^2) from measures of the cyclization probability of *Eco*RI restriction fragments as a function of DNA twist. The writhing force constant B_0^2 might be estimated from those estimates of $\beta\kappa\equiv\beta[\omega_0^2B_0^2/(\omega_0^2+B_0^2)]$ and $\beta\omega_0^2$; $\beta B_0^2\simeq3700$ from $\beta\kappa/2\simeq920$ and $\beta\omega_0^2\simeq3750$, and $\beta B_0^2\simeq5700$ from $\beta\kappa/2\simeq1560$ and $\beta\omega_0^2\simeq6840$. On the other hand, Eq. (73) indicates that the effective twisting force constant of a closed circular DNA is equal to $(\omega_0^2+B_0^2)$ rather than ω_0^2. Therefore, the values of the twisting force constant obtained by Millar *et al.*[37] for the linear and circular DNAs indicate $\beta B_0^2\simeq1510$ which is significantly smaller than the estimates from the values of $\beta\kappa$ and $\beta\omega_0^2$. Vologodskii *et al.*[41] calculated the equilibrium distribution of the writhing number in randomly generated closed chains consisting of Kuhn segments and then claimed that the variance of the writhing number equaled approximately half the observed variance of the linking number, indicating that the writhing force constant is the same order as the twisting force constant; from the variance of the twisting number, the twisting force constant was estimated to be $\beta\omega_0^2=4700$. On the other hand, it was reported[36] that the twisting force constants for synthetic DNA fragments with different base compositions were in the range of $\beta\omega_0^2=880$ (9×10^{-13} erg) to 39 000 (4×10^{-11} erg), significantly depending on the base composition.

In the following, three parameters $\beta(e_Z-e_B)$, $\beta e_{J'}$, and $\beta\kappa$ will be estimated by analyzing experimental data, and the characteristics of B-to-Z transitions induced by changing the linking number will be discussed in detail. The standard twists of the B and Z forms τ_B and τ_Z will be assumed in the following to be 1/10 and $-1/12$ that were both determined from single-crystal x-ray analyses.[4–7]

A. Analyses of experimental data; estimation of $\beta(e_Z-e_B)$, $\beta e_{J'}$, and $\beta\kappa$

Peck and Wang[25] analyzed supercoiling-induced B-to-Z transitions by two-dimensional gel electrophoresis of topoisomers containing an alternating G–C segment $d(pCpG)_n\cdot d(pCpG)_n$ inserted at BamHI site of the plasmid pBR332. In their experiments, DNA topoisomers with different linking numbers were resolved by first running a mixture of topoisomers in one dimension and then electrophoresis in the direction perpendicular to that of the first dimension was carried out after the gel was equilibrated in the buffer containing chloroquine in which Z forms in the topoisomers revert to the right-handed structure. A pair of topoisomers that had the same mobility but either one of which contained Z forms in the alternating G–C insert were obtained; the linking numbers of these topoisomers can be identified. Let us represent the linking numbers and twisting numbers of this pair of topoisomers by L_k and T_w for topoisomers with Z forms, and by L_k' and T_w' for topoisomers whose conformations are the B form. Then, analyses can be done by assuming that a pair of topoisomers with the same gel mobility have the same writhing number.

$$L_k-\langle T_w\rangle=L_k'-\langle T_w'\rangle. \quad (104)$$

The statistical average $\langle T_w'\rangle$ in the B-form DNA and the statistical average $\langle T_w\rangle$ are represented by Eq. (67). Therefore, the quantity (L_k-L_k') observed in their experiment is represented as follows:

$$L_k - L'_k = \langle T_w \rangle - \langle T'_w \rangle = \frac{\omega_0^2 \langle (N_B \tau_B + N_Z \tau_Z + 2 n_J \tau_{J'}) \rangle + B_0^2 L_k}{\omega_0^2 + B_0^2} - \frac{\omega_0^2 N \tau_B + B_0^2 L'_k}{\omega_0^2 + B_0^2}$$

$$= \frac{\omega_0^2 + B_0^2}{\omega_0^2} (\langle T_w \rangle - \langle T_w \rangle_B) = \langle N_z \rangle (\tau_Z - \tau_B) + 2 \langle n_J \rangle \tau_{J'}, \quad (105)$$

where $\langle T_w \rangle_B$ is the statistical average of the total twist over only B conformations and given by Eq. (65); $\langle T_w \rangle$, $\langle N_Z \rangle$, and $\langle n_J \rangle$ are statistical averages over all conformations. Equation (105) indicates that the observed quantity $(L_k - L'_k)$ will be proportional to the statistical average $\langle N_Z \rangle$ of the number of Z forms, if $\tau_{J'}$ is equal to zero as in the present model; this quantity that is a function of L_k varies in the range from zero to $N_2(\tau_Z - \tau_B)$. Thus, the quantity $(L_k - L'_k)$, which was measured by Peck and Wang,[25] serves as a measure for supercoiling-induced B-to-Z transitions.

Peck and Wang[25] analyzed such B-to-Z transition curves on the basis of an Ising model, in which the number of Z regions in alternating B–Z conformations was simply approximated to be one. They treated all of $\tau_{J'}$, $e_{J'}$, and $(e_Z - e_B)$ as independent parameters, and obtained $\tau_{J'} = -0.4$, which is almost twice the value of $(\tau_Z - \tau_B)$, by fitting theoretical transition curves to the experimental data; $\beta e_{J'} = 8.6$ (5.0 kcal/mol), and $\beta(e_Z - e_B) = 0.57$ (0.33 kcal/mol) were also obtained with $\beta\kappa/2 = 1100$ determined from other experiments. The large negative value of $\tau_{J'}$ is inconsistent with the present model in which $\tau_{J'}$ is approximated to be zero. Here it should be noted that $\tau_{J'}$ is not the mean twist of a B–Z junction and that the twist per base in a B–Z junction is equal to τ_J, which is nonzero in the present model; see Table I and Eq. (19) for the definition of $\tau_{J'}$. Peck and Wang[25] have claimed that there must be a substantial unwinding at the B–Z junctions, because the maximum value of $[\langle N_Z \rangle (\tau_Z - \tau_B) + 2 \langle n_J \rangle \tau_{J'}]$ approaches a value significantly larger than $N_2(\tau_Z - \tau_B)$, the value expected when the inserted G–C segment wholly changes from the B form to the Z form. However, in other similar experiments, the maximum value of $[\langle N_Z \rangle (\tau_Z - \tau_B) + 2 \langle n_J \rangle \tau_{J'}]$ is almost equal to the expected value $N_2(\tau_Z - \tau_B)$, indicating $\tau_{J'} \simeq 0$; see Table I in Ref. 15. Peck and Wang[25] assumed that the number of base pairs that could take the Z form, i.e., N_2, was equal to the length of the inserted G–C segment. Figure 2 shows the base sequence[42] of pBR322 from 351th to 400th base position near the cleavage site of the restriction enzyme BamHI where the G–C segment was inserted. Four bases of alternating pyrimidine–purine d(pTpGpTpG), which may take the Z form,[8,9] are located just before the cleavage site of BamHI. Therefore, it might be reasonable to assume that N_2 is equal to the total length of the inserted G–C segment and the neighboring segment d(pTpGpTpG). Then, the transition curves obtained by Peck and Wang[25] might be consistent with $\tau_{J'} = 0$; see Eq. (105).

Figures 3(a) and 3(b) show the experimental data of Peck and Wang[25] and B-to-Z transition curves of Eq. (105) that have been calculated by using the partition function of Eqs. (61), (63), and (64b) with $m_B = 1$ and $m_Z = 2$. In Fig. 3(a), a parameter $\beta\kappa/2$ is assumed to be equal to 1100 and three parameters $\tau_{J'}$, $\beta(e_Z - e_B)$, and $\beta e_{J'}$ have been optimized as done by Peck and Wang[25] by curve fitting to their experimental data; $\tau_{J'} = -0.16$, $\beta(e_Z - e_B) = 0.60$, and $\beta e_{J'} = 7.51$ are obtained with the root mean square error 0.29. The value -0.16, which is about half of -0.4 reported by them, has been obtained for $\tau_{J'}$, chiefly because of the difference of the value of N_2. On the other hand, if $\beta\kappa$ is regarded as a variable to be optimized, one will obtain almost same goodness of fit, even though $\tau_{J'}$ is fixed to be zero. Figure 3(b) shows the case in which $\tau_{J'}$ is fixed to be zero and three parameters including $\beta\kappa$ instead of $\tau_{J'}$ are optimized; $\beta(e_Z - e_B) = 0.84$, $\beta e_{J'} = 8.28$, and $\beta\kappa/2 = 1490$ are obtained with the root mean square error 0.25. The standard twists of the B and Z forms τ_B and τ_Z are assumed here to be $1/10$ and $-1/12$ which were both determined from single-crystal x-ray analyses[4–7]; Peck and Wang[25] assumed τ_B to be $1/10.5$. In these analyses, it is assumed that each Z region consists of even number of base pairs. If Z helices consisting of odd numbers of base pairs are permitted, one will obtain slightly but insignificantly different values for the parameters. If Eqs. (60), (63), and (64b) are employed with $m_B = 1$ and $m_Z = 1$, $\tau_{J'} = 0.17$, $\beta(e_Z - e_B) = 0.60$, and $\beta e_{J'} = 7.91$ will be obtained with the root mean square error 0.28 for the assumed $\beta\kappa/2 = 1100$, and $\beta(e_Z - e_B) = 0.88$, $\beta e_{J'} = 8.88$, and $\beta\kappa/2 = 1550$ will be optimized with the root mean square error 0.25 for $\tau_{J'} = 0$. Although a definite conclusion cannot be drawn from these limited experimental data, the present model in which $\tau_{J'}$ is assumed to be zero might be a good approximation, giving another interpretation of the experimental data reported by Peck and Wang.[25]

Here it should be noted that DNAs with the insertion or deletion of a few base pairs that are not in an alternating G–C block are treated[25] as if their length were the same. As a result, the linking differences $(L_k - N\tau_B)$ of plasmids in Fig. 3 take values as if the linking number could take nonintegral values. On the other hand, in all theoretical curves of B-to-Z transitions calculated here, the linking number is treated as if it could take nonintegral values.

In the following, the characteristics of B-to-Z transitions will be examined by numerical calculations; mainly

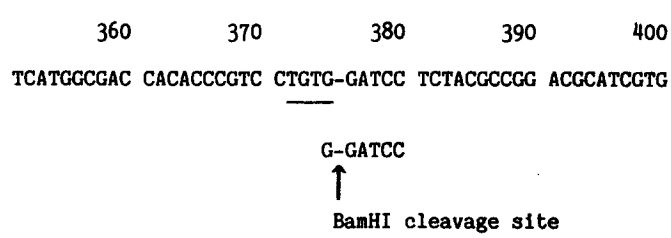

FIG. 2. Base sequence (Ref. 42) of the plasmid pBR322 from 351 to 400 near the cleavage site of the restriction enzyme BamHI. Plasmids used by Peck and Wang (Ref. 25) were generated by inserting an alternating G–C segment $d(pCpG)_n \cdot d(pCpG)_n$ into the BamHI cleavage site that is shown by arrow together with the recognition site. Four bases of alternating pyrimidine–purine d(pTpGTpG), which may take the Z form (Refs. 8 and 9) and are located just before the BamHI cleavage site, are underlined.

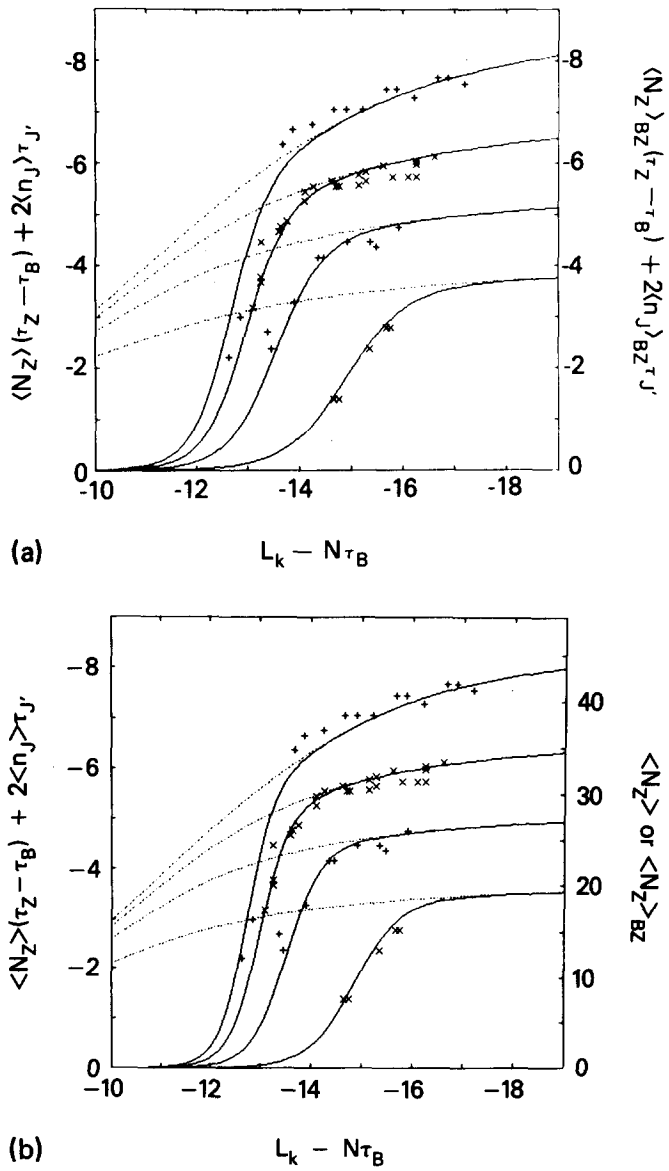

FIG. 3. Supercoiling-induced B-to-Z transitions in closed circular DNAs of plasmids containing $d(pCpG)_n \cdot d(pCpG)_n$. The quantity measured for those plasmids by Peck and Wang (Fig. 2 of Ref. 25), which is proved by Eq. (105) to be equal to $[\langle N_Z\rangle(\tau_Z-\tau_B)+2\langle n_J\rangle\tau_{J'}]$ in the present model, is plotted against the linking difference $(L_k - N\tau_B)$ by $\times$ or $+$ mark; n is equal to 21, 16, 12, and 8 for upper to lower plots in both figures. The statistical average $[\langle N_Z\rangle(\tau_Z-\tau_B)+2\langle n_J\rangle\tau_{J'}]$ over all conformations has been calculated by using the partition function of Eqs. (61), (63), and (64b) with $m_B = 1$ and $m_Z = 2$ and fitted to the experimental data; each Z-conformational region is assumed to consist of even number of Z forms. In Fig. 3(a), three parameters $\tau_{J'}$, $\beta(e_Z - e_B)$, and $\beta e_{J'}$ are optimized wth $\beta\kappa/2 = 1100$ as done by Peck and Wang (Ref. 25); $\tau_{J'} = -0.16$, $\beta(e_Z - e_B) = 0.60$, and $\beta e_{J'} = 7.51$ with the root mean square error 0.29. In Fig. 3(b), $\tau_{J'}$ is taken to be zero according to the present model, and then three parameters including $\beta\kappa$ instead of $\tau_{J'}$ are optimized; $\beta(e_Z - e_B) = 0.84$, $\beta e_{J'} = 8.28$, and $\beta\kappa/2 = 1490$ with the root mean square error 0.25. In both figures, the statistical average over all conformations $[\langle N_Z\rangle(\tau_Z-\tau_B)+2\langle n_J\rangle\tau_{J'}]$ and the statistical average over alternating B–Z conformations $[\langle N_Z\rangle_{BZ}(\tau_Z-\tau_B)+2\langle n_J\rangle_{BZ}\tau_{J'}]$ are shown by solid and dotted lines, respectively. The standard twists of the B and Z forms are assumed to be $\tau_B = 1/10$ and $\tau_Z = -1/12$. N_2 is assumed to be equal to the total length of the inserted G–C segment and the neighboring segment d(pTpGpTpG), which may take the Z form (Refs 8 and 9); N_2 is equal to 46, 36, 28, and 20 for upper to lower curves. N_1 is assumed to be equal to the length of pBR322 (Ref. 42) subtracted by the length of the segment d(pTpGpTpG); $N_1 = 4358$.

dependencies on N_2 and the total length N of DNA will be examined. It will be assumed that Z helices consist of even numbers of base pairs; Eqs. (61), (63), and (64b) are used with $m_B = 1$ and $m_Z = 2$ to calculate partition functions. The values of parameters are assumed to be $\tau_B = 1/10$, $\tau_Z = -1/12$, $\tau_{J'} = 0$, $\beta(e_Z - e_B) = 0.84$, $\beta e_{J'} = 8.28$, and $\beta\kappa/2 = 1490$.

B. Characteristics of B-to-Z transitions; dependencies on N_2

The characteristics of B-to-Z transitions are shown in Fig. 4 for DNAs containing a segment with various lengths N_2 that can take the Z form. The transition curves are shown in Fig. 4(a). The order parameter of B-to-Z transitions considered here is $\langle N_Z\rangle/\langle N_Z\rangle_{BZ}$. The transition point is defined to be where the free energy of B comformations is equal to that of alternating B–Z conformations; it should be noted that Eq. (78) is an approximate relationship because the free energy of alternating B–Z conformations is approximated by that of the conformations with N_Z and n_J equal to their most probable values. The dependencies of the transition linking number $L_{k,t}$ on N_2 is shown in Fig. 4(b). As already proved by Eq. (92), the transition linking number increases as N_2 increases. Figure 4(c) shows the statistical average and the most probable value of N_Z and the average number $\langle n_J\rangle_{BZ}$ of Z regions in alternating B–Z conformations at the transition point $(L_k = L_{k,t})$. The free energies of B–Z conformations at the transition point are plotted against N_Z in Fig. 4(d) and against n_J in Fig. 4(e). In Fig. 4(f), the average energy and the configurational entropy of B–Z conformations and the average energy of B conformations at the transition point are plotted against N_2. Also, each energy component at the transition point is plotted in Fig. 4(g) as a function of N_Z; the total free energy is divided into three terms, the average of the short range interaction energy $[N_Z(e_Z - e_B) + 2n_J e_{J'}]$, the average of the long range interaction energy $[\kappa/(2N)][L_{k,t} - N\tau_B - N_Z(\tau_Z - \tau_B) - 2n_J\tau_{J'}]^2$, and the configurational entropy. In Figs. 4(d)–4(g), the energy e_B of the B form is taken to be zero, and the configurational entropy of B conformations is defined to be zero.

The statistical averages of the number of base pairs in the Z form, $\langle N_Z\rangle$ shown by solid lines and $\langle N_Z\rangle_{BZ}$ by dotted lines in Fig. 4(a), indicate that the transition proceeds with a decrease in the population of B conformations and an increase of alternating B–Z conformations rather than a gradual increase of base pairs of the Z form. Free energy curves in Figs. 4(d) and 4(g) clearly show that there is a large free energy barrier between overall B conformations and B–Z conformations with a certain number $\langle N_Z\rangle_{BZ}$ of Z forms, indicating a cooperative transition between them. Figure 4(g) shows the contribution of each energy component to the total free energy. In the present case, this energy barrier is attributable to the unfavorable energies $e_{J'}$ of the formation of a B–Z junction and $(e_Z - e_B)$ associated with the conformational change of a base pair from the B to the Z form; the increase of $e_{J'}$ and/or $(e_Z - e_B)$ will make the free energy barrier higher and then transitions more cooperative; as proved by Eqs. (93) and (94), the transition linking number

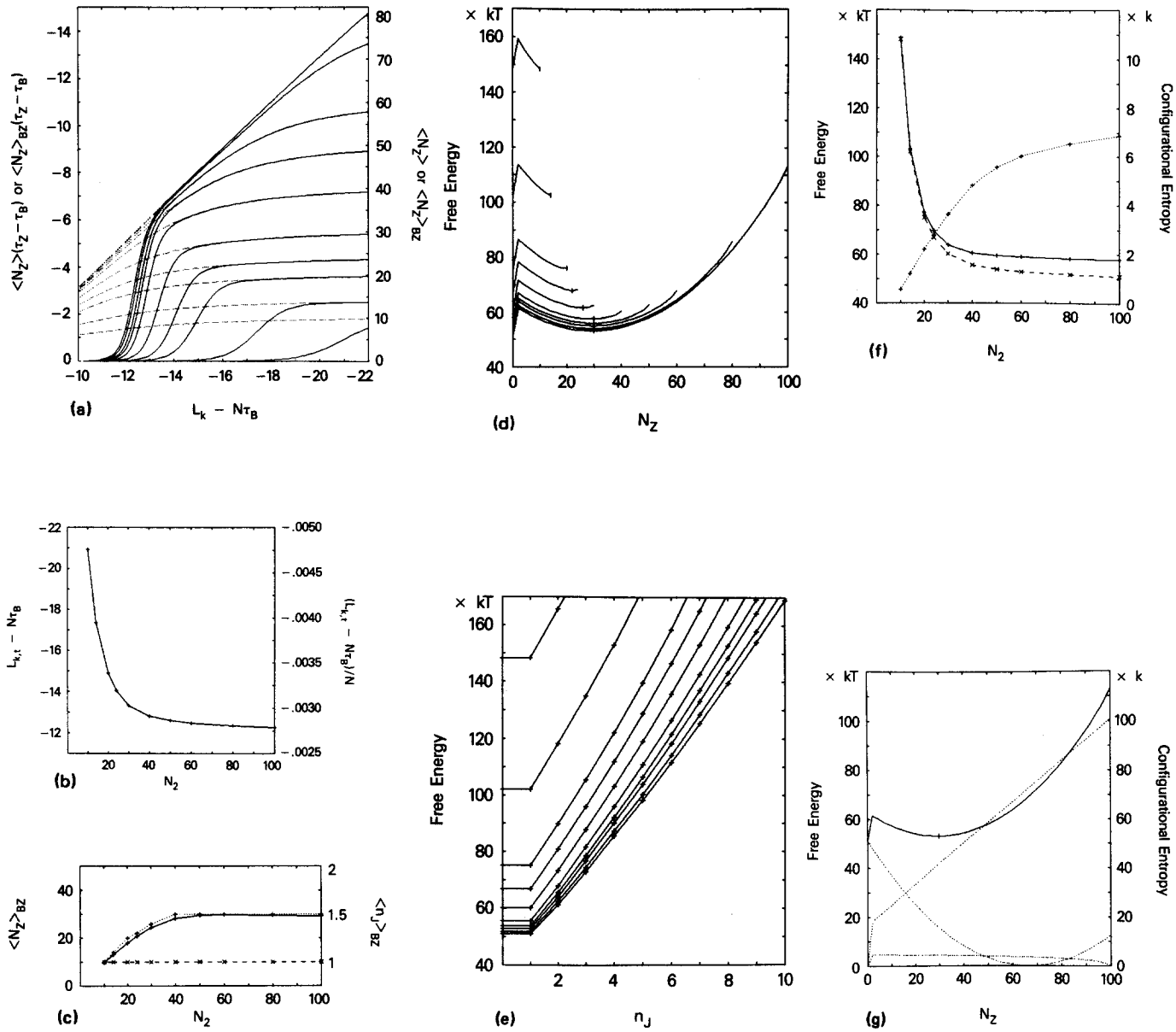

FIG. 4. Supercoiling-induced B-to-Z transitions of closed circular DNAs; dependencies on the length N_2 of a segment that can take the Z form. B-to-Z transitions of DNAs with different N_2 but the same total length $N = 4400$ are calculated by using the partition function of Eqs. (61), (63), and (64b) with $m_B = 1$ and $m_Z = 2$; each Z-conformational region consists of even number of Z forms. The values of parameters are $\tau_B = 1/10$, $\tau_Z = -1/12$, $\tau_{J'} = 0$, $\beta(e_Z - e_B) = 0.84$, $\beta e_{J'} = 8.28$, and $\beta\kappa/2 = 1490$. In Figs. 4(d)–4(g), the energy e_B of the B form is taken to be zero, and the configurational entropy of B conformations is defined to be zero. (a) B-to-Z transition curves; the values of N_2 are 100, 80, 60, 50, 40, 30, 24, 20, 14, and 10 for upper to lower curves. The statistical averages, $\langle N_Z\rangle$ over all conformations and $\langle N_Z\rangle_{BZ}$ over alternating B–Z conformations, of the number of Z forms are plotted against the linking difference $(L_k - N\tau_B)$ by solid and dotted lines, respectively. (b) Transition linking difference $(L_{k,t} - N\tau_B)$ is plotted against N_2. (c) Statistical average $\langle N_Z\rangle_{BZ}$, the most probable values of N_Z, and the statistical average $\langle N_J\rangle_{BZ}$ in alternating B–Z conformations at the transition point $(L_k = L_{k,t})$ are plotted against N_2 by solid, dotted, and broken lines, respectively. (d) Free energies of conformations at the transition point $(L_k = L_{k,t})$ are plotted against the number N_Z of Z forms; the values of N_2 are 100, 80, 60, 50, 40, 30, 24, 20, 14, and 10 for lower to upper curves. The bars represent the most probable values of N_Z. (e) Free energies of conformations at the transition point $(L_k = L_{k,t})$ are plotted against the number n_J of Z regions; the values of N_2 are 100, 80, 60, 50, 40, 30, 24, 20, 14, and 10 for lower to upper curves. (f) Average energy and the configurational entropy of alternating B–Z conformations and the average energy of B conformations at the transition point $(L_k = L_{k,t})$ are plotted against N_2 by solid, dotted, and broken lines, respectively. Because of the transition point, the average energy of B–Z conformations subtracted by the configurational entropy is equal to the average energy of B conformations. (g) Statistical averages of the short range interaction energy $[N_Z(e_Z - e_B) + 2n_J e_{J'}]$ and long range interaction energy $[\kappa/(2N)][L_{k,t} - N\tau_B - N_Z(\tau_Z - \tau_B) - 2n_J\tau_{J'}]^2$ and the configurational entropy at the transition point $(L_k = L_{k,t})$ are plotted by dotted lines as functions of the number N_Z of Z forms; a solid line represents the total free energy. The values of parameters are $N_2 = 100$, $N = 4400$, and $(L_{k,t} - N\tau_B) = -12.27$. The bar represents the most probable value of N_Z.

decreases as $\beta e_{J'}$ or $\beta(e_Z - e_B)$ increases. On the other hand, the concave part of free energy curves in Figs. 4(d) and 4(g) is attributable to the long range interactions that favor base pairs to take the Z form. In other words, the cooperativity of the transition results from the unfavorable short range interactions and favorable long range interactions for the formation of Z forms. Although the combinatory entropy favors the formation of many, short regions of the Z form rather than a long stretch of the Z form, most of alternating B–Z conformations have only one Z region in this specific set of parameter values, because of the large positive value of $e_{J'}$; see Figs. 4(c) and 4(e). Thus, in Fig. 4(g), the configurational entropy is almost equal to the combinatory entropy $[k \ln(N_2 - N_Z + 1)]$ with $N_Z > 0$.

Until N_2 becomes sufficiently large, about 20 in this specific set of parameter values, the most probable value of N_Z is equal to N_2 and also the slope of the free energy curve at that point is negative, indicating that a DNA segment which can take the Z form is not long enough to make a preferable, long stretch of the Z form; see Fig. 4(d). Equation (102) gives $N_{2,M} \simeq 2\beta e_{J'} = 16.56$ that is smaller than 20; the difference is attributable to the fact that the continuous approximation for N_Z is used to derive Eq. (102). Equation (103) gives the explicit functional form of $L_{k,t}$ for the case of $N_2 \lesssim N_{2,M}$, i.e., $\langle N_Z \rangle_{BZ} \simeq N_2$ and $\langle n_J \rangle_{BZ} \simeq 1$. In this case, the whole segment will take the Z form in compensation for taking a highly writhed and twisted structure. Decreasing the linking number beyond the transition point causes further writhing of DNA as well as twisting, depending on the relative strength of the writhing force constant B_0^2 to the twisting force constant ω_0^2; see Eqs. (66) and (67). As N_2 increases, the transition linking number increases because it becomes possible to make a preferable, long stretch of the Z form; see Eq. (92).

Figure 4(c) shows that the statistical average and the most probable number of N_Z in alternating B–Z conformations appear to have a maximum, while N_2 increases beyond $N_{2,M}$. In this specific set of parameter values, $\langle n_J \rangle_{BZ}$ is almost equal to one irrespectively of N_2. Therefore, the second factor in the right-hand side of Eq. (96) can be evaluated from Eq. (81) with the approximation of $C_{b_2}(\langle N_B \rangle_{BZ}, \langle N_Z \rangle_{BZ}, 2\langle n_J \rangle_{BZ}) \simeq (N_2 - \langle N_Z \rangle_{BZ} + 1)$. If $(N_2 + 1) < 2\langle N_Z \rangle_{BZ}$, then $\partial^2 \Delta L_{k,t} / \partial N_2 \partial \langle N_Z \rangle_{BZ}$ will be positive, otherwise it will be negative. It is obvious from Eq. (96) that as N_2 increases, $\langle N_Z \rangle_{BZ}$ increases while $(N_2 + 1) < 2\langle N_Z \rangle_{BZ}$ and then it begins to decrease; therefore, $\langle N_Z \rangle_{BZ}$ has a maximum at $(N_2 + 1) \simeq 2\langle N_Z \rangle_{BZ}$. If the combinatory entropy were to be ignored, $\partial^2 \Delta L_{k,t} / \partial N_2 \partial \langle N_Z \rangle_{BZ}$ would be zero and therefore, on the contrary to the fact, it would be expected that if $N_2 > N_{2,M}$, then $\langle N_Z \rangle_{BZ} = N_{2,M}$. The combinatory entropy is also responsible for the increase of the transition linking number associated with the increase of N_2 in the case of $N_2 > N_{2,M}$; Eq. (81) represents the dependence of $L_{k,t}$ on the combinatory factor C_b. Figure 4(f) shows that the contribution of the configurational entropy of B–Z conformations to the total free energy is negligible in the case of $N_2 < N_{2,M} \simeq 20$, but gradually increases with the increase of N_2. In the case of $N_2 > 40 > N_{2,M}$, the configurational entropy increases logarithmically with N_2; in this specific set of parameter values, it is approximately equal to the combinatory entropy $[k \ln(N_2 - \langle N_Z \rangle_{BZ} + 1)]$ because $\langle n_J \rangle_{BZ} \simeq 1$ at the transition point.

In case that a DNA segment that can take the Z form is sufficiently long, i.e., at least $N_2 > N_{2,M}$, decreasing the linking number beyond the transition point causes a gradual increase of base pairs in the Z form before further writhing and twisting of DNA; see Fig. 4(a). Equation (69) indicates that if the combinatory entropy were to be ignored, the linking number change would wholly be transformed into the conformational change of base pairs from the B form to the Z form. Also, Eq. (72b) indicates that the statistical average of the writhing number of B–Z conformations changes only because the differential coefficient of the conformational entropy changes. In other words, after the transition, the linking number change is transformed primarily into the change of the twisting number. Here it should be noted that such B–Z conformations are not relaxed, but significantly writhed; at the transition point, only part of the linking difference is transformed into the conformational change of base pairs from the B form to the Z form; see Figs. 4(a) and 4(b).

C. Characteristics of B-to-Z transitions; dependencies on the total length N

Next, let us consider the effects of the total length N of DNA on B-to-Z transitions. It should be recalled here that increasing N is equivalent to decreasing $\beta\kappa$ with regard to their effects on the transition linking difference $(L_{k,t} - N\tau_B)$; $(L_{k,t} - N\tau_B)$ is a function of $N/(\beta\kappa)$; see Eqs. (81) and and (90). Transition curves for DNAs with various lengths are shown in Fig. 5(a). The transition linking difference decreases as N increases; the right-hand side of Eq. (90) is negative. However, Fig. 5(b) shows that as proved by Eq. (91), the density of the transition linking difference $(L_{k,t} - N_{\tau B})/N$ asymptotically increases to a constant with the increase of N; the second term in Eq. (81) is the asymptotic form of $\Delta L_{k,t}$ in the limit of large N. Since it is shown in Fig. 5(c) that $\langle N_Z \rangle_{BZ} \simeq N_2$ and $\langle n_J \rangle_{BZ} \simeq 1$ in the case of $N_2 = 20$, Eq. (103) can be applied to that case. On the other hand, Figs. 5(c) and 5(d) show that in the case of $N_2 = 80 > N_{2,M}$, as N_2 increases, the concave part of the free energy curve plotted against N_Z becomes shallow and the most probable number of N_Z at the transition point increases. This is attributable to the change, caused by the increase of N, of the long range potential $[\beta\kappa/(2N)][L_k - N\tau_B - N_Z(\tau_Z - \tau_B) - 2n_J\tau_{J'}]^2$. As a result, the most probable number of N_Z at the transition point gradually increases as N increases, as proved by Eq. (101); however, since $N_2 > N_{2,M}$, the most probable number of N_Z does not reach N.

VII. CONCLUSION

A configurational partition function for transitions between the right-handed B helix and the left-handed Z helix in a closed circular double stranded DNA has been formulated in an idealized model. The formulation of the partition function has been discussed rather in detail, because this simple system may be worth analyzing as a one-dimensional

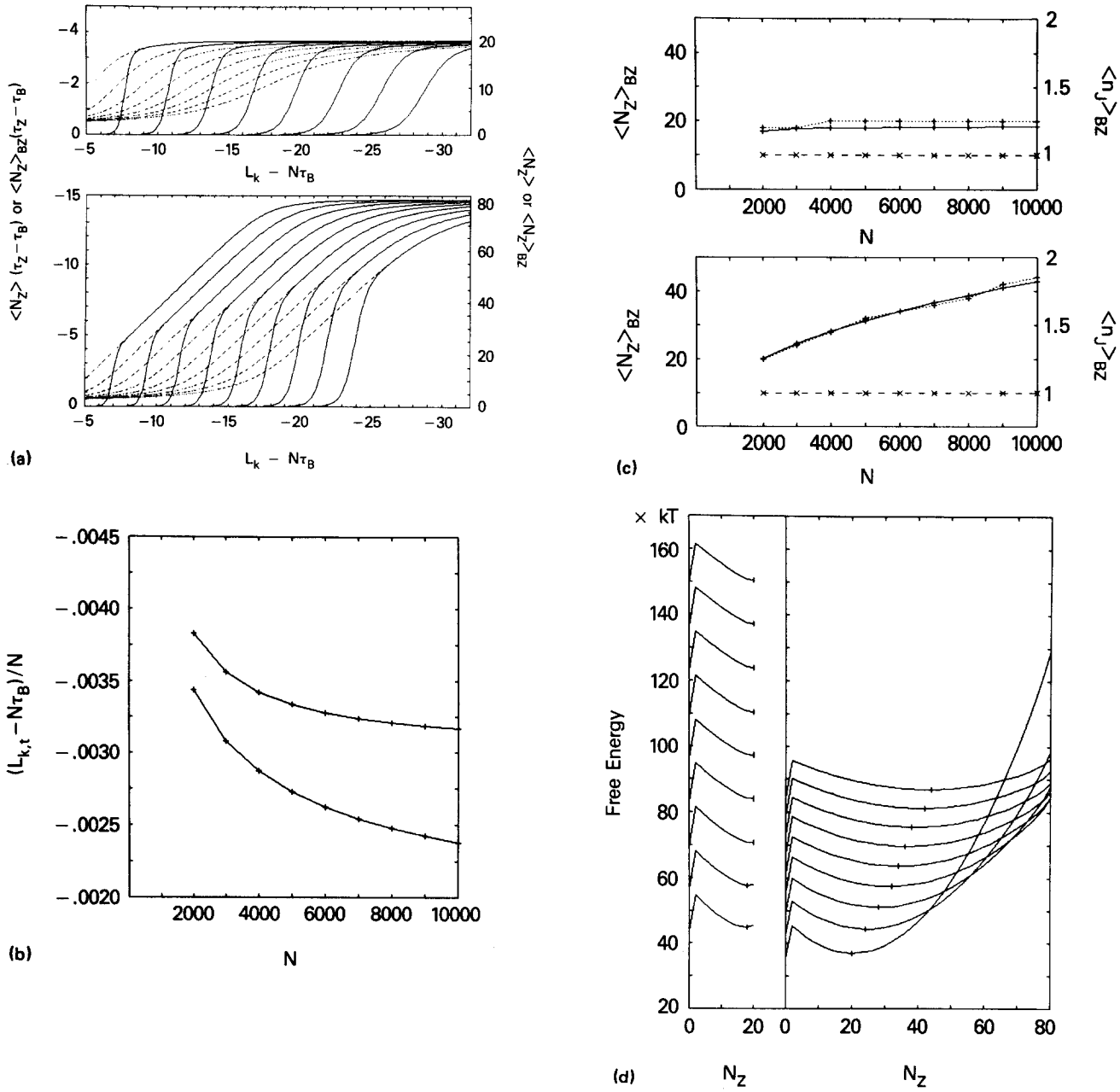

FIG. 5. Supercoiling-induced B-to-Z transitions of closed circular DNAs; dependencies on the length N of DNA. B-to-Z transitions of DNAs with different values of N_1 but fixed vlaues of N_2 are calculated by using the partition function of Eqs. (61), (63), and (64b) with $m_B = 1$ and $m_Z = 2$; each Z-conformational region consists of even number of Z forms. The values of parameters are $\tau_B = 1/10$, $\tau_Z = -1/12$, $\tau_{J'} = 0$, $\beta(e_Z - e_B) = 0.84$, $\beta e_{J'} = 8.28$, and $\beta\kappa/2 = 1490$. In Figs. 5(c) and 5(d), the energy e_B of the B form is taken to be zero, and the configurational entropy of B conformations is defined to be zero. (a) B-to-Z transition curves; the upper figure is for $N_2 = 20$ and the lower for $N_2 = 80$. The total length N of DNA is from 2000 to 9000 or 10000 for left to right curves in increments of 1000. The statistical averages, $\langle N_Z \rangle$ over all conformations and $\langle N_Z \rangle_{BZ}$ over alternating B–Z conformations, of the number of Z forms are plotted against the linking difference $(L_k - N\tau_B)$ by solid and dotted lines, respectively. (b) Density of the transition linking difference $(L_{k,t} - N\tau_B)/N$ is plotted against the total length N of DNA. The upper curve is for $N_2 = 20$ and the lower for $N_2 = 80$. (c) Statistical averages $\langle N_Z \rangle_{BZ}$, the most probable values of N_Z, and the statistical average $\langle n_J \rangle_{BZ}$ in alternating B–Z conformations at the transition point $(L_k = L_{k,t})$ are plotted against N by solid, dotted, and broken lines, respectively. The upper figure is for $N_2 = 20$ and the lower for $N_2 = 80$. (d) Free energies of conformations at the transition point $(L_k = L_{k,t})$ are plotted against the number N_Z of Z forms; the left figure is for $N_2 = 20$ and the right for $N_2 = 80$. The values of N are from 2000 to 10000 for the lower to upper curves in increments of 1000. The bars represent the most probable values of N_Z.

model system in which a displacement potential is strongly anharmonic and also long range interactions are included, and which undergoes structural transitions by the change of long range interactions. Because interactions between phonons and junctions are neglected, the present formulation of the configurational partition function would be appropriate for the regime in which the on-site potential is comparable with or stronger than interactions between sites; in other words, $d_J \equiv C_0/\omega_0 \lesssim 0(1)$. In the case of DNA, the width of a junction is uncertain, but at least shorter than ten base pairs; because 16 base pairs of d(pCpG) are long enough to allow a Z conformation in a closed DNA.[25] Thus, d_J would be the order of one. On the other hand, the double quadratic function employed here for the twisting potential and the har-

monic potential for nearest neighbor interactions between twists might be too simple to represent a double stranded DNA. For practical use such as experimental analyses, the formula of the partition function that is based on the Ising limit $d_J \equiv C_0/\omega_0 \to 0$ and presented in Appendix B might be useful; the formula of the partition function, Eq. (B3) with $\omega_0 \equiv \omega_B = \omega_Z$, is identical with Eq. (64) except for the librational factor that does not depend on conformational types.

The second order approximation [Eq. (9)] for the writhing potential would be adequate; because the result of the configurational partition function is consistent with the experimental fact[22,23] that the equilibrium ensemble of topoisomers with different linking numbers, which is generated by using nicking-closing enzymes, obeys a Gaussian distribution of the linking number whose variance is almost proportional to the total length of DNA; the variance of the linking number is indicated to be equal to $N/(\beta\kappa)$. The writhing force constant B_0^2 can be estimated from the twisting force constant ω_0^2 and $\kappa \equiv [\omega_0^2 B_0^2/(\omega_0^2 + B_0^2)]$. Also, B_0^2 might be obtained from the twisting force constant ω_0^2 for a linear DNA and the effective twisting force constant $(\omega_0^2 + B_0^2)$ for a closed circular DNA; long range interactions due to the conservation of linking number that is specific to closed circular DNAs reduce the variance of the total twist as if the twisting force constant increased from ω_0^2 to $(\omega_0^2 + B_0^2)$. However, at present those experimental data appear to be insufficient to obtain a reliable value for B_0^2.

It was claimed[26] that in the limit of a long DNA, a segment of the DNA that could take the Z form would wholly change from the B form to the Z form at the transition point. However, it has been proved that although the most probable number of Z forms in alternating B–Z conformations at the transition point increases as DNA becomes long, it can never attain the whole segment unless the segment that can take the Z form is extremely short. Therefore, on the contrary to the claim in Ref. 26, the expression Eq. (103) for the transition linking number, in which a segment of the DNA that can take the Z form is assumed to wholly change from the B form to the Z form at the transition point, is much limited to use.

In the present analyses of experimental data,[25] $\tau_{J'}$ is taken to be zero according to the model. The estimates of the relative energy of the Z form to the B form and the energy of the formation of a B–Z junction are not significantly different from those by Peck and Wang[25]; however, $\beta\kappa$, optimized instead of $\tau_{J'}$, has been estimated to be larger than the value used by them. The present estimation would be another interpretation of their data.

It should be noted that although B-to-Z transitions induced by changing the linking number have been discussed, the partition function formulated here can also be applied to analyzing B-to-Z transitions induced by changing the relative energy $(e_Z - e_B)$ of the Z form to the B form. In the present analyses, we have completely neglected other structural changes that may take place as well as B-to-Z transitions when the linking number is reduced, such as the cruciform formation that may occur only at inverted repeats in base sequences and loss of base pairing or melting. Both structural changes can decrease the twisting number such that the requirement for writhe is reduced. Developing theories to take account of such conformational changes remains to be studied.

ACKNOWLEDGMENTS

I wish to thank Dr. A. Sarai that I was inspired to do the present work by his manuscript[27] in which B–Z junctions were considered as kinks or domain walls in nonlinear systems. Also I thank Dr. R. L. Jernigan for his encouragement during this work.

APPENDIX A. CONFIGURATIONAL PARTITION FUNCTION Z_{CP}^0 FOR PHOTONS

The classical partition function for harmonic lattice vibrations is easily calculated.

$$\int\int \prod_{i=1}^{N} dP_i\, d\Delta\tau_i \exp\left[-\beta\left(\sum_i \tfrac{1}{2}P_i^2 + \sum_i\sum_j \frac{\Delta\tau_i \Delta\tau_j}{2} V^0_{\tau_i\tau_j}\right)\right] = \left(\frac{2\pi}{\beta}\right)^{N/2} Z^0_{CP} \tag{A1}$$

or

$$\begin{aligned} &= \exp\left\{ -\sum_k \ln\left[\beta\omega_k/(2\pi)\right]\right\} \\ &= \exp\Big\{ -N\ln[\beta\omega_0/(2\pi)] \\ &\quad - \sum_k (1/2)\ln\left[1 + 4(C_0^2/\omega_0^2)\sin^2(k/2)\right]\Big\} \\ &\simeq \exp\Big\{ -N\ln[\beta\omega_0/(2\pi)] \\ &\quad - \frac{N}{2\pi}\int_{-\pi}^{\pi} dk\,(1/2)\ln\left[1 + 4(C_0^2/\omega_0^2)\sin^2(k/2)\right]\Big\} \\ &= \left(\frac{2\pi}{\beta}\right)^{N/2}\left[\left(\frac{2\pi}{\beta\omega_0^2}\right)^{1/2}\frac{2}{\{1 + [1 + 4(C_0^2/\omega_0^2)]^{1/2}\}}\right]^N. \end{aligned} \tag{A2}$$

The dispersion relation used to derive Eq. (A2) is

$$\omega_k^2 = \omega_0^2 + 4C_0^2 \sin^2(k/2), \quad k = (2\pi n/N) \text{ with } n = 0 \ldots N-1. \tag{A3}$$

A periodic boundary condition $\Delta\tau_{i\pm N} = \Delta\tau_i$ is assumed to derive the above equation. By comparing Eq. (A1) with Eq. (A2), the configurational partition function Z_{CP}^0 for phonons or harmonic lattice vibrations [Eq. (43)] is derived.

APPENDIX B. FORMULATION OF THE CONFIGURATIONAL PARTITION FUNCTION BASED ON AN ISING MODEL; THE LIMIT OF $d_J \equiv C_0/\omega_0 \to 0$

In this Appendix, on the basis of an Ising model, the configurational partition function is formulated for the case that the Z and B forms have different twisting force constants. The twisting force constant is assumed to be equal to ω_B^2 for the B form and ω_Z^2 for the Z form

$$V_{1i}(\tau)\simeq\begin{cases} e_B + (\omega_B^2/2)(\tau-\tau_B)^2 & \text{for } \tau\simeq\tau_B \\ e_Z + (\omega_z^2/2)(\tau-\tau_Z)^2 & \text{for } \tau\simeq\tau_Z \end{cases}. \tag{B1}$$

Each twist is assumed to fluctuate independently. However, it is assumed that the formation of a B–Z junction accompanies an additional energy $e_{J'}$ and twist $\tau_{J'}$, which are both treated as parameters; $\tau_{J'}$ may be treated to be zero. In other words, nearest neighbor coupling between twists V_{2i} is assumed to be negligible compared with the twisting potentials; $C_0/\omega_B\to 0$ and $C_0/\omega_Z\to 0$ are assumed.

Under these assumptions, the statistical weight of conformations that consist of N_B B forms, N_Z forms, and j B–Z and Z–B junctions is represented by

$$\begin{aligned} W_I(N_B,N_Z,j) &= C_I(N_B,N_Z,j)\exp[-\beta(N_Be_B+N_Ze_Z+je_{J'})] \\ &\times\int d\Delta\lambda\left(\sum_i\frac{1}{\omega_i^2}\right)\int\prod_i d\Delta\lambda_i\left(\frac{1}{\omega_i^2}\right)\exp\left[-\beta\sum_i\frac{\omega_i^2}{2}\left(\frac{\Delta\lambda+\Delta\lambda_i}{\omega_i^2}\right)^2\right]\delta\left(\sum_i\frac{\Delta\lambda_i}{\omega_i^2}\right) \\ &\times\exp\left[-\frac{\beta B_0^2}{2N}\left(L_k-N_B\tau_B-N_Z\tau_Z-j\tau_{J'}-\sum_i\frac{\Delta\lambda}{\omega_i^2}\right)^2\right] \\ &= C_I(N_B,N_Z,j)\exp[-\beta(N_Be_B+N_Ze_Z+je_{J'})] \\ &\times\int\prod_i d\Delta\lambda_i\left(\frac{1}{\omega_i^2}\right)\exp\left[-\beta\sum_i\frac{\omega_i^2}{2}\left(\frac{\Delta\lambda_i}{\omega_i^2}\right)^2\right]\delta\left(\sum_i\frac{\Delta\lambda_i}{\omega_i^2}\right) \\ &\times\int d\Delta\lambda\left(\sum_i\frac{1}{\omega_i^2}\right)\exp\left[-\beta\sum_i\frac{\omega_i^2}{2}\left(\frac{\Delta\lambda}{\omega_i^2}\right)^2\right]\exp\left[-\frac{\beta B_0^2}{2N}\left(L_k-N_B\tau_B-N_Z\tau_Z-j\tau_{J'}-\sum_i\frac{\Delta\lambda}{\omega_i^2}\right)^2\right], \end{aligned} \tag{B2}$$

where ω_i^2 takes either ω_B^2 or ω_Z^2, depending on whether ith base pair is in the B form or in the Z form. After the librational factor is evaluated, the above equation is transformed to

$$\begin{aligned} W_I(N_B,N_Z,j) &= \left(\frac{N/B_0^2}{(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}\right)^{1/2}C_I(N_B,N_Z,j) \\ &\times\exp[-\beta(N_Bf_B+N_Zf_Z+je_{J'})]\left(\frac{\beta(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}{2\pi(N/B_0^2)(N_B/\omega_B^2+N_Z/\omega_Z^2)}\right)^{1/2} \\ &\times\int dT_w\exp\left\{-\frac{\beta(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}{2(N/B_0^2)(N_B/\omega_B^2+N_Z/\omega_Z^2)}\right. \\ &\times\left.\left[T_w-\frac{(N/B_0^2)(N_B\tau_B+N_Z\tau_Z+j\tau_{J'})+(N_B/\omega_B^2+N_Z/\omega_Z^2)L_k}{(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}\right]^2\right\} \\ &\times\exp\left[-\frac{\beta}{2(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}(L_k-N_B\tau_B-N_Z\tau_Z-j\tau_{J'})^2\right] \end{aligned} \tag{B3a}$$

$$\begin{aligned} &= \left(\frac{N/B_0^2}{(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}\right)^{1/2}C_I(N_B,N_Z,j)\exp[-\beta(N_Bf_B+N_Zf_Z+je_{J'})] \\ &\times\exp\left[-\frac{\beta}{(N/B_0^2+N_B/\omega_B^2+N_Z/\omega_Z^2)}(L_k-N_B\tau_B-N_Z\tau_Z-j\tau_{J'})^2\right], \end{aligned} \tag{B3b}$$

where the free energy of the B or Z form including the librational entropy is defined as

$$f_\sigma\equiv e_\sigma-\frac{1}{\beta}\ln\left[\left(\frac{2\pi}{\beta\omega_\sigma^2}\right)^{1/2}\right]\quad\text{with }\sigma\in\{B,Z\}. \tag{B4}$$

The partition function is represented by Eqs. (62) and (63) with Eq. (B3) instead of Eq. (64); the combinatory factors C_I are given by Eqs. (57), (58), (60), and (61).

[1]Abbreviations for bases in DNA (deoxyribonucleic acid) are A, adenine; C, cytosine; G, guanine; T, thymine; and U, uracil. The corresponding deoxyribonucleosides are dA, deoxyadenosine; dC deoxycytidine; dG, deoxyguanosine; dT, deoxythymidine. Also, the nucleotides are pA, adenosine 5′-phosphate; pC, cytidine 5′-phosphate; pG, guanosine 5′-phosphate; pT, thymidine 5′-phosphate. $d(pCpG)_n\cdot d(pGpC)_n$ is a complementary double stranded polymer of alternating deoxyribocytidine 5′-phosphate and deoxyriboguanosine 5′-phosphate.

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