Theoretical Analysis of Disruptions in DNA Minicircles

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ABSTRACT Under sufficient bending stress, which appears in DNA minicircles and small DNA loops, the double helix experiences local disruptions of its regular structure. We developed a statistical-mechanical treatment of the disruptions in DNA minicircles, studied experimentally by Du et al. The model of disruptions used in our Monte Carlo simulation of minicircle conformations specifies these conformations by three parameters: DNA bend angle at the disruption, θ_d; local DNA unwinding caused by the disruption; and the free energy associated with the disruption in the unstressed double helix, G_d. The model is applicable to any structural type of disruption, kinks or opening of single basepairs. The simulation shows that accounting for both torsional and bending deformation associated with the disruptions is very important for proper analysis. We obtained a relationship between values of G_d and θ_d under which the simulation results are compatible with the experimental data. The relationship suggests that the free energy of basepair opening, which includes flipping out both bases, is significantly higher than the generally accepted value. The model is also applied to the analysis of *j*-factors of very short DNA fragments.

INTRODUCTION

It seems clear that under a sufficient bending stress, the DNA double helix has to experience local disruptions of the secondary structure. These disruptions destroy only the helical structure of the molecule, preserving all covalent bonds, and therefore, they are completely reversible. Since a strong bending deformation often appears in DNA-protein complexes and small DNA loops, these disruptions were for a long time the subject of biophysical studies. Two major types of disruption can serve as bending hinges. Kinks of the double helix that preserve the basepairing but eliminate the stacking in the basepair step represent the first type. Kinks were first suggested by Crick and Klug (1) and later found in many DNA-protein complexes (2–4). They have also been observed in molecular dynamics simulations (5). The second type of disruption is basepair opening, which destroys at least one basepair and two surrounding stacking interactions (6,7). Recently, a third type of disruption was found in the molecular dynamics simulation of small DNA circles (5). It involves one broken basepair whose bases are stacked with the 5′ bases of the corresponding strands. It is quite possible that more structural disruptions will be found.

Despite the recent interest in DNA disruptions, many of the structural and energetic features associated with them remain unknown. We need to know the ranges of bend angles and the double helix unwinding associated with each kind of disruption, as well as the free energy cost of a disruption appearance in an unstressed DNA molecule. Simple molecular modeling (1) and molecular dynamics simulations (5) show that the double helix can be bent through up to 100° at kinks. However, kink angles >60° have not been observed in DNA-protein complexes (8,9). Large bend angles at the kinks are always associated with the double helix unwinding by 10–20° in these complexes (1,5,8). There are no data on the free energy of kink formation, although the stacking energy between adjacent basepairs was recently determined for all 10 types of the basepair steps containing the single-stranded nick (10). The free energy of basepair opening, determined in hydrogen exchange experiments, equals 7–9 kcal/mol (11,12). However, there are no data on the possible bend angles and the helix unwinding caused by this disruption. It is possible that the third type of disruption mentioned above played a major role in the hydrogen exchange, and that, correspondingly, its free energy was determined in the proton-exchange experiments. Still, it seems reasonable to assume that basepair opening creates a more flexible disruption than kinks, since the former involves two basepair steps. Therefore, kinks can compete with basepair opening for reducing bending stress only if the free energy of their formation is lower than that of basepair opening.

A natural way to address the formation of DNA disruptions under bending stress is to study DNA minicircles, and cyclization of short DNA fragments has attracted a lot of attention recently (6,13–16). Cloutier and Widom suggested that sharp local bends can significantly facilitate cyclization of such short fragments, and subsequent theoretical analysis has confirmed this suggestion (6,15,16). Still, in cases where cyclization efficiency could be reliably measured, the disruptions do not affect this efficiency due to their low probability of occurrence (15). To overcome this difficulty, Du et al. (17) suggested an alternative approach to the problem. They developed a method for obtaining very small, covalently closed DNA minicircles up to 60 bp in length and probed their structure by single-strand-specific endonucleases. These enzymes were widely used in studies of DNA disruptions in torsionally stressed DNA (18–23). Du et al. (17) detected

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the presence of disruptions in minicircles of 64–65 bp but not in minicircles of 85–86 bp. These and other data obtained by Du et al. can be used to deduce some quantitative conclusions about the disruptions. However, the information can be obtained only by comparing the experimental results with statistical-mechanical analysis of the disruptions in DNA minicircles. Such an analysis was performed in this study. Although the analysis has some common features with the theoretical analysis of cyclization of short DNA fragments (6,15,16), it uses a more elaborate model of the disruptions in covalently closed minicircles. One of the most important distinctive features of our analysis is that it accounts for changes of both bending and torsional free energies caused by the disruption. A substantial torsional stress is nearly always present in DNA minicircles and it is absolutely necessary to account for the changes of this stress caused by the disruptions. Available data show that the disruption formation is always associated with local unwinding of the double helix (see, e.g., (24,25).

In the next sections, we describe a model of the disruptions in covalently closed DNA minicircles and the corresponding statistical-mechanical simulation. This model accounts for changes of both bending and torsional free energies of the minicircles that result from the disruptions. Comparison between the computer simulation and the experimental data by Du et al. (17) allowed us to establish a strong correlation between possible values of the bend angles and the free energy of the disruption. This result suggests that either the bend angle at the disruption is much smaller than that predicted for DNA kinks (1,5), or the free energy of disruption is substantially higher than the generally accepted value for basepair opening. We conclude that basepair opening, detected in the proton exchange experiments (11,12), eliminates the stacking interaction in one DNA strand only and therefore creates very limited flexibility at the disruption. Correspondingly, disruptions that provide larger bending flexibility should be energetically more costly. We obtained very good agreement between the experimentally observed rate of minicircle digestion by single-strand-specific endonuclease (17) and calculated the average number of disruptions in the minicircles. The model was also used to analyze the cyclization efficiency of short DNA fragments.

THEORY

DNA model

Closed circular DNA of N basepairs in length was modeled as a discrete wormlike chain composed of n rigid segments. The bending energy of the chain, E_b, was computed as

$$E_b = RT\sum_{i=1}^{n} U(\theta_i), \tag{1}$$

where $U(\theta)$ is a bending potential, θ_i is the angular displacement of segment $i+1$ relative to segment i, R is the gas constant, and T is the absolute temperature. The potential $U(\theta)$ consisted of two parts and was specified as

$$U(\theta) = \min\left[\frac{1}{2}g_1\theta^2,\ h + \frac{1}{2}g_2(\theta-\theta_d)^2\right], \tag{2}$$

where g_1 is the DNA bending rigidity and h, θ_d, and g_2 specify the disruption (Fig. 1 *A*). The first part of $U(\theta)$, which is proportional to θ^2, corresponds to the energy of the chain elastic deformation. Correspondingly, the value of g_1 is directly related to the DNA persistence length, a:

$$g_1 = a/l, \tag{3}$$

where l is the length of one straight segment of the model chain. The second part of $U(\theta)$ corresponds to the disruption. The average bend angle at the disruption is specified by θ_d. If the value of θ_d is chosen, g_2 and h specify the probability that the disruption will appear, p_d, at a particular vertex of the linear model chain:

$$p_d = \frac{\int_{\beta}^{\pi}\exp(-U(\theta)/RT)\sin\theta d\theta}{\int_{0}^{\pi}\exp(-U(\theta)/RT)\sin\theta d\theta}, \tag{4}$$

where angle β separates two parts of the potential (see Fig. 1 *A*). We checked that the simulation results depend on p_d rather than on a particular pair of g_2 and h if $g_2 \geq 200$. We used a g_2 value of 250, and the value of h was adjusted to have a chosen value of p_d. It was checked that the potential provides a relatively narrow distribution of the disruption bend angles centered in the close vicinity of θ_d (see Fig. 1 *B*).

Each straight segment of the model chain represents 1–4 bp, depending on the value of θ_d. We tested that the simulation results do not depend on the segment length if the total number of segments in the minicircle exceeds 20. If the length of the segments of the model chain exceeds the length of one basepair step, l_{bp}, it has to be accounted for in the calculation of the free energy of the disruption appearance at a particular basepair step of linear DNA, G_d. In this case, the value of G_d depends on both the probability of a disruption appearing in a particular vertex of the model chain, p_d, and the value of l:

$$G_d = -RT\ln\left(\frac{l_{bp}}{l}p_d\right). \tag{5}$$

We also accounted for the torsional energy of the circular molecules, which was necessary, because this energy was affected by disruptions. In the absence of disruptions, the free energy, $G_t(\Delta Lk, 0)$, was calculated as

$$G_t(\Delta Lk, 0) = \frac{2\pi^2 C}{L}(\Delta Lk)^2, \tag{6}$$

where C is the DNA torsional rigidity, L is the DNA contour length, and ΔLk is the linking number difference. The latter value is specified by the equation

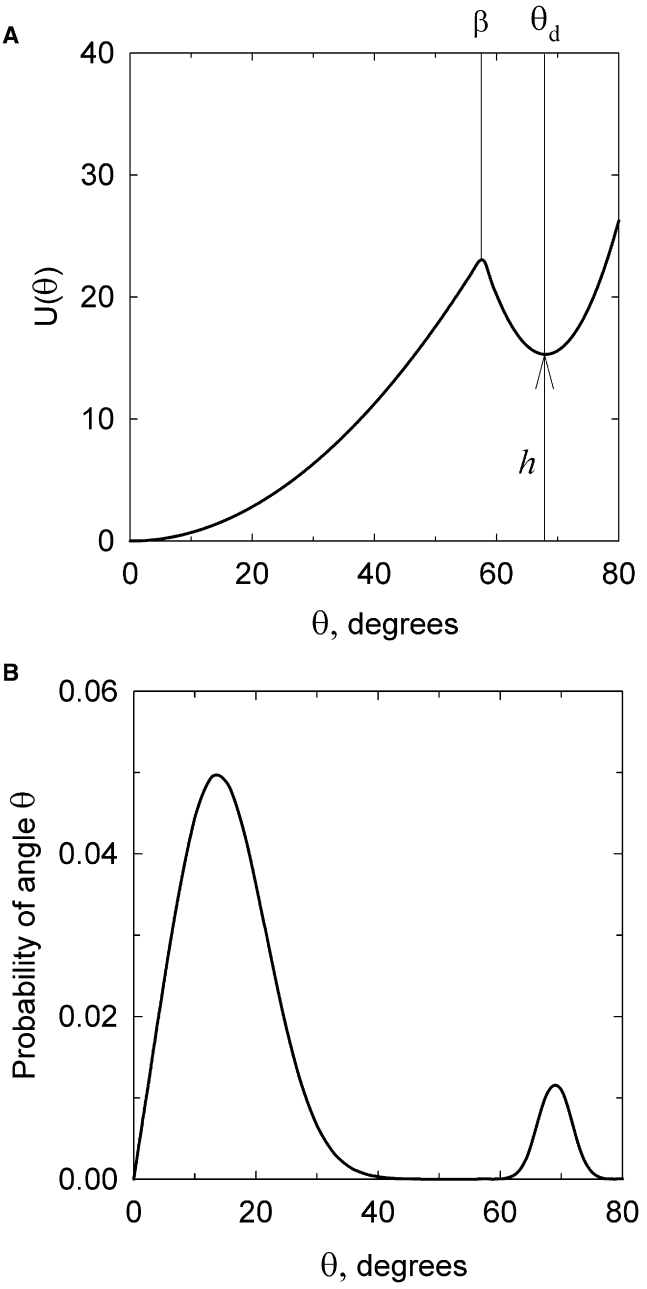

FIGURE 1 (*A*) Bending potential of the model chain, $U(\theta)$, which accounts for the possibility of disruptions. The second minimum of the potential, at $\theta = 68^\circ$, corresponds to a disruption probability of 3.6×10^{-7} in unstressed linear DNA. Each segment of the model chain corresponds to 3 bp of the double helix. Angle β separates the two parts of the potential. (*B*) Typical distribution of bend angles in negatively stressed DNA minicircles of 65 bp in length.

$$\Delta Lk = Lk - N/\gamma, \tag{7}$$

where Lk is the linking number of the complementary strands and γ is the number of basepairs per turn in the unstressed double helix, which is equal to 10.5 (26). Equation 6 presumes that the circle conformation is planar regardless of the value of ΔLk, which is true for small circular DNA molecules if $|\Delta Lk| < 1.5$ (27).

We assumed that each bend is associated with a specific change of the DNA twist, δtw_d (measured in units of helix turns). Therefore, after the appearance of i disruptions, the number of helix turns in torsionally unstressed molecules is $N/\gamma + i\times\delta tw_d$ rather than N/γ, so the elastic torsional deformation is specified as $Lk - (N/\gamma + i\times\delta tw_d)$, or $\Delta Lk - i\times\delta tw_d$. Correspondingly, the energy of elastic torsional deformation in the presence of i disruptions, $G_t(\Delta Lk, i)$, depends on $\Delta Lk - i\times\delta tw_d$ rather than on ΔLk:

$$G_t(\Delta Lk, i) = \frac{2\pi^2 C}{L}(\Delta Lk - i\times\delta tw_d)^2. \tag{8}$$

To summarize the above, the overall free energy of a chain conformation, G, can be expressed as

$$G = E_b + G_t(\Delta Lk, i). \tag{9}$$

Parameters of the model

Our model of disruptions in minicircles has three parameters whose values are essentially unknown. These parameters are the free energy of the disruption, G_d, the bend angle associated with the disruption, θ_d, and the change in DNA twist introduced by the single disruption, δtw_d. It has been shown, by measuring the rate of proton exchange in unstressed DNA, that the probability of an AT basepair opening is close to 10^{-5}, which corresponds to a G_d value of 7 kcal/mol (11,12). It is not clear, however, what kind of basepair opening is detected in these experiments, so we consider the above value as a first approximation for G_d. Simple molecular modeling has shown that the double helix can be bent at kinks up to 100° (1), and a recent molecular dynamics simulation confirmed this estimation (5). There are no data on the value of θ_d associated with basepair opening. Therefore, we did not use any preliminary restrictions on the value of θ_d. Some assumptions can be made about the value of δtw_d. It seems clear that δtw_d should be negative, which means that the disruption results in the local unwinding of the double helix. The structures of DNA-protein complexes confirm this assumption (8). On the other hand, it seems clear that $|\delta tw_d|$ cannot exceed 0.2/basepair step involved in the disruption. The latter conclusion is confirmed by data on local unwinding of the double helix in supercoiled DNA (25). Thus, $|\delta tw_d|$ should not exceed 0.2 for kinks and 0.4 for basepair opening, although most probable values should be essentially lower. We use the above consideration in the analysis that follows.

Throughout this study, DNA persistence length and its torsional rigidity were assumed to be 48 nm and 3×10^{-19} erg·cm, respectively (28).

METHODS

The Metropolis Monte Carlo procedure was used to sample the equilibrium ensemble of the minicircle conformations. This sampling did not account for the free energy of torsional deformation, and therefore it allowed us to calculate the probability of conformations with i disruptions under conditions of zero torsional rigidity, $P_d^0(i)$. Subsequent accounting for torsional stress was achieved using the equation

$$P_d(i) = \frac{P_d^0(i)\exp(-G_t(\Delta Lk,i)/RT)}{\sum_j P_d^0(j)\exp(-G_t(\Delta Lk,j)/RT)}. \quad (10)$$

The Metropolis procedure consisted of consecutive rotations of an arbitrary number of adjacent segments around the straight line connecting two randomly chosen vertices. The random rotation angle was uniformly distributed within one of two intervals, either $(-\pi/2, +\pi/2)$ or $(-\phi_0, \phi_0)$. The former interval was needed to facilitate the formation and disappearance of disruptions with large bend angles, and the latter interval served to enhance the efficiency of sampling for small bend angles. Each of the two intervals was used with a probability of 0.5. The trial conformations created by the rotations were accepted or rejected according to the rules of the Metropolis procedure (29). The value of ϕ_0 that specified the second interval was chosen to have ~50% of the corresponding trial conformations accepted.

The computational procedure described above provides a good sampling of the minicircle conformation only if $\theta_d\sqrt{g_1}$ does not exceed 10, so that the separation between two peaks of the bend angle distribution is not too large (see Fig. 1 *B*). To address a wide range of θ_d, we changed the length of straight segments in the model chain. Increasing the segment length corresponds to a reduction of g_1, so that the above condition can be satisfied. The segment lengths used for different θ_d are shown in Table 1. Up to 10^{10} trial moves were performed to obtain sufficient statistical accuracy in the simulations.

To calculate the j-factor for our model chain, we used the algorithm based on a chain of conditional probabilities (30). To save computer time, we used the algorithm to calculate the j-factors without accounting for torsional alignment, j_0. The total j-factor accounting for torsional alignment was calculated as

$$j = j_0 j_{tw} = j_0 \sum_i P_d^0(i) j_{tw}(i), \quad (11)$$

where $j_{tw}(i)$ is the torsional component of j-factor that accounts for conformations with i disruptions. Calculating the value of $j_{tw}(i)$ was similar to the calculation of j_{tw} without disruptions (31,32):

$$j_{tw}(i) = \frac{\sum_{Lk=-\infty}^{\infty} P(\Delta Lk,i)}{\int_{-\infty}^{\infty} P(x,i)dx} \approx \sqrt{\frac{2\pi C}{LRT}} \sum_{Lk=-\infty}^{+\infty} \exp\left(-\frac{2\pi^2 C(\Delta Lk - i\times\delta tw)^2}{LRT}\right). \quad (12)$$

The values of $P_d^0(i)$ were calculated for closed chains as described above.

RESULTS

Disruption appearance under bending stress

In general, both bending and torsional deformations of the double helix affect the formation of disruptions in DNA minicircles. However, under certain conditions, the influence of torsional deformation on the disruptions can be negligible.

TABLE 1 Parameters of the bending potential used in the Monte Carlo simulation

θ_d	40°	54°	68°	90°
l (bp)	1	2	3	4
g_1	69.9	34.9	23.1	17.3
g_2	250	250	250	250
h	13.4	13.6	15.3	16.9

The potential is specified by Eq. 2. The length of the segment of the model chain, l, had different values depending on the bend angle, θ_d.

The total change in the torsional free energy, ΔG_t, resulting from the formation of i disruptions in the minicircle can be found from Eqs. 6 and 8:

$$\Delta G_t = -\frac{4\pi^2 C}{L} i\delta tw_d(\Delta Lk - i\delta tw_d/2). \quad (13)$$

It follows from Eq. 13 that $\Delta G_t \approx 0$ if ΔLk is close to $i\delta tw_d/2$. Although we do not know the value of δtw_d, we can assume that the unwinding angle of a DNA kink is between −0.05 and −0.2 in units of helix turns. The maximum unwinding could be larger for an opened basepair, presumably as much as −0.4. Therefore, the value of ΔG_t associated with the formation of the first disruption should be close to 0 for minicircles with small negative values of ΔLk. The value of ΔLk is specified by the minicircle length, which could be chosen to minimize ΔG_t. The results shown in this section were obtained without accounting for ΔG_t and therefore are applied to minicircles with specific lengths: 64 bp, 74–75 bp, 85 bp, etc.

The calculated probability of minicircle conformations with at least one disruption, P_d, versus minicircle length is shown in Fig. 2 *A* for specific values of θ_d and G_d. As expected, P_d increases when minicircle length decreases, since the bending deformation is larger in smaller minicircles. We analyzed the distribution of the number of disruptions in the different-sized minicircles. The calculated probabilities of minicircle conformations with a particular number of disruptions, $P_d^0(i)$, are shown in Fig. 2 *A* as components of P_d. We found that if $P_d^0(i)$ is plotted as a function of P_d (Fig. 2 *B*), the results weakly depend on chosen values of θ_d and G_d. We see from the figure that conformations with a single disruption are predominant among all conformations when $P_d < 0.3$. The conformations with two disruptions prevail for values of P_d between 0.5 and 0.95, and only beyond this range conformations with a larger number of disruptions become important.

It seems clear, and the calculation confirms, that the disruption-induced reduction of the total energy of elastic deformation is larger for larger values of θ_d, so that the disruptions start appearing in larger minicircles. On the other hand, increasing G_d should reduce the probability of disruptions appearing in the minicircles. The influence of θ_d and G_d on the disruption appearance is quite strong and allows us to make an important conclusion by comparing the calculation with the experimental data by Du et al. According to the

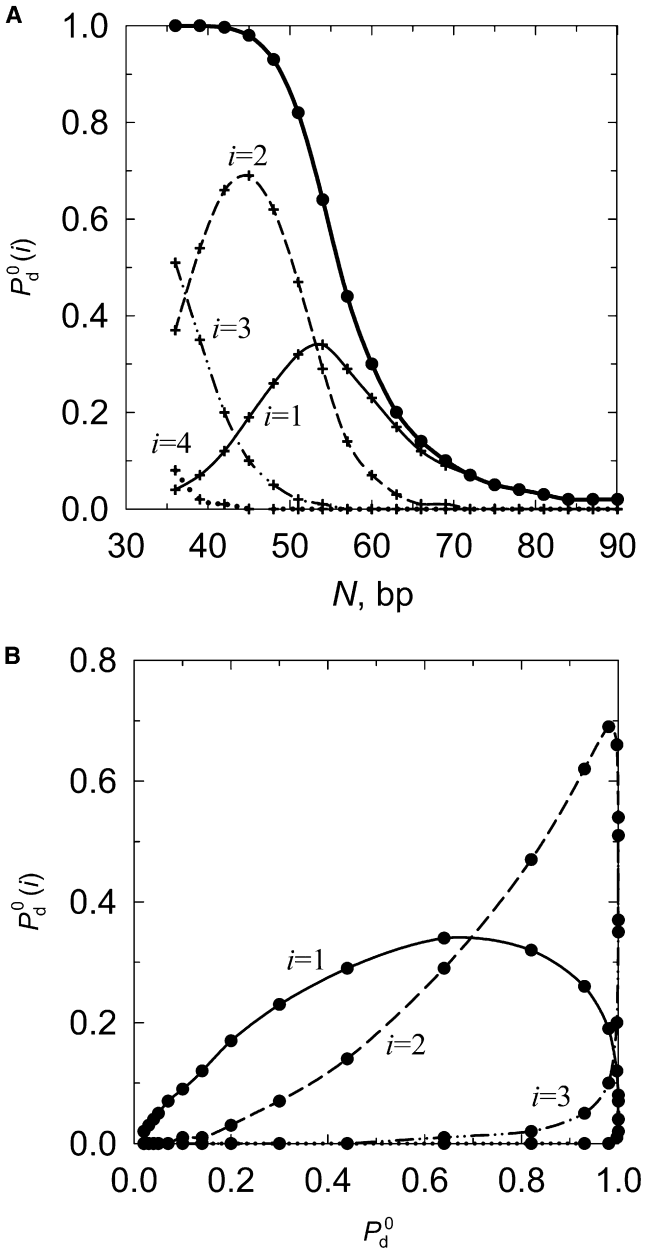

FIGURE 2 Probability of minicircle conformations with disruptions, P_d^0, and its component probabilities of i disruptions, $P_d^0(i)$. The values of i are shown near the corresponding dashed and dotted lines, and P_d^0 is shown by a solid line. (*A*) The values of P_d^0 and $P_d^0(i)$ are plotted as a function of minicircle length. (*B*) Plot of $P_d^0(i)$ as a function of P_d^0, which is relatively insensitive to the choice of G_d and θ_d. The data shown were calculated for $G_d = 8.7$ kcal/mol and $\theta_d = 68°$. The torsional free energy of the minicircles was not taken into account in this calculation.

experimental results, the disruptions were hardly detected in minicircles of 85–86 bp, but they were very well pronounced in minicircles of 64–65 bp. Thus, we assumed that the average number of disruptions in the calculation for 63-bp minicircles should be equal to 0.25 (without accounting for torsional stress). We calculated the pairs of G_d and θ_d that satisfied this condition (Fig. 3). One can see from the figure

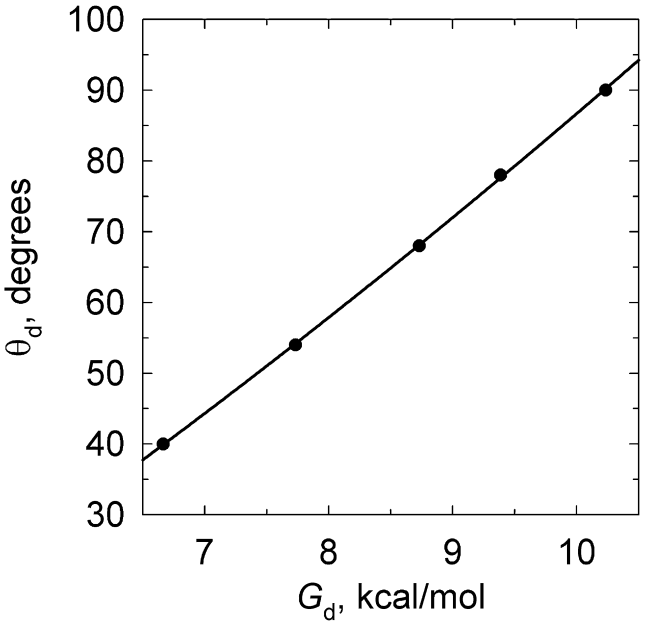

FIGURE 3 Disruption parameters that fit the experimental data on minicircle digestion by single-strand-specific endonucleases (17). The pairs of θ_d and G_d that provide 0.25 probability of disruption appearance in 63-bp minicircles are shown on the plot. The calculated data did not account for torsional deformation in the minicircles.

that for G_d of 7 kcal/mol the bend angle satisfying the experimental data should be around 45°. This value of θ_d is much smaller than that predicted by molecular modeling for DNA kinks (1,5). On the other hand, we found that for a bend angle around 90°, the angle predicted in these studies, the value of G_d should be close to 10 kcal/mol (see Fig. 3). Thus, it is difficult to choose a pair of G_d and θ_d unambiguously. In the subsequent calculations, we used one pair of these parameters, G_d of 8.7 kcal/mol and θ_d of 68°. We checked, however, that the simulation results for other pairs of G_d and θ_d from the curve in Fig. 3 do not differ substantially from those obtained for the chosen pair.

Influence of torsional deformation on the disruption appearance

The change in torsional energy due to disruptions, $G_t(\Delta Lk, i)$, is comparable to, and may even exceed, the corresponding change in bending energy. Of course, the effect strongly depends on ΔLk of the minicircle, which changes with its length. This is illustrated in Fig. 4, which shows the average number of disruptions, $\langle n_d \rangle$, for DNA minicircles of 62–67 bp in length. The computation was performed for different values of δtw_d. We assumed here that the DNA helical repeat, γ, equals 10.5; thus, the ΔLk of the minicircles gradually decreases from 0.095 to −0.38 as their length increases. We see from the figure that $\langle n_d \rangle$ does not change notably over this range of the minicircle sizes if we assume that δtw_d equals zero. Clearly, the unwinding at the disruptions has to suppress their appearance

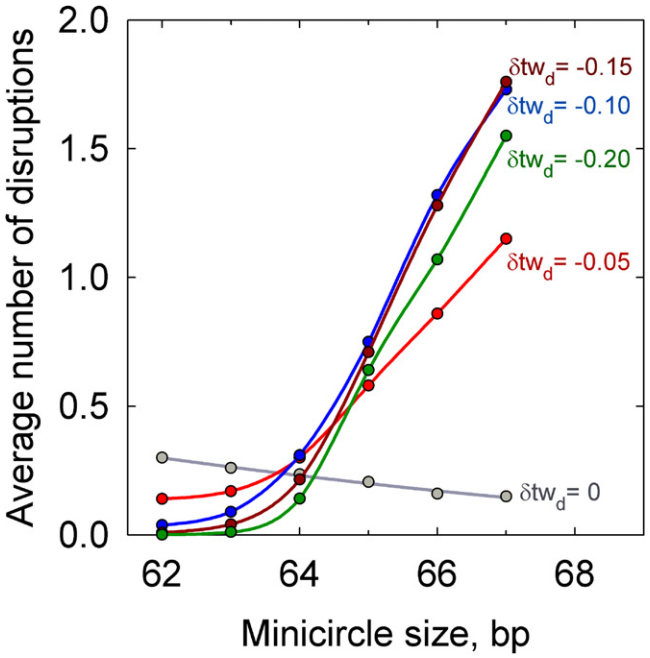

FIGURE 4 Average number of disruptions, $\langle n_d \rangle$, as a function of ΔLk. The values of ΔLk change with minicircle size from 0.095 for 62-bp minicircles to −0.38 for 67-bp minicircles. The calculation was performed for different values of the double helix unwinding associated with the disruption, δtw_d. For different values of δtw_d, the calculated values of $\langle n_d \rangle$ are shown by different colors.

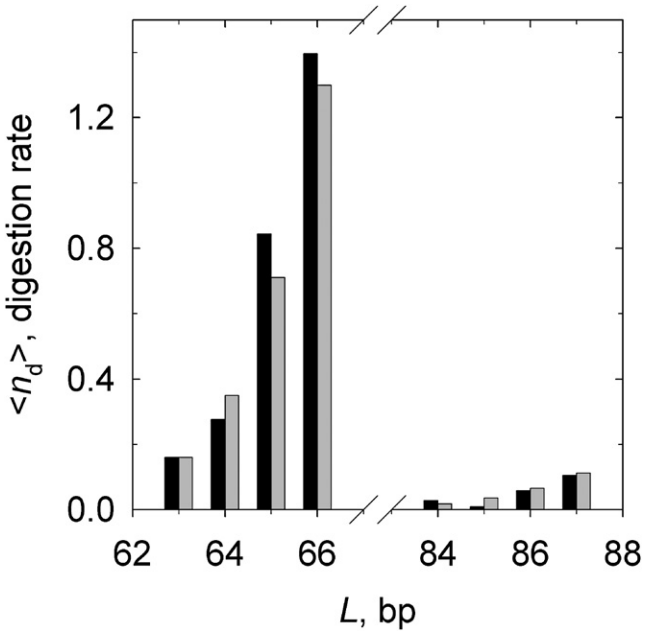

FIGURE 5 Comparison of simulation results with experimental data by Du et al. (17). The experimental data correspond to the rate of minicircle digestion by single-strand-specific endonuclease. We assume that the digestion rate (*black bars*) is proportional to the average number of disruptions in the minicircles, $\langle n_d \rangle$. The calculated values of $\langle n_d \rangle$ are shown by gray bars. The scaling of the two sets of data was selected to have the perfect match for 63-bp minicircles.

for initially positively supercoiled (62 bp) and relaxed minicircles (63 bp), and this is what we see in Fig. 4. The effect of torsional energy is relatively small for the minicircles with small negative supercoiling (64 bp in length), as we suggested in the previous section. However, the effect is large for minicircles with larger negative supercoiling (65- to 67-bp minicircles). It is also interesting that for all these minicircles $\langle n_d \rangle$ hardly changes when we decrease δtw_d from −0.1 to −0.2. This is because when we increase $|\delta tw_d|$ over the range, the fraction of conformations with smaller numbers of disruptions also increases at the expense of the conformations with a larger number of disruptions (data not shown). Thus, the total reduction of torsional stress remains nearly the same over this range of δtw_d.

Du et al. studied how the rate of minicircle digestion by single-strand-specific endonucleases depends on ΔLk (17). We fitted these data by the calculation results to evaluate our model of disruptions. Comparing the simulation results with these experimental data, we assumed that the digestion rate is proportional to the average number of disruptions in the minicircles. Therefore, the comparison used an additional adjustable parameter, a coefficient in the proportionality. The results of this comparison are shown in Fig. 5. We found that good agreement is obtained for δtw_d between −0.075 and −0.15, when the calculated results only weakly depend on δtw_d. Still, the agreement is slightly better for $\delta tw_d = -0.075$, which was used for the plot. We see remarkable agreement between the experimental and calculated data for minicircles of 63–66 bp and 84–87 bp in length.

The effect of disruption on *j*-factor oscillations

In agreement with earlier theoretical studies, we found that disruptions should facilitate cyclization of short DNA fragments even without accounting for the torsional stress (6,15,16). However, for the pairs of θ_d and G_d found here from the comparison with the experimental data (see Fig. 3), a substantial increase in *j*-factor was obtained in calculations only for fragments <50 bp in length (Fig. 6 *A*). However, Fig. 6 *A* does not account for the necessity of torsional alignment of the fragment ends. Our primary goal in this section was to study the effect of this requirement on the *j*-factors of short DNA fragments.

It was found experimentally that strong negative torsional stress in the DNA minicircles promotes change in the disruption type, supposedly from kinks to basepair openings (17). The unwinding angle should be larger for the latter kind of disruption. Thus, we used larger values of $|\delta tw_d|$ in this section, between 0.2 and 0.3 turns. The calculated dependence of *j*-factor on DNA fragment length, which accounts for torsional deformation, is shown in Fig. 6 *B*. One can see from the figure that accounting for the possibility of disruptions reduces the amplitude of *j*-factor oscillations even for fragments of ~100 bp. This reduction in amplitude is in agreement with experimental observation for fragments

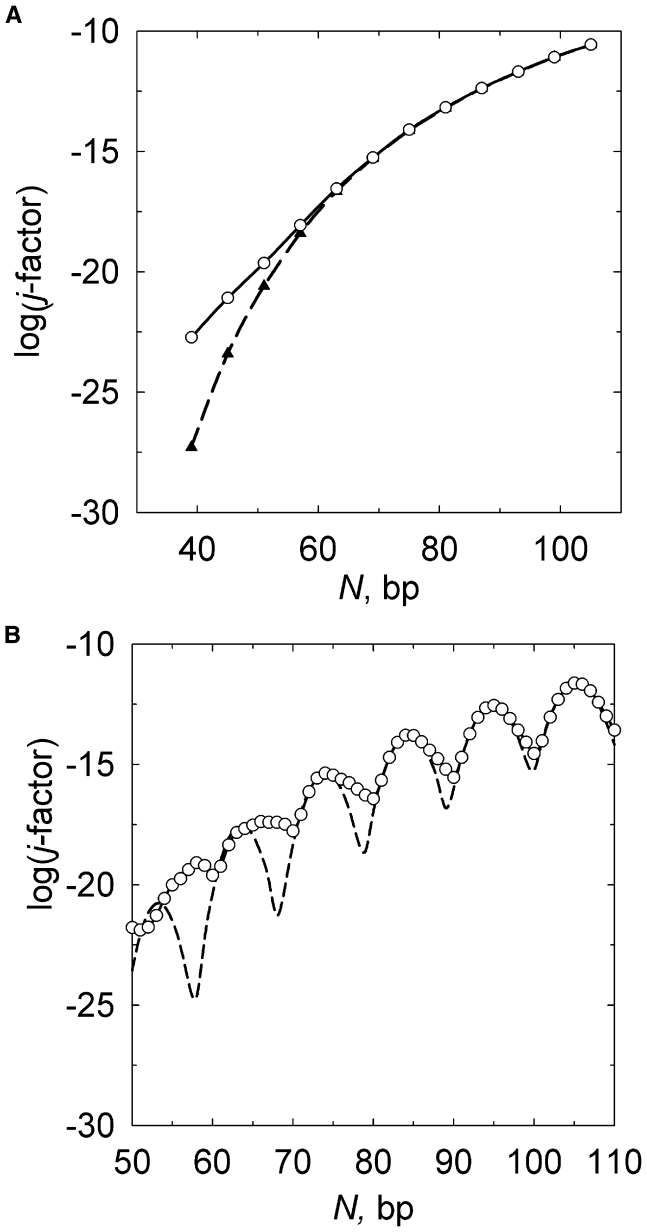

FIGURE 6 Calculated *j*-factors for small DNA fragments. To emphasize the effect of disruptions, the *j*-factor calculation was performed with (*solid line*, *circles*) and without (*dashed line*, *triangles*) the possibility of disruptions. (*A*) Calculated *j*-factors without accounting for the torsional alignment of the fragment ends. (B) The effect of disruptions on *j*-factor oscillations, which result from the requirement of torsional alignment of the fragment ends.

of 95–105 bp by Cloutier and Widom (14). According to the calculations for fragment lengths in this range, the disruptions do not play any role in the formation of torsionally unstressed DNA minicircles, but they play a key role for the formation of minicircles of this size with ΔLk values of ~ -0.5. In other words, in minicircles of this size, the disruptions appear with large probability only if they can release large negative torsional stress. This conclusion is in full agreement with the high rate of nuclease digestion for minicircles 100 bp in length ($\Delta Lk \approx -0.5$) observed by Du et al. (17).

Cyclization of even shorter fragments, 50–70 bp in length, involves disruptions in nearly all minicircles, regardless of ΔLk. As a result, the amplitude of *j*-factor oscillations reduces even further for these DNA fragments (see Fig. 6 *B*). What is even more interesting is that for fragment lengths of 60–100 bp, *j*-factor oscillations change their character. Although the oscillation maxima correspond to the same fragment lengths, the minima are more and more shifted toward larger lengths (Fig. 6 *B*). Both of these effects reflect a larger role of topoisomers with negative ΔLk as products of the fragment cyclization. Formation of these topoisomers results in the reduction of both bending and torsional stress, so topoisomers with lower Lk and a few disruptions can compete with topoisomers with no disruptions and higher values of Lk. This increasing competition of topoisomers was brightly illustrated experimentally when the topoisomers with ΔLk of -1 and 0 appeared with very close probability in minicircles of 63 bp in length (17). The *j*-factor oscillations are further reduced over this range of fragment lengths (see Fig. 6 *B*).

DISCUSSION

In this study, we developed a statistical-mechanical model to analyze conformational properties of DNA minicircles related to formation of local disruptions. In many aspects, our model oversimplifies the conformational properties of actual DNA. The most important simplification is the assumption that unwinding of the double helix resulting from disruptions has a unique magnitude. A more accurate model would use a specific torsional potential for each disruption type, which would provide a variety of torsional angles at the disruption. However, this model would also involve additional adjustable parameters that are absolutely unknown at the moment. Clearly, we wanted to minimize the number of such parameters. Still, the results obtained in this study show that the model is capable of describing disruptions in DNA minicircles with good accuracy. A comparison between the Monte Carlo simulation of minicircle conformations, based on this model, and experimental data obtained by Du et al. (17) allowed us to make important predictions about the disruption properties.

Our model also ignores the sequence dependence and anisotropy of DNA bending rigidity. It has been shown, however, that the latter factor is well averaged over short stretches of the double helix (33) and therefore has a very small effect on the disruption appearance. The sequence dependence of the free energy of disruption, G_d, which was also ignored in this model, is more important. We analyzed the model, which accounts for the sequence dependence of G_d, assuming that the variations of G_d follow the variations of the stacking energy found at the single-stranded DNA nicks (10). As one can expect, in this model, the

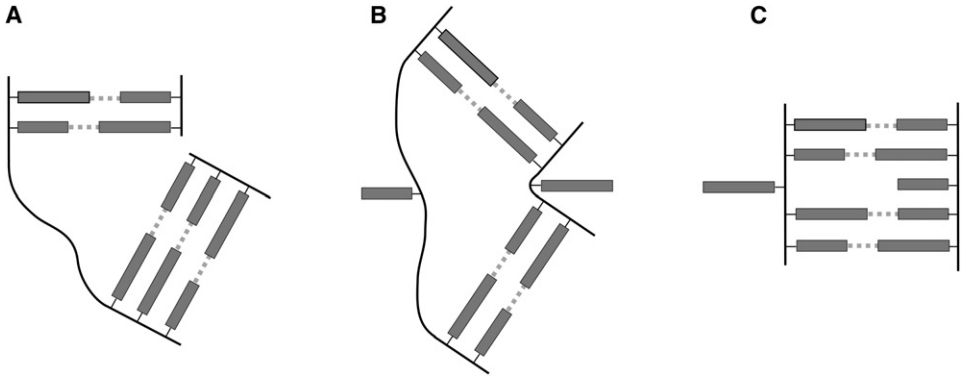

FIGURE 7 Diagrams of the three types of disruption discussed in this study. (*A*) A kink of the double helix keeps all basepairing but eliminates stacking between two adjacent basepairs. It is associated with a large bend angle. (*B*) Basepair opening eliminates stacking in two consecutive basepair steps and disrupts one basepairing. It is associated with even larger bending of the double helix. (*C*) Flipping of a base from the double helix. Only the stacking of the flipped base with the adjacent pairs is eliminated in this case. The other base of the pair maintains stacking with adjacent basepairs, preventing substantial bending at the disruption.

disruptions were mainly localized at the weakest basepair steps of the minicircles (data not shown). To a reasonable approximation, the value of G_d considered here corresponds to the basepair steps with the weakest stacking energy.

There are two important features of our model that better describe the DNA properties and distinguish this model from those used in previous analyses (6,16). First, we considered restricted bend angles at the disruptions, whereas previous studies assumed that the disruptions form free joints or segments with ample flexibility. Our results clearly show that the value of bend angle is very important. Second, our analysis considers, for the first time that we are aware of, the change of torsional energy in DNA minicircles caused by the disruptions. Both the experimental data (17) and our theoretical results show the great importance of torsional energy in disruptions.

Only two parameters of our model, the free energy of the disruption appearance in unstressed linear DNA, G_d, and the bend angle associated with the disruption, θ_d, were varied to fit the experimental data by Du et al. (17). From this fitting we deduced a strong restriction on the pairs of θ_d and G_d compatible with the experimental data (see Fig. 3). It is interesting to analyze how this result agrees with existing data on DNA disruptions.

It was predicted by simple molecular modeling, and found in the molecular dynamics simulation, that the value of the bend angle in DNA kinks can be close to 90° (1,5). Although such large bends have not been observed at kinks in the crystal structures of DNA-protein complexes (8), the theoretical analysis leaves no doubt that there are no serious obstacles to bending the double helix at kinks by angles of ~90°. Still, there are no data on the value of G_d for DNA kinks. Such data were obtained only for basepair openings. They are based on measurements of the proton exchange rate (11,12) and the rate of DNA unwinding by formaldehyde (34). According to these results, G_d is ~7 kcal/mol for opening AT basepairs. However, there are no conformational data for opened basepairs detected in these studies. If we assume that the opening eliminates the stacking interaction at two consecutive basepair steps (7), bend angles >90° should be possible at such disruptions. Since our analysis predicts a G_d of 10 kcal/mol for θ_d of 90°, we have to conclude that a G_d of 7 kcal/mol corresponds to a more restricted disruption of the DNA helical structure (Fig. 7). Indeed, both methods of the disruption detection cited above require only that a base is exposed to the surrounding solution. Such basepair opening with only one base flipped out from the helix has been observed in the crystal structure of a DNA-protein complex (35) and obtained in molecular dynamics simulations (36,37). This kind of conformation does not provide a large bend angle but makes the flipped-out base available for chemical modifications (36). Since half of the stacking interactions are preserved in this disruption, the corresponding free energy should be lower than for a disruption capable of providing θ_d close to 90°.

The experimental data on minicircle digestion by single-strand-specific endonucleases showed that the bending stress alone causes disruptions only for minicircles <~75 bp in length (17). We chose the parameters of the disruptions to fit these experimental data. Correspondingly, our calculations showed that only for DNA fragments <~75 bp in length the cyclization efficiencies (*j*-factors) are increased by disruptions relative to the theoretical values deduced for the wormlike chain model of DNA (see Fig. 6 *A*). However, this is true only if the corresponding minicircles have only minor negative supercoiling. DNA minicircles whose lengths correspond to semi-integer numbesr of helix turns (close to 80, 90, 100, or 110 bp) have strong negative supercoiling that greatly stimulates disruptions in the minicircles (17). The *j*-factors of the fragments with corresponding lengths are strongly increased by the disruptions. Therefore, the amplitude of the *j*-factor oscillations due to the double-helix periodicity (31) has to be many times smaller for DNA fragments in the size range 80–110 bp than what is predicted by the wormlike chain model. Such reduced amplitude of the *j*-factor oscillations was observed experimentally (14), although the absolute values of *j*-factors were not measured correctly in that study (15). Our simulations also

showed that the oscillation minima are shifted to larger fragment lengths, so for very short DNA molecules, the oscillation minima do not correspond to linear fragments with a semi-integer number of helix turns. The latter effect has not been observed experimentally, and it is not easy to do so, since the corresponding values of *j*-factors are too small to be measured by currently employed methods.

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