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Effect of Supercoiling on the Juxtaposition and Relative Orientation of DNA Sites

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ABSTRACT There are many proteins that interact simultaneously with two or more DNA sites that are separated along the DNA contour. These sites must be brought close together to form productive complexes with the proteins. We used Monte Carlo simulation of supercoiled DNA conformations to study the effect of supercoiling and DNA length on the juxtaposition of DNA sites, the angle between them, and the branching of the interwound superhelix. Branching decreases the probability of juxtaposition of two DNA sites but increases the probability of juxtaposition of three sites at branch points. We found that the number of superhelix branches increases linearly with the length of DNA from 3 to 20 kb. The simulations showed that for all contour distances between two sites, the juxtaposition probability in supercoiled DNA is two orders of magnitude higher than in relaxed DNA. Supercoiling also results in a strong asymmetry of the angular distribution of juxtaposed sites. The effect of supercoiling on site-specific recombination and the introduction of supercoils by DNA gyrase is discussed in the context of the simulation results.

INTRODUCTION

Supercoiling has critical consequences for the biological functions of DNA. It changes the efficiency of many transcriptional promoters (Menzel and Gellert, 1994) and the replication of many replicons (Wang and Liu, 1990). Supercoiling is also necessary for a number of site-specific recombination systems (Kanaar and Cozzarelli, 1992; Stark and Boocock, 1995). The mechanisms of these effects are not completely understood.

There are two basic ways that supercoiling can influence biological processes. First is the local changes of DNA structure by supercoiling. These can be changes in DNA secondary structure, such as the formation of open regions, cruciform structures, and the A, Z, and H forms of DNA (reviewed in Wang, 1986; Wells, 1988; Frank-Kamenetskii, 1990; Johnston, 1992; Murchie and Lilley, 1992; Vologodskii, 1992). Such transitions usually occur at specific nucleotide sequences and result from supercoiling alone or from supercoiling and the binding of specific proteins. For example, DNA replication commonly initiates in a particular A-T-rich region near the binding site of a replication initiator (Benham, 1992; Kornberg and Baker, 1992; Natale et al., 1993). Supercoiling can also influence local changes of DNA tertiary structure that result from complexes with proteins. Supercoiling has this effect whenever the DNA axis adopts a helical form in the complex, as in nucleosomes, (Germond et al., 1975; Wolffe, 1992) or is sharply bent, as in transcription complexes (Ten Heggeler-Bordier et al., 1992; Kahn and Crothers, 1993) and recombination complexes (Nash, 1990).

The second way that DNA supercoiling influences biological reactions is based on global changes of DNA conformations. These global changes affect the relative orientation of DNA sites that are far apart along the chain contour but close in space, as well as the probability of multiple DNA site juxtapositions (Vologodskii et al., 1992). The global consequences of supercoiling are important in processes where two or more DNA sites are joined by protein bridges. Numerous examples of such multisite complexes are found in DNA replication, transcription, site-specific recombination, and transposition (Echols, 1990; Kanaar and Cozzarelli, 1992; Kornberg and Baker, 1992; Tjian and Maniatis, 1994). This second category of supercoiling effects is the subject of our study.

It is not easy to study experimentally the conformational properties of supercoiled DNA. The difficulties arise from the great length and conformational flexibility of DNA molecules that can be supercoiled. Electron microscopy (EM) is the most suitable method for studying conformations on this scale. EM studies have shown that supercoiled DNA is an interwound superhelix that can be branched, and have revealed basic parameters of the superhelix (see Rhoades and Thomas, 1968; Laundon and Griffith, 1988; Adrian et al., 1990; Boles et al., 1990; Bednar et al., 1994, and, for a review, Vologodskii and Cozzarelli, 1994). However, the most labile features of conformations may be changed during sample preparation for EM. Moreover, the conformational properties of supercoiled DNA depend strongly on ionic conditions, which are undefined in the dehydrated samples used for conventional microscopy.

In light of the limitations of experimental studies of supercoiled DNA conformations, computer simulation is a very useful alternative (Vologodskii and Cozzarelli, 1994; Schlick, 1995). It allows the construction of an equilibrium

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distribution of DNA conformations (Klenin et al., 1991, 1995; Vologodskii et al., 1992; Gebe et al., 1995). Using such a set, we can estimate many different conformational properties of supercoiling. Comparison of calculated and measured properties allows us to judge how well the simulated conformations reflect the conformations of actual molecules. The available data show that the simulations describe well many of the properties of DNA supercoiling (Vologodskii et al., 1992; Langowski et al., 1994; Vologodskii and Cozzarelli, 1994; Gebe et al., 1995). In this report, we used simulation to calculate the conformational properties of supercoiled DNA that are particularly relevant for its biological functions. We studied the distribution of the relative orientations of juxtaposed sites as a function of superhelix density. We also investigated the DNA length dependence of superhelix conformations and found that the branching has a strong influence on the probability of DNA site juxtaposition.

METHODS OF CALCULATIONS

The DNA model we used has been described in detail previously (Vologodskii et al., 1992). A closed DNA molecule of n Kuhn statistical lengths is modeled as a closed chain of kn rigid cylinders of equal length and diameter d. The elastic energy of the chain is computed as the sum of the bending and torsional elastic energies. The bending rigidity constant between adjacent cylinders is chosen so that the Kuhn statistical length corresponds to k rigid segments. It was shown that the simulation results do not depend on the value of k, if $k \geq 10$ (Vologodskii et al., 1992). We make the standard assumption that the torsional energy is a quadratic function of the displacement of the chain twist from equilibrium. The connection between the model chain and an actual DNA molecule is specified by three parameters. One is the Kuhn statistical length for DNA, which is twice the persistence length. It is equal to 100 nm for the ionic conditions we modeled in the simulations (Hagerman, 1988). We used a value of 3×10^{-19} erg·cm for the second parameter, the torsional rigidity constant (Hagerman, 1988; Klenin et al., 1989). Although a lower value of C, 2×10^{-19} erg·cm, is often used in the literature (see Gebe et al., 1995, for example), we wish to emphasize that the simulation results are rather insensitive to such changes in C (Vologodskii et al., 1992). The third parameter, the DNA effective diameter (d), specifies the electrostatic interaction between DNA segments (Stigter, 1977; Vologodskii and Cozzarelli, 1995). d is the diameter of an uncharged polymer chain that mimics the conformational properties of actual, electrically charged DNA. We have used a value of 5 nm for d, which corresponds to a sodium ion concentration of 0.2 M (Stigter, 1977; Brian et al., 1981; Yarmola et al., 1985; Rybenkov et al., 1993; Shaw and Wang, 1993). Adding up to 10 mM magnesium ions to this solution does not change the value of d (Rybenkov et al., unpublished data). Thus, the chosen value of d corresponds to physiological ionic conditions.

The model used in the work approximates the electrostatic interaction between DNA segments in terms of a hard-core potential. Although this potential is a relatively rough approximation of the actual electrostatic potential of the double helix, we showed that it gives practically the same results for conformations of supercoiled DNA as a more accurate approximation of the potential based on the Poisson-Boltzmann equation (Vologodskii and Cozzarelli, 1995).

We used the Metropolis-Monte Carlo procedure for the preparation of the equilibrium set of DNA conformations (Vologodskii et al., 1992). Up to 10^8 elementary movements were employed to construct each set. We saved for further analysis only one conformation after every 10,000 moves because of the strong correlation between successive simulated conformations.

We used the following method to calculate the angle ϕ between juxtaposed DNA segments (Fig. 1). We assigned an arbitrary direction to the chain to define vectors corresponding to each chain segment. For a pair of such vectors, $\mathbf{u}_a$ and $\mathbf{u}_b$, we then calculated the angle ϕ' as $\arccos[(\mathbf{u}_a\mathbf{u}_b)/u_a u_b]$. This angle varies between 0° and 180°. Its value is the same for the two symmetric orientations of the vectors shown in Fig. 1, which we need to distinguish. Thus, we calculated the box product $(\mathbf{u}_a \times \mathbf{u}_b)(\mathbf{r}_b - \mathbf{r}_a)$, where $\mathbf{r}_a$ and $\mathbf{r}_b$ are the vectors from the coordinate system origin to the beginnings of $\mathbf{u}_a$ and $\mathbf{u}_b$. The product has different signs for the two conformations shown in Fig. 1. We took the angle ϕ as equal to ϕ' if the box product was positive, and ϕ as equal to $360° - \phi'$ if the box product was negative.

RESULTS

Branching of superhelical DNA as a function of chain length

Typical simulated conformations of a circular DNA molecule change from a random coil to an interwound superhelix

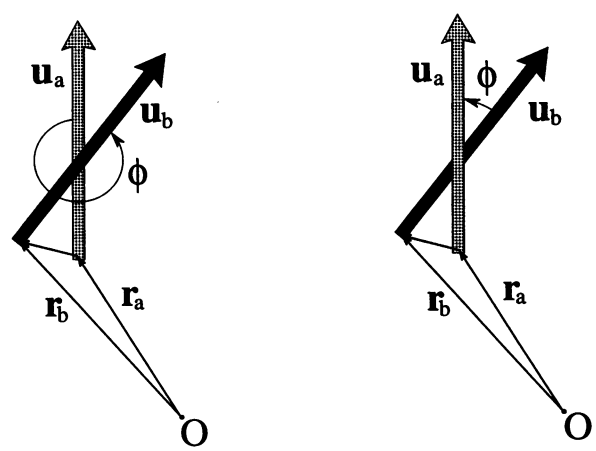

FIGURE 1 Definition of the angle between juxtaposed segments of DNA. The DNA segments are represented by vectors $\mathbf{u}_a$ and $\mathbf{u}_b$. The angle ϕ between the vectors is measured in the counterclockwise direction from the underlying vector to the overlying one in the plane that is parallel to both vectors. $\mathbf{r}_a$ and $\mathbf{r}_b$ are vectors from the origin of the coordinate system, O, to the beginning of $\mathbf{u}_a$ and $\mathbf{u}_b$. The box product $(\mathbf{u}_a \times \mathbf{u}_b)(\mathbf{r}_b - \mathbf{r}_a)$, used in the calculation of ϕ, is negative for the configuration on the left and positive for the one on the right.

as the superhelix density, σ, increases. This progression is illustrated in Fig. 2 for a 7-kb molecule. The interwound conformation begins to emerge at a σ of 0.02, but there remains some coil form and perhaps solenoidal superhelices. The plectonemic structure can be clearly identified for a σ of about -0.03 and becomes more regular in appearance for $\sigma = -0.04$. Its regularity grows as $-\sigma$ increases.

For a given σ, the local superhelix structure does not change over the size range investigated, 2 to 20 kb (Fig. 3). As illustrated in Fig. 3 *B*, the larger molecules are compact in overall shape because of the bending of the superhelix axis and particularly its branching.

Branching is an important feature of the interwound conformations of superhelices. Branch points correspond to junctions of three or more segments of a superhelix and are a major way of bringing three DNA sites close together in space. Branch points also introduce new superhelix ends, and this characteristic is easier to follow in the simulations than the number of branch points. However, because most branch points are three-way junctions, the number of ends is approximately equal to the number of branch points plus 2.

Shown in Fig. 4 is the DNA length dependence of the number of superhelix ends. Branches are very rare in DNA less than 2 kb in length but clearly present in molecules 3 kb in length, and for lengths of more than 5 kb nearly all molecules have a few branch points. Beyond about 3 kb, the average number of ends of interwound superhelix increases nearly linearly with DNA length. Over this range, there are, on average, 0.6 branch ends per kilobase.

Branched interwound conformations of supercoiled DNA have been observed by EM (Vinograd et al., 1965; Rhoades and Thomas, 1968; Sperrazza et al., 1984; Laundon and Griffith, 1988; Adrian et al., 1990; Boles et al., 1990; Bednar et al., 1994). However, the diameter of the superhelix has varied significantly in different EM studies. At one extreme, cryo-EM studies found collapsed conformations of superhelix at ionic conditions corresponding to the current simulations (Adrian et al., 1990; Bednar et al., 1994). In the collapsed form, there is no visible space between the opposing segments of the interwound superhelix, so that the superhelix diameter is just twice the double helix diameter. The existence of such collapsed structures would certainly contradict the simulation results. We have undertaken intensive investigation of supercoiled DNA conformations in solution (Rybenkov et al., manuscript in preparation). These studies showed no collapse of the interwound superhelix in physiological ionic conditions. Thus we believe that the collapse is an artifact of the sample preparation procedure in cryo-EM, and that simulation results reflect the properties of actual supercoiled DNA at room temperature.

One study with 3.5-kb and 7.0-kb molecules found that the average number of branches was proportional to DNA length (Boles et al., 1990), as demonstrated more systematically by the simulations. The absolute number of branches per unit length as observed by EM varied widely between two studies (Laundon and Griffith, 1988; Boles et al., 1990) and brackets the results of the simulations. We have noted elsewhere that differences in microscopy procedures and nucleotide sequence contribute to this variation (Vologodskii and Cozzarelli, 1994).

Effect of supercoiling on site juxtaposition

We showed previously that the probability that a pair of DNA sites separated along the chain contour are juxtaposed is about two orders of magnitude greater in supercoiled DNA ($\sigma = -0.04$ to -0.07) than in relaxed molecules (Vologodskii et al., 1992). The juxtaposition of distant DNA sites takes place mainly when the sites are across the interwound superhelix, and nearby sites can be juxtaposed if they are near the apices of the superhelix. Here we did a more comprehensive study of site juxtaposition, and, in particular, we studied the effect of DNA length L.

We define two segments of the model chain as juxtaposed if the distance between their centers does not exceed 1 nm. We studied previously the probability of site juxtaposition for a 3.5-kb supercoiled DNA (Vologodskii et al., 1992) and did not observe any clear dependence of the probability of site juxtaposition on the distance l between sites measured along the chain contour. The only exception was for very short distances, $l <$ 100 nm, because of the bending energy required for the formation of small loops. The independence of the probability of site juxtaposition of l is reasonable if there is no branching of an interwound superhelix, because any two sites that are more than 100 nm apart should have the same probability of being across the superhelix axis from each other. In this paper we consider only sites separated by more than this minimum distance.

However, because branching is a constant feature for DNA more than 5 kb in length (Fig. 4), the picture is more complex. To illustrate the influence of branching,

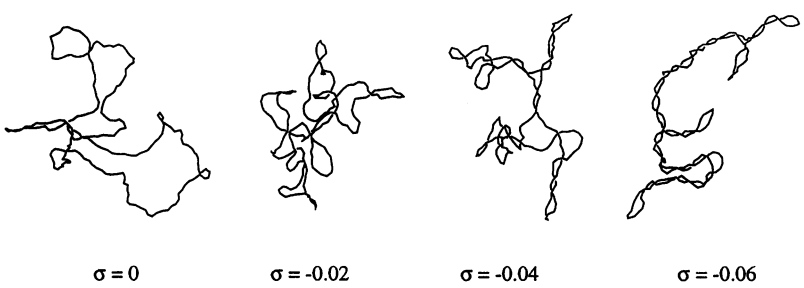

FIGURE 2 Typical simulated conformations of relaxed and supercoiled DNA 7 kb in length. The values of DNA superhelix density, σ, are indicated. The simulations employed an electrostatic repulsion between DNA segments that would be found in a 0.2 M monovalent salt solution.

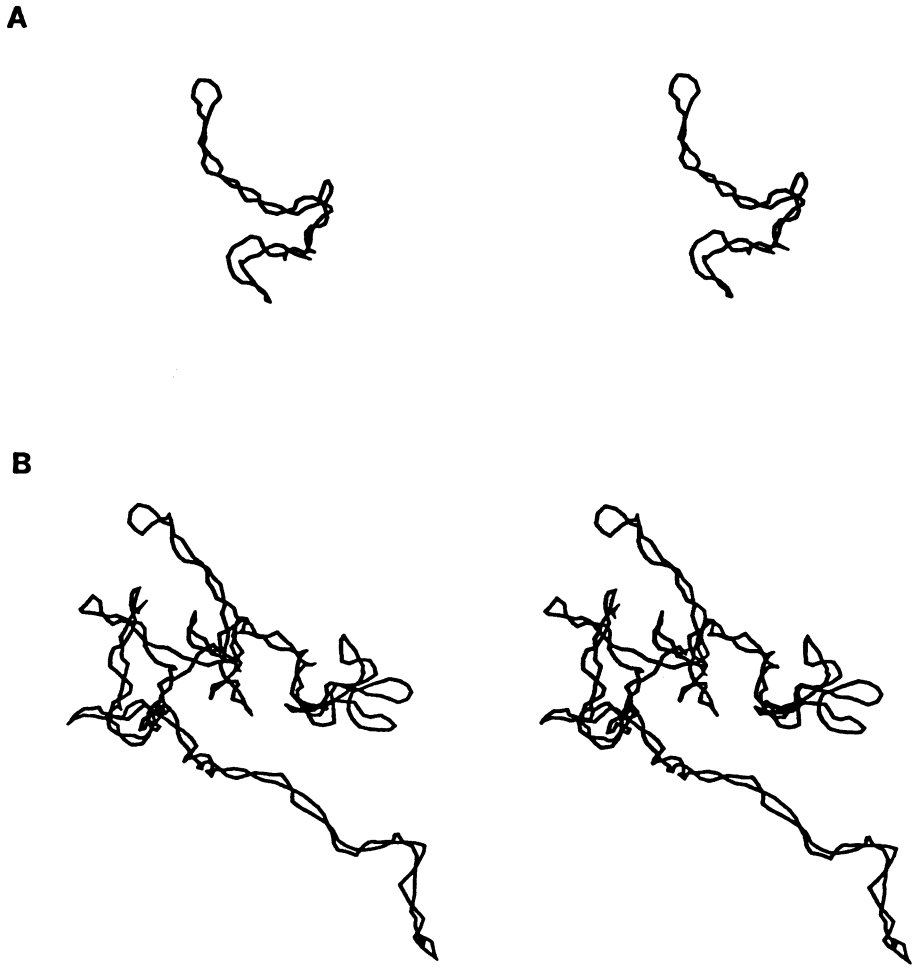

FIGURE 3 Stereoscopic views of supercoiled DNA. The DNA in *A* is 3.5 kb in length, and in *B* it is 15 kb long. σ is -0.05 in both cases.

let us consider a regular superhelix with one branch point and three branches of equal length. Two sites never juxtapose across the superhelix axis if $1/3L < l < 2/3L$. Moreover, the probability of site juxtaposition will be the same for all pairs with $l < L/3$. Indeed, in this case each segment is juxtaposed to a distant segment three times as it completes one cycle of sliding along the chain conformation. Therefore, the length of a branch is the critical distance for site juxtaposition in the regular model of the superhelix.

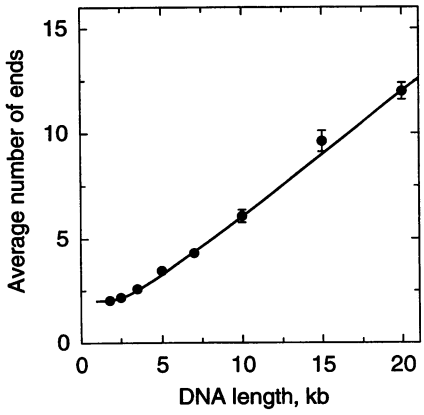

FIGURE 4 The branching frequency of supercoiled DNA as a function of molecular length. The average number of superhelix ends was computed for a σ of -0.05. The error bars represent the standard deviation and, where not shown, are less than the diameter of the symbol.

There is, however, no critical value of l for an equilibrium conformational set because the amount, length, and relative arrangement of branches vary strongly. As a result, the average probability of site juxtaposition decreases as l increases (Fig. 5). Comparison of the probabilities of site juxtaposition for relaxed and supercoiled DNA shows interesting features. The probability of site juxtaposition for relaxed DNA decreases with l at about the same rate as the probability for supercoiled DNA (Fig. 5). Therefore, the ratio of the probabilities of site juxtaposition for supercoiled and relaxed DNA does not vary with l and remains at about 100. Thus, the superhelix makes a major contribution to site juxtaposition for all sites, no matter how far apart they are. Conversely, the juxtaposition of sites not across the superhelix axis is rare for any l. The probability of such juxtaposition, taking place by the close approach of different branches, should be about equal to the probability of site juxtaposition in relaxed DNA.

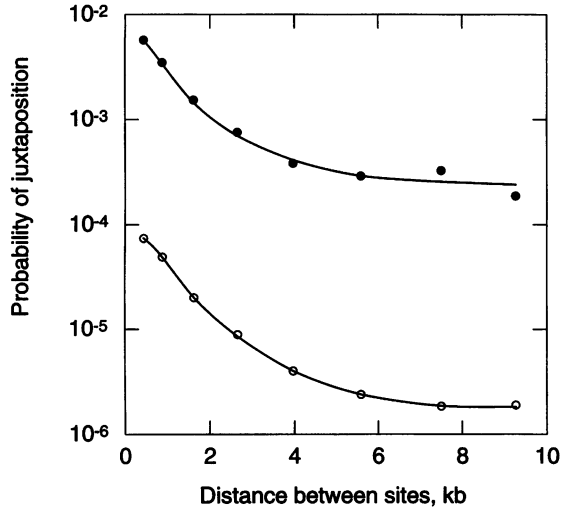

FIGURE 5 The probability of site juxtaposition as a function of site separation along the chain contour. The calculations were done for DNA 20 kb in length. The probabilities for relaxed DNA (○) and supercoiled (●) DNA with a σ of -0.05 are shown.

The effect of supercoiling on relative segment orientation

The binding surfaces in a protein complex for two DNA sites are relatively fixed in spatial orientation. Consequently, for two sites to form a DNA-protein complex, they must, in general, be brought close together at a particular angle. Thus we must consider the effect of supercoiling on the angular distribution of sites as well as on site juxtaposition. Let us first consider which relative orientations of two sites are possible and introduce some nomenclature.

Because DNA sites that interact with proteins are short in comparison with the DNA persistence length, they can be approximated by straight segments. Formation of the complex can then cause bending, wrapping, or unwinding of DNA, but we need not consider this stage now. Because specific sites also have a directionality provided by their sequence, we can consider sites as vectors. For any two vectors there is a plane parallel to both of them. We define the angle between the sites as the angle between the projections of the vectors onto this plane. We measure this angle, ϕ, from the underlying vector in a counterclockwise direction to the overlying vector, as shown in Fig. 1.

We note three properties of this definition:

1. The value of the angle is unchanged when viewed from the other side of the plane.
2. The value of the angle is not changed if the directions of both vectors are switched.
3. The orientation of the vectors is physically distinguishable for ϕ in the range of 0° to 360°.

We note that in an a review (Vologodskii and Cozzarelli, 1994) we restricted the range of angles from 0° to 180° to analyze the site orientation. The current procedure involving a 360° range is more accurate.

It has been shown by electron microscopy that the superhelix winding angle averages about 55° (the value of ϕ equals 125°), independently of σ over the range -0.03 to -0.06 (Boles et al., 1990). This suggests that the relative orientation of juxtaposed sites in the interwound superhelix might have a rather narrow distribution. To determine this, we calculated the distribution as a function of superhelix density.

In calculating the angular distributions between juxtaposed segments, all segments were given the same direction relative to the contour. For each segment of the chain we found the closest segment to it. If the distance between the segment centers was less than 10 nm, we calculated the angle between the segments according to the algorithm described in Methods of Calculations. Each value found contributed to the angular distribution, $P(\phi)$, which is shown in Fig. 6 for relaxed and supercoiled DNA. Also shown in this figure is the angular distribution for independent vectors. Because these are oriented randomly in space, their distribution is symmetrical. This symmetry is disturbed a little for juxtaposed segments of a relaxed DNA. For supercoiled DNA the distribution is strikingly asymmetric. It has a maximum in the range of 120°, which corresponds to an average superhelix winding angle of 60°, very close to the value calculated from EM.

Fig. 6 also shows the dependence of $P(\phi)$ on superhelix density. It is interesting that even very low supercoiling ($\sigma = -0.01$) results in a notable asymmetry of the distribution, even though a more or less regular interwound superhelix requires a σ of -0.03 or less. Thus, $P(\phi)$ is a very sensitive indicator of the change in conformational properties induced by supercoiling. It also suggests that even low levels of supercoiling can have significant biological consequences through effects on DNA conformation.

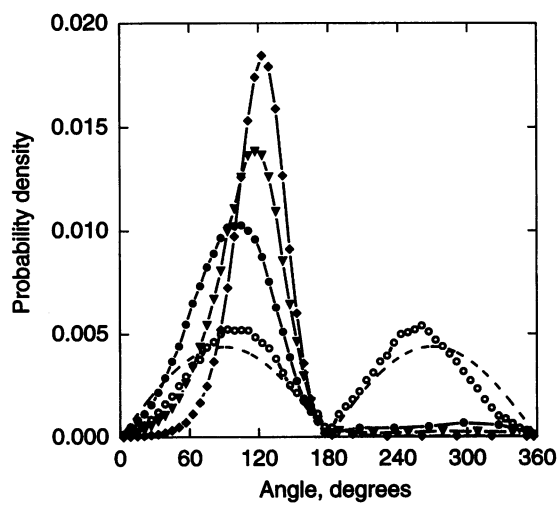

FIGURE 6 The angular distribution between juxtaposed segments of circular relaxed and supercoiled DNA. The angle between all segments within 10 nm of each other but separated by more than 1 kb along the DNA contour was calculated. The data were computed for a circular DNA 3.5 kb in length that was either supercoiled (σ of -0.01 (●), -0.03 (▼), -0.05 (◆)) or relaxed (○). For comparison, the distribution for random vectors is shown by the thin dashed line.

The relative segment orientation in supercoiled DNA is nearly independent of DNA length and of the distance between sites, l (data not shown). This is because for all values of l, sites across the superhelix axes contribute mainly to site juxtaposition, and the local structure of an interwound superhelix does not depend on DNA length.

DISCUSSION

Conformations of supercoiled DNA are sensitive to ionic conditions (Bednar et al., 1994; Vologodskii and Cozzarelli, 1994). We did almost all of the calculations for a solution containing 200 mM monovalent salt and up to 10 mM magnesium ions. In this range of ionic conditions, the parameters of the DNA model used in the simulations have practically the same values (Rybenkov et al., unpublished data). These are the ionic conditions that are usually used for enzyme reactions involving DNA. We did almost all of the calculations for a solution containing 200 mM monovalent salt, a concentration in the physiological range. We found that the effect of supercoiling on site juxtaposition decreased only slightly if the salt concentration was reduced to 10 mM (data not shown). Therefore, changes in ionic conditions will not significantly affect site juxtaposition as long as the DNA remains an interwound superhelix.

Relative site positioning is important for transactions in which two or more DNA sites participate simultaneously. In light of our results, we will consider next two types of such reactions, site-specific recombination and the change of DNA topology by topoisomerases.

Site-specific recombination involves the cleavage of selective DNA sequences and their religation in a rearranged order. The related process of transposition involves the movement of a particular sequence, a transposon, from one chromosomal location to another. These processes are usually studied with specially constructed short circular DNA molecules with specific sites and topology (Wasserman and Cozzarelli, 1986). In many of these cases, supercoiling is essential for formation of the synaptic complex, in which the specific DNA sites are held together by recombination proteins (Nash, 1990; Kanaar and Cozzarelli, 1992). Synapsis involves the juxtaposition of either two specific sites (resolvase, Int, Cre, Flp, and Fis-independent mutant Gin recombination) or three specific sites, two recombination sites, and an enhancer (Gin and Hin recombination, Mu transposition). The general rule is that supercoiling is essential for synapsis in some of the systems with two sites, but it is always necessary for the synapsis of three specific sites. To analyze these data, let us consider in more general terms why synapsis can be dependent on DNA supercoiling.

Synapsis requires an alignment of DNA sites in a particular local conformation. If the free energy of formation of the appropriate site conformation, ΔF_s, in linear DNA is not too high, then synapsis will result with linear molecules. This occurs in the simplest cases in which only two specific sites participate in synapsis and the conformational requirements are not extensive. Although supercoiling can increase the rate of synapsis in these cases, it is not necessary for complex formation. Site-specific recombination by Cre, mutant Gin, and Int (excision) are examples (Nash and Pollock, 1983; Klippel et al., 1988; Hoess and Abremski, 1990). There is evidence that synapsis does not require the wrapping of DNA around such proteins. The local conformation of DNA sites required for synapsis may, however, be quite complex, even when only two sites participate in synapsis. For example, the resolvase synaptic complex contains three interwound negative supercoils (Wasserman et al., 1985). As a result, ΔF_s will be high and the probability that the sites take the required conformation will be low. Indeed, resolvase synapsis is not observed for linear or nicked circular DNA (Benjamin and Cozzarelli, 1988).

The value of ΔF_s is expected to be higher if three specific DNA sites participate in synapsis, because the probability of their random juxtaposition in linear or relaxed DNA is very low. It is not surprising, therefore, that synapsis is not observed in such cases for linear DNAs (Heichman and Johnson, 1990). The mutation in the Gin system that reduces the requirement from three sites to two simultaneously makes recombination independent of supercoiling (Klippel et al., 1988; Crisona et al., 1994).

Our simulation results show two ways that supercoiling can decrease the free energy cost of synapsis. The first gain comes from the increase in probability of site juxtaposition. We showed that moderate and high supercoiling increases the probability of juxtaposition of two sites by about a factor of 100. Our initial calculations of the effect of supercoiling on the juxtaposition of three sites have given an increase of more than three orders of magnitude with supercoiled DNA ($\sigma = -0.06$) over that with relaxed DNA (data not shown). The second gain is from changing the local conformations of the juxtaposed sites by supercoiling. We calculated the angular distribution between two juxtaposed sites as a function of supercoiling. The increase of the probability over relaxed DNA for a particular range of the angles never exceeds a factor of 5 (Fig. 6). Thus, the effect of supercoiling on angular dependence cannot greatly decrease the free energy cost of synapsis. However, the conformational requirements for synapsis can be much more complex than only a certain value of the angle between the juxtaposed sites. For example, the local conformation of the sites in Gin synapsis may be close to that at a branch point in an interwound superhelix (Kanaar et al., 1988).

The asymmetric angular distribution between juxtaposed sites induced by supercoiling might play an important role in the specificity of certain DNA transactions. All nonpalindromic DNA sites have an intrinsic orientation. This orientation is usually important because it imposes an orientation on bound proteins. Two identical sites within the same DNA molecule can have either a direct or an inverse relative orientation (Fig. 7 *A*). Some enzymes, such as resolvase, recombine directly repeated sites but not inverse sites (Stark et al., 1989; Bliska et al., 1991); others such as phage mu Gin are competent only with inverse sites (Stark

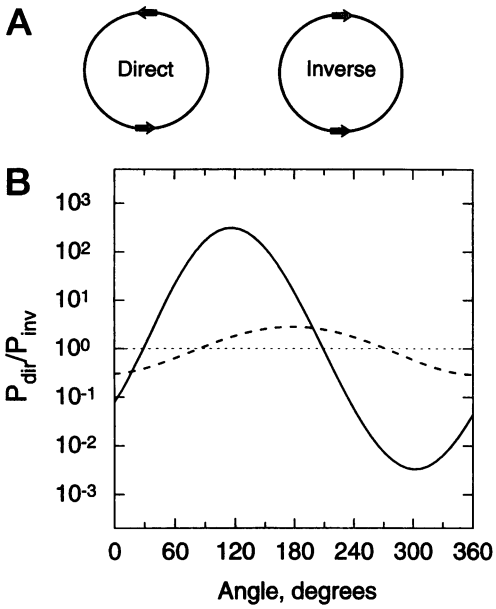

FIGURE 7 Comparison of the angular distributions between juxtaposed segments for direct and inverse site orientations in a DNA chain. (*A*) Definition of the direct and inverse site orientations. (*B*) The ratio of the probabilities that direct and inverse sites will be found at a given angle is equal to and is plotted for supercoiled DNA, σ of -0.05 (*solid line*), and relaxed DNA (*bold dashed line*). For comparison, the ratio for random vectors is shown by the thin dashed line. The data of Fig. 6 were used to calculate these ratios.

and Boocock, 1995). There is good evidence that the discrimination in both classes depends on the restriction of DNA conformations imposed by negative supercoiling (Craigie and Mizuuchi, 1986; Kanaar and Cozzarelli, 1992; Stark and Boocock, 1995).

Using the data of Fig. 6, we can now compute the effect of supercoiling on the discrimination between direct and inverse sites as a function of the angle between these sites. Let us suppose that the orientation required corresponds to the angle θ between the sites. The angle θ corresponds to ϕ for direct sites and to $\phi + 180°$ for inverse sites. Therefore, the ratio of probabilities of having an angle θ between sites in direct and inverse orientation equals $P(\phi)/P(\phi + 180°)$. These are plotted in Fig. 7 *B*. For random vectors, this ratio is 1 for all θ. For relaxed DNA, the ratio remains close to 1. For supercoiled DNA the ratio reaches 300 and is strongly dependent on θ. It is greatest at 120° and 300°. This result clearly shows that substrate DNAs with direct and inverse site orientation can be easily distinguished if the DNAs are supercoiled.

Even with supercoiled DNA the ratio is not large enough to make synapsis impossible for unfavorable site orientations. The magnitude of the ratio is restricted by the contribution of juxtaposed sites located in different branches of the superhelix and juxtaposition from looping of a branch. The juxtaposition of such sites has a nearly symmetric distribution of relative orientations, and their contribution is about 1/300 of sites within a branch (data not shown). The random collision of such sites provides, probably, the required structures for the systems with rather simple geometrical requirements. Cre, mutant Gin, and excisive Int recombination are examples (Nash, 1983; Klippel et al., 1988; Hoess and Abremski, 1990; Crisona et al., 1994). For these systems, recombination occurs with sites in direct or inverse orientations in both supercoiled and relaxed DNA.

The effect of the site orientation along the DNA contour should be more important when there are more complex structural requirements than just a certain angle between the sites, as for Tn3/$\gamma\delta$ resolvase (Wasserman et al., 1985; Benjamin and Cozzarelli, 1990; Stark and Boocock, 1995), Gin-mediated inversion (Kanaar et al., 1988, 1990), and Hin-mediated inversion (Heichman et al., 1991). Random collisions of sites on different DNA branches in a supercoiled molecule cannot provide such structures with detectable frequency. Thus, only one orientation of the sites provides a substrate for these reactions.

There is one special case, λ Int integrative recombination (Landy, 1989; Nash, 1990). As in the simpler systems, direct and inverse orientations of the recombination sites *att*B and *att*P are equally suitable for the reaction. Although supercoiling is required for the synapsis in this case, it is needed only for assembling the complex at *att*P, by promoting DNA bending at the site (Landy, 1989; Nash, 1990). Because such bends are usually located at superhelix apices (Laundon and Griffith, 1988; Kremer et al., 1993; Klenin et al., 1995), juxtaposition with *att*B should take place mainly through collision with a different branch of the superhelix. Therefore the angular distribution between the juxtaposed sites should be nearly symmetric, even in supercoiled DNA, and direct and inverse sites are equally productive.

Our simulation results are also relevant for the mechanism of negative supercoiling of circular DNA by DNA gyrase. According to the current model for supercoiling by gyrase, DNA is wrapped around the protein, thereby introducing a positive writhe (Fig. 8 *A*) (Reece and Maxwell, 1991). Gyrase then cleaves the wrapped DNA segment, passes another DNA segment through the break site, and reseals the break (Fig. 8 *A*). The consequence is a change in linking number of -2 (Brown and Cozzarelli, 1979). However, it was noted by Kirchhausen et al. (1985) that additional information must be present to account for the topological specificity of gyrase. Our results illustrate the problem quantitatively. It follows from Fig. 6 that any two orientations of the passing segment that differ by 180° should have a similar probability in relaxed DNA. As a consequence, gyrase should knot DNA (as shown in Fig. 8 *B*) as well as supercoil it. Because knotting is not observed experimentally, we conclude that gyrase controls the orientation of the passing segment relative to the wrapped segment. The only easy way to do so is to wrap both DNA segments around the enzyme (Kirchhausen et al., 1985). We also must suppose that there is a less favored mode of enzyme action that does not require full contact of the passing segment. This mode provides for such reactions as catenation, decatenation, and unknotting of circular DNA.

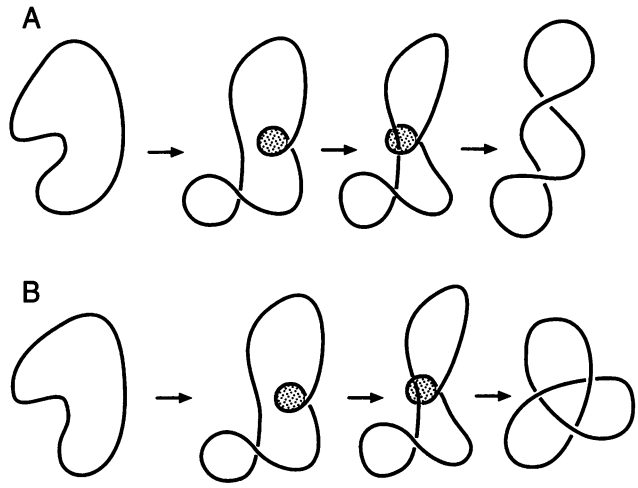

FIGURE 8 DNA topology changes by DNA gyrase. The DNA is indicated by a bold line and the enzyme by the stippled ball. In the first step the DNA winds around the enzyme in a positive supercoil. Both (−) supercoiling (*A*) and knotting (*B*) will result if the enzyme cannot distinguish the relative orientation of the passing and gate segments.

Indeed, direct measurements show that these last three reactions by gyrase are far less rapid than the introduction of negative supercoils (Marians, 1987).

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