Computational Analysis of DNA Gyrase Action

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ABSTRACT DNA gyrase introduces negative supercoiling into circular DNA by catalyzing the passage of one DNA segment through another. The efficiency of the reaction is many times higher than that of other topological transformations. We analyze, by a computer simulation, the reaction selectivity for a model of DNA gyrase action that assumes existence of a free loop between the G- and T- DNA segments participating in the reaction. A popular model of this type assumed that the selectivity can be provided by the conformation of the DNA segment wrapped around the enzyme into the right-handed helix turn (G-segment). We simulated the distribution of the reaction products for this model. Equilibrium sets of DNA conformations with one segment of the double helix wrapped around the enzyme were constructed. From these sets we selected conformations that had a second segment properly juxtaposed with the first one. Assuming that the juxtapositions result in the strand-passing reaction, we calculated the reaction products for all these conformations. The results show that different products have to be formed if the enzyme acts according to the model. This conclusion can be extended for any model with a free loop between the G- and T-segments. An alternative model that is consistent with the major experimental observations and the computational analysis, is suggested.

INTRODUCTION

DNA gyrase is a bacterial enzyme that introduces negative supercoiling into circular DNA (Gellert et al., 1976). It belongs to the type II DNA topoisomerases that catalyze passing one double-stranded DNA segment through another one. The free energy required for supercoiling comes from ATP hydrolysis coupled with the strand-passing reaction. The enzyme is important for maintaining a certain level of DNA supercoiling inside bacterial cells and for DNA replication (Levine et al., 1998; Reece and Maxwell, 1991; Wang, 1996).

The active form of the *Escherichia coli* enzyme consists of two A and two B subunits (reviewed by Reece and Maxwell, 1991). For many years it was accepted that the tetramer binds a segment of double-stranded DNA ~140 bp in length that is supposed to wrap around the enzyme forming approximately one turn of the right-handed helix (Fisher et al., 1981; Kirkegaard and Wang, 1981; Liu and Wang, 1978a). The bound DNA segment, named the gate (G) segment, is cleaved approximately in the middle, and the 5′ ends of the broken strands are covalently attached to the protein (Fig. 1 *A*). Another DNA segment, the so-called transporting (T) segment, then passes through the double-stranded break and the break is resealed (Reece and Maxwell, 1991; Wang, 1998). This model of the G-segment conformation is supported by the results from nuclease protection experiments (Liu and Wang, 1978a; Morrison and Cozzarelli, 1981) as well as by DNase I (Fisher et al., 1981; Kirkegaard and Wang, 1981) and hydroxyl radical footprinting (Orphanides and Maxwell, 1994). It has been shown that binding DNA gyrase to nicked DNA in the absence of ATP increases the linking number of the DNA strands, measured after ligation of the nicks (Kampranis et al., 1999; Liu and Wang, 1978b; Peng and Marians, 1995). Quantitative analysis of these experiments showed that each complex between the enzyme and DNA increases the linking number in the ligated molecules by 0.5–0.8. These data are considered to provide additional support for the model. If both the G- and T-segments belong to the same closed circular DNA, each strand-passing reaction results in the change of the linking number (Lk) of DNA complementary strands by two (Brown and Cozzarelli, 1979). If the T-segment belongs to second circular DNA, the reaction can link or unlink the molecules.

There are data, however, that do not agree with this model. The strongest result of this kind, obtained by Bates and Maxwell (1989), is the ability of the enzyme to introduce supercoiling in the very small DNA circles, 174 bp in length. Clearly, in this case, the strand passing cannot follow the model simply because the contour length of the circles is insufficient to form the starting conformation (Fig. 1 *A*). Indeed, if ~140 bp are wrapped around the enzyme, there are only ~40 bp available to make the external loops. Recent data obtained by atomic force microscopy are also in certain disagreement with the model (Heddle et al., 2004). For us, however, this is an example of a model in which the G- and T-segments are separated by a free DNA loop. The major conclusion of the work will be essentially the same for any model of this type. In the alternative type of models the G- and T-segments belong to one DNA stretch whose conformation is completely specified by the interaction with DNA gyrase.

Although DNA gyrase can catenate, decatenate, and unknot circular DNA molecules (Kreuzer and Cozzarelli, 1980; Mizuuchi et al., 1980), the efficiency of these reactions is very low (Marians, 1987; Ullsperger and Cozzarelli, 1996). The major catalytic activity of the enzyme is selective

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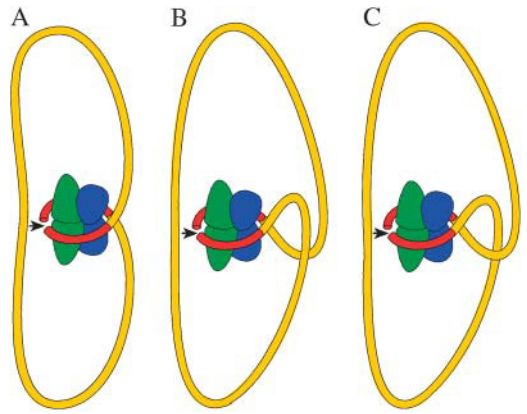

FIGURE 1 The model of DNA gyrase action. The enzyme wraps a DNA segment, G-segment (*red*), around itself. Thermal motion brings another segment, T-segment, into the entrance gate of the enzyme and the segment passes through the temporary cleaved G-segment, then the break is resealed (Wang, 1998). Depending on DNA conformation that precedes the T-segment entering, the reaction can result in creating two negative supercoils (from conformation in panel *A*), two positive supercoils (*B*), or creating positive trefoil knot (*C*) and even more complex products.

introduction of negative supercoils into circular DNA. The right-handed helix turn, formed by G-segment, has been assumed to provide this selectivity (Fig. 1 *A*). However, DNA molecules of a few thousand base pairs in length adopt many different conformations in solution, and it is hard to believe that a small chiral loop can provide the required selectivity. Indeed, other types of conformations (with a potential T-segment juxtaposed with the enzyme gate) would result in different topological changes. In particular, the outcome from two conformations shown in Fig. 1, *B* and *C*, would be the introduction of two positive supercoils and formation of a positive trefoil. None of these products has been observed as a reaction product under conditions optimal for supercoiling. Thus, we suggested that the model might not account for the experimentally observed selectivity of the enzyme action. In this article we analyze this question quantitatively by computer simulation.

There is convincing evidence that computer simulations provide accurate quantitative description of the large-scale conformational properties of DNA. Simulations reproduce experimental data on hydrodynamic properties of DNA molecules (Hagerman, 1981; Hagerman and Zimm, 1981; Rybenkov et al., 1997d), DNA cyclization (Hagerman, 1990; Levene and Crothers, 1986; Taylor and Hagerman, 1990; Vologodskaia and Vologodskii, 2002), equilibrium distributions of topological states (Klenin et al., 1988, 1989; Rybenkov et al., 1993, 1997c; Shaw and Wang, 1993; Vologodskii and Cozzarelli, 1993), elasticity of single molecules (Vologodskii, 1994; Vologodskii and Marko, 1997), and light and neutron scattering data on supercoiled DNA (Gebe et al., 1996; Hammermann et al., 1998, 1997; Klenin et al., 2000). The simulations are based on the statistical-mechanical treatment of a well-established model of the double helix. There are only three parameters in the model and all of them have been reliably determined for various solution conditions. Thus, the simulations are able to provide reliable quantitative information on many DNA properties that are hard to measure experimentally. The simulations are very useful for the analysis of enzymatic reactions that involve formation of DNA loops (Grainge et al., 2002; Klenin et al., 2002; Vologodskii et al., 2001). Here we apply the simulation to estimate the distribution of the reaction products corresponding to the model of DNA gyrase shown in Fig. 1. The results leave no doubt that the model is inconsistent with selective formation of negative supercoils by DNA gyrase.

The simulation results allow us to formulate a general requirement for a correct model of the enzyme. Models that satisfy this requirement were suggested by Kampranis et al. (1999) and recently by Corbett et al. (2004). We discuss and elaborate important details of such a model.

METHODS OF COMPUTATION

DNA model and simulation procedure

Circular DNA was modeled as a discrete worm-like chain consisting of N rigid cylinders of equal length with harmonic potentials with respect to the angles between adjacent segments and the value of torsional deformation, ΔTw (Klenin et al., 1991; Vologodskii et al., 1992). Electrostatic interactions between the segments were taken into account through a hard core potential specified by DNA effective diameter (Vologodskii and Cozzarelli, 1995; Vologodskii et al., 1992). The equilibrium ensemble of chain conformations was simulated by the Metropolis procedure (Vologodskii et al., 1992). Each simulation was performed for the chains with a particular topology and ΔLk value. To preserve the chain topology during a simulation run we used a topological invariant, the Alexander polynomial, $\Delta(t)$. A topological invariant has the same value over all conformations with a particular topology. We calculated $\Delta(t)$ for $t = -1$ and $t = -2$ after each move of the Metropolis procedure (Vologodskii et al., 1974). If the value of $\Delta(-1)$ or $\Delta(-2)$ were different for the current conformation and trial conformations, the trial conformation was rejected.

A rigid subchain of five straight segments that form nearly one turn of the right-handed helix corresponded to the G-segment bounded with DNA gyrase (Fig. 2 *A*). The geometry of the subchain did not change during the simulation.

The subsets of conformations, having a segment juxtaposed with the G-segment, were selected from the constructed equilibrium sets for the analysis of potential outcome of the strand-passing reaction.

Parameters

All computations were performed for a DNA persistence length of 50 nm (Hagerman, 1988), a torsional rigidity, C, of 3.0×10^{-19} erg·cm (Horowitz and Wang, 1984; Klenin et al., 1989), and a DNA effective diameter, d, of 5 nm. The later value of d corresponds to a 0.2 M solution of NaCl (Brian et al., 1981; Rybenkov et al., 1993, 1997b; Stigter, 1977). The length of the straight segments was equal to 10 nm.

Analysis of the simulated conformations

A segment of the chain was considered to be juxtaposed with the G-segment if all of the following conditions were satisfied:

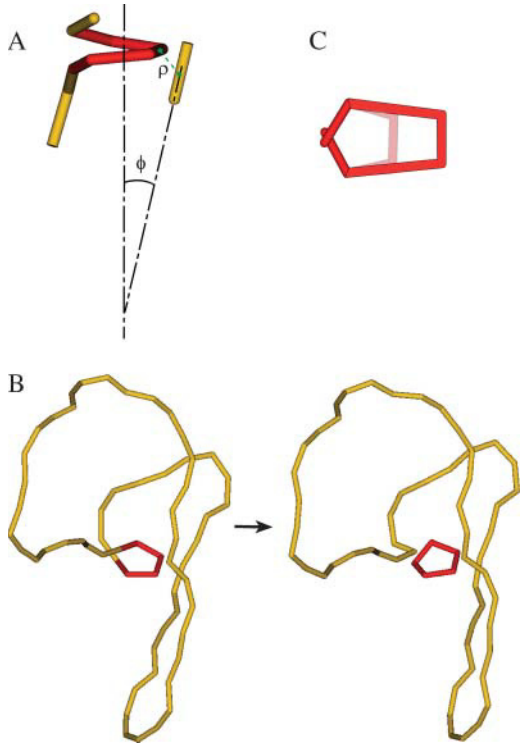

FIGURE 2 Analysis of the simulated conformations. (*A*) Juxtaposition of the G-segment with a potential T-segment. It is assumed that the G-segment is bounded with the enzyme and thus has a rigid conformation. The G-segment consists of five straight segments (*red*) that form approximately one turn of the right-handed helix. A segment (*yellow*) is treated as a potential T-segment if the distance ρ and the angle ϕ are less than ρ_o and ϕ_o, correspondingly. (*B*) Topological test of the juxtaposition. Two closed contours were formed by closing the ends of the G-segment and by closing the remaining part of the model chain. The closings of both parts of the chain were done by small deformations of the end segments. It was then tested that the contours are not linked. (*C*) Extension of the G-segment during the analysis of simulated conformations. The conformation of the G-segment was extended by doubling the length of the second and fourth straight segments of the loop and keeping the length and direction of the third straight segment. The extended and original conformations are shown by bright and dim red, correspondingly.

1. The distance between the middle of the testing segment and the middle of the G-segment contour was less than a distance ρ_o (Fig. 2 *A*);
2. The angle ϕ ($0° \leq \phi \leq 90°$) between the tested segment and the axis of the helix, which specifies the orientation of the G-segment, did not exceed ϕ_o (Fig. 2 *A*);
3. There is no linkage between small closed contour formed by closing the ends of the G-segment and the contour formed by closing the rest of the chain without the G-segment (Fig. 2 *B*). To test this we calculated the Alexander polynomial for these two closed contours, $\Delta(s,t)$, for $s = -1$, $t = -1$ (Vologodskii et al., 1975). This test excluded conformations when the tested segment was inside the loop formed by the G-segment.
4. To determine the product that could be obtained by the strand-passing reaction from a particular conformation of the chain, the G-segment was extended as shown in Fig. 2 *C*. We tested that two circular contours, created by closing the extended G-segment and by closing the chain with the excluded G-segment, form the simplest topological link. This was done by calculating $\Delta(-1, -1)$. If the link did not appear, we considered that there is no juxtaposition. Such cases were very rare, however.

Although the probabilities of juxtaposition depend on the values chosen for ρ_o and ϕ_o, for sufficiently small ρ_o and ϕ_o the product distributions do not depend on their choice. In most of the calculations we used $\rho_o = 7.5$ nm and $\phi_o = 35°$.

If a particular conformation of the chain passed all tests for the juxtaposition, we determined the potential reaction product by calculating the change of chain writhe, δWr, and the values of $\Delta(-1)$ or $\Delta(-2)$ after the extension of the G-segment shown in Fig. 2 *C*. The Alexander polynomial, however, does not distinguish between a knot and its mirror image. To solve this problem, we used δWr (Kleninet al., 2002). It can be proven that, if the substrate is an unknotted conformation and the product is a trefoil, then the ''sign'' of the product coincides with the sign of δWr. Similarly, if the substrate is a trefoil and the product is an unknot, then the ''sign'' of the substrate is opposite to the sign of δWr (see, e.g., Murasugi, 1996; in particular Example 6.4.2 and Theorem 6.4.7).

RESULTS

We assume that the reaction catalyzed by gyrase is not diffusion limited, and thus can be analyzed in terms of the equilibrium distribution of DNA conformations. This assumption seems valid for the majority of enzymatic reactions. An initial state for the simulation corresponds to the complex between gyrase and DNA in which a segment of circular DNA is wrapped around DNA gyrase. We assume that the complex is ready to absorb another DNA segment, the T-segment. We also assume that the gate for the T-segment is located in the middle of the wrapped G-segment. The conformation of the G-segment is kept unchanged during the simulation. Conformations of the rest of the circular DNA are sampled to simulate the equilibrium distribution. We select from the constructed set of conformations those that have a segment properly juxtaposed with the assumed gate located in the middle of the G-segment (Fig. 3). Each of these conformations has equal probability to become a subject of the strand-passing reaction. This is true because the distribution of DNA conformations is a continuous function and, thus, the probabilities to find a segment in any two very close positions are nearly equal. We imitate the strand-passing reaction for each of the selected conformations and calculate the topological state of the product (see ''Methods of Computation''). The topological state of circular DNA is specified by two parameters, the linking number difference of the complementary strands, ΔLk, and by the topology of DNA axis, which can be unknotted or form various knots. Averaging the results of such analysis over a large volume of sampled conformations gives us the desired distribution of the reaction outcomes.

First, we calculate the distributions for relaxed ($\Delta Lk = 0$) unknotted chains. Fig. 4 *A* shows the results obtained for DNA molecules of different lengths. One can see from the figure that introduction of two (−) supercoils would be the dominant reaction outcome only for DNA molecules shorter than 2000 bp. For molecules of 3000 bp or longer, formation of positive trefoils should be comparable with the introduction of negative supercoiling. Clearly, for the relaxed molecules of these lengths the chirality of the G-segment

FIGURE 3 Typical simulated conformation of DNA 3.5 kb in length with a segment forming a complex with DNA gyrase. The G-segment, shown by red, is assumed to be wrapped around the enzyme (not shown). The conformation of the segment was kept unchanged during the simulation whereas conformations of the rest of the model chain were sampled according to the equilibrium distribution. There is another segment in the shown conformation, which is juxtaposed with the break (not shown) in the middle of the G-segment. If the juxtaposed segment were a T-segment, the strand-passing reaction for the shown conformation would result in the introduction of two negative supercoils.

does not provide the selectivity of reaction outcomes comparable with what is observed experimentally.

One might suggest that the positive trefoils formed after the first strand-passing reaction will be unknotted in the second round of the process. To test this possibility we simulated the product distribution formed from positive trefoils (Fig. 4 *B*). The results of this simulation show that the second strand-passing reaction would rarely return the model chains to an unknotted state. In most cases the second reaction results in formation of more complex knots.

Fig. 5 shows the product distributions obtained from negatively supercoiled molecules. The results were obtained for a circular DNA 3500 kb in length. Although for this DNA length negative supercoiling is the major reaction outcome for all tested initial values of supercoiling, the total fraction of other products remains close to 30%. The distribution of the other products changes, however, with the increase of (−) supercoiling. Surprisingly, the fraction of positive trefoils, which was the second largest fraction for the relaxed chains (Fig. 4 *A*), declines rapidly as (−) supercoiling increases. Instead, an increase of *Lk* by (+2) becomes the second major outcome of the reaction.

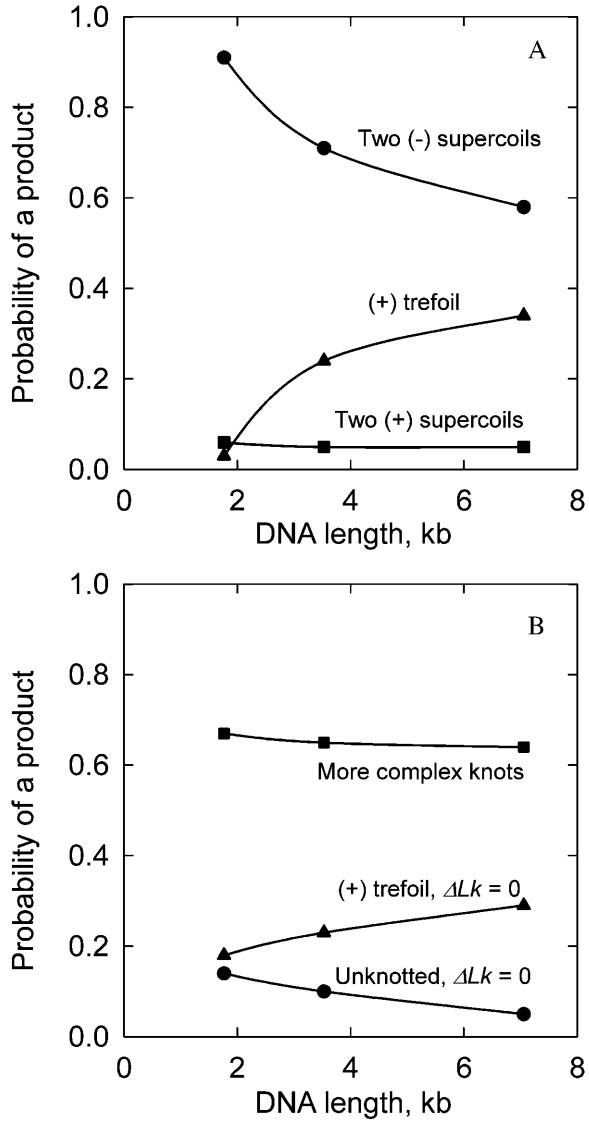

FIGURE 4 Computed distributions of the reaction products for the gyrase model. The data correspond to one strand-passing reaction in each DNA molecule. (*A*) The product distributions for initially relaxed unknotted molecules of different lengths. (*B*) The product distributions for the molecules whose initial state corresponds to positive trefoil with $\Delta Lk = 2$. This state is the second major product obtained from relaxed unknotted molecules.

DISCUSSION

The simulation results clearly show that the model of DNA gyrase action shown in Fig. 1 cannot explain the high selectivity of the strand-passing reaction catalyzed by the enzyme. If DNA gyrase were to act according to this model, it would produce a large fraction of knotted molecules. Because negatively supercoiled molecules overwhelmingly dominate the population of DNA gyrase products, we have to conclude that the model presented in Fig. 1 is incorrect. The failure of the model to provide a unique reaction outcome results from the assumption that there are free loops between the G- and T-segments that do not interact with protein. Conformations of these loops have to depend on the properties of the double helix only. Because DNA is a sufficiently flexible molecule on the scale of thousands of basepairs, the loops accept many different conformations that correspond to different products of the strand-passing reaction. Negative supercoiling can represent the overwhelming reaction outcome only if the

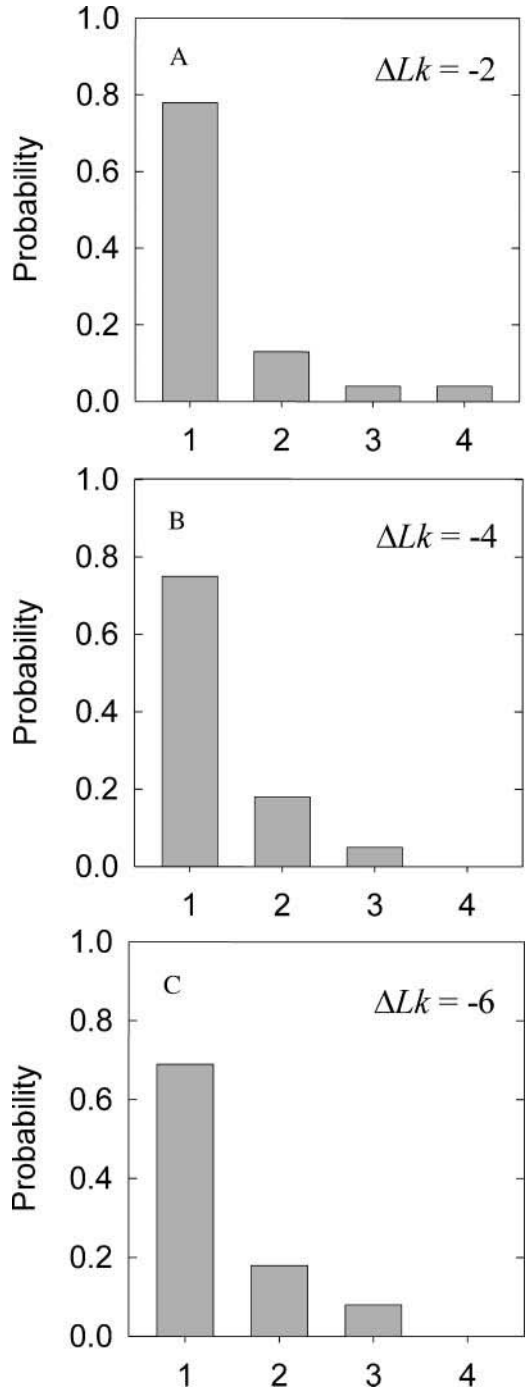

FIGURE 5 Computed distributions of the reaction products for the gyrase model. The distributions were obtained for initially unknotted molecules 3.5 kb in length with ΔLk of -2 (*A*), -4 (*B*), and -6 (*C*). In each panel the bars correspond to adding two (−) supercoils and keeping the unknotted state (bar *1*), adding two (+) supercoils and keeping the unknotted state (bar *2*), forming negative trefoil and decreasing *Lk* by two (bar *3*), and forming positive trefoil and increasing *Lk* by two (bar *4*). The product distributions correspond to one strand-passing reaction in each molecule.

G- and T-segments belong to one DNA stretch whose conformation is completely specified by the interaction with DNA gyrase. Models of this kind were suggested by Kampranis et al. (1999) and recently by Corbett et al. (2004). Below we elaborate on a version of such a model with some additional features.

What reliable experimental data have to be explained by a realistic model of the enzyme action? Wrapping the G-segment around the enzyme seems to be an established feature of the complex. A model also has to account for the fact that the double-stranded break is introduced nearly in the middle of DNA segment bound to the enzyme (reviewed in Reece and Maxwell, 1991). Additional strong restriction for a model imposes the finding that the enzyme is able to introduce supercoiling into very small DNA circles, 174 bp in length (Bates and Maxwell, 1989). We believe that the simplest and perhaps the only way to fit all of the above requirements for DNA minicircles corresponds to the diagram shown in Fig. 6. Here the T-segment enters the gate from inside rather than from outside the loop formed by the G-segment. This allows the G- and T-segments to be combined into a single stretch that interacts with the enzyme along its entire length. If the enzyme has twofold symmetry, as is usually assumed, the pathway of the short DNA circle keeps (or nearly keeps) this symmetry during the strand-passing reaction in the model. To generalize the model for longer DNA molecules we extend one of the two short internal loops formed by the G- and T-segments outside the enzyme surface (Fig. 7). Conformations of the DNA stretch interacting with the protein during the strand-passing reaction are completely specified by this interaction, and this provides a unique reaction outcome. It is important to note that even a small free loop between the G- and T-segments suggested by Kampranis et al. (1999) as an alternative to the bound loop, cannot exclude other outcomes of the reaction. Indeed, there is no way to restrict the size and conformations of the free loop if its pathway is not controlled by DNA-protein interaction.

The model shown in Fig. 7 *A* does not fit the requirement of the symmetry in the complex, however. More than this, half of the G-segment does not play an active role in the reaction, and it is not clear why its extensive interaction with the protein is needed. To overcome this problem we suggest that either of two halves of the enzyme can interact with the stretch of G- and T-segments (see Fig. 7). This would make both sides of the bound segment equivalent for footprinting or nuclease digestion. Of course, in any particular DNA stretch bound with the enzyme, the DNA sequence can determine preferential formation of one of two possible loops. Therefore the level of the symmetry observed in footprinting or nuclease digestion experiments should depend on the G-segment sequence, and this could explain certain discrepancy between different nuclease protection and footprinting experiments (Fisher et al., 1981; Heddle et al., 2004; Kirkegaard and Wang, 1981; Liu and Wang,

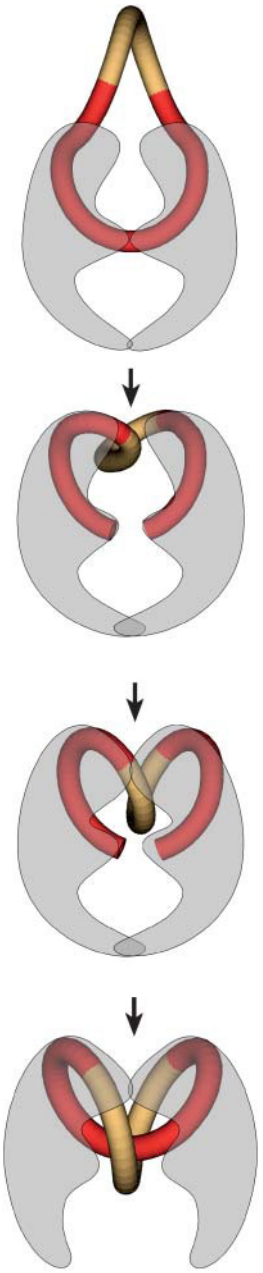

FIGURE 6 Introduction of two negative supercoils into a DNA minicircle by DNA gyrase. The model assumes that DNA-protein interaction causes the motion of the upper part of the DNA circle (*yellow*) through the break introduced in the G-segment (*red*). The diagram addresses only conformational changes in DNA during the strand-passing reaction.

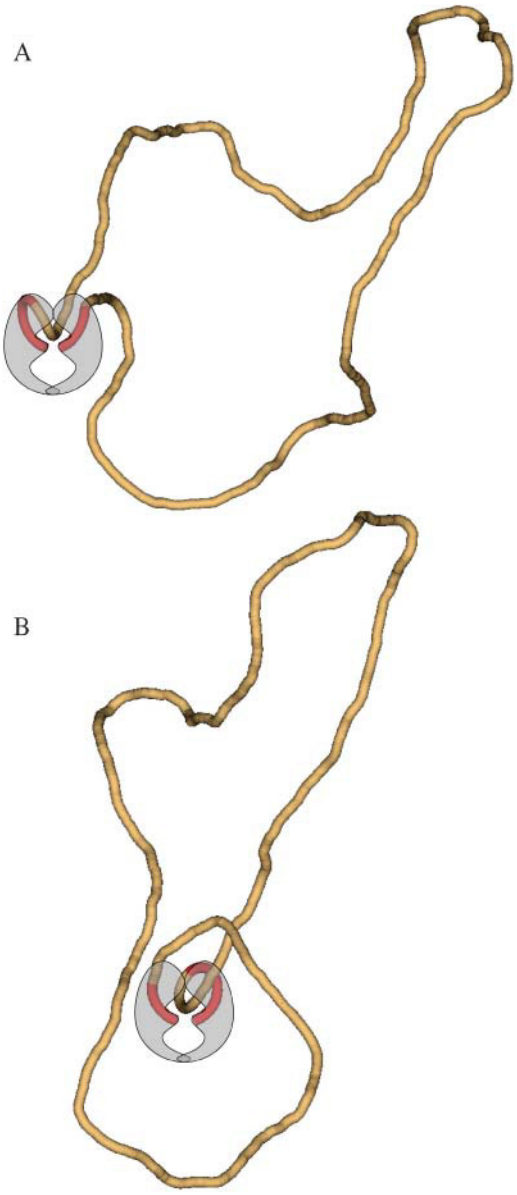

FIGURE 7 The model of DNA gyrase action. A continuous stretch of the double helix interacting with the protein includes both the G- and T-segments, and the DNA-protein interaction specifies the conformational changes in the stretch. Introduction of negative supercoiling is the only possible outcome of the reaction in this model. To keep the symmetry of the complex we assume that the required DNA-protein interaction can occur at either right (*A*) or left side (*B*) of the enzyme. The diagram does not address conformational changes in DNA gyrase.

1978a; Morrison and Cozzarelli, 1981; Orphanides and Maxwell, 1994).

A model that assumes that both the G- and T-segments belong to a continuous DNA stretch interacting with the enzyme cannot explain, however, catalysis of the catenation/decatenation reaction by DNA gyrase. Indeed, in this case the G- and T-segments belong to different DNA molecules and cannot form a continuous stretch of the double helix. Thus, we have to conclude that there are two modes of the enzyme action. In the major mode DNA gyrase interacts with a continuous stretch of DNA and introduces negative supercoiling into closed circular DNA molecules. In the minor mode the second part of the stretch, which corresponds to the T-segment, is substituted by another DNA segment. The later segment can belong to a remote part of the same DNA or another molecule. In this second mode the enzyme is able

to knot/unknot DNA molecules and catenate/decatenate DNA circles. The catalytic activity of the major mode is at least 1000 times greater than that of the minor mode. It cannot be excluded, however, that the activity of the minor mode is related with a small fraction of damaged enzymes.

Formation of knots in circular DNA has never been observed in the reaction catalyzed by DNA gyrase. According to the computer simulations performed for the model shown in Fig. 1, trefoils have to make up a large fraction of reaction products if relaxed circular molecules serve as a substrate (see Fig. 4 *A*). On the other hand, the fraction of trefoils that corresponds to the thermodynamic equilibrium does not exceed 3% for 7-kb DNA molecules under close to physiological ionic conditions (Rybenkov et al., 1993). Thus, if DNA gyrase works according to this model, it would greatly increase the fraction of knots over the equilibrium level on nicked circular DNA (see Fig. 4 *A*). This effect would be the opposite of the property of other type II topoisomerases that strongly decrease the steady-state fraction of knots relative to the equilibrium level (Rybenkov et al., 1997a). This is not surprising because the model shown in Fig. 1 is exactly opposite to one that was suggested to explain topology simplification by the topo II enzymes (Vologodskii et al., 2001). The computational analysis showed that a topoisomerase reduces the fraction of knots and links below the equilibrium level if the T-segment passes through the G-segment from inside to outside the loop formed by the G-segment upon binding with the enzyme (Vologodskii et al., 2001). Conversely, the fraction of knots and links will be increased above the equilibrium level if the T-segment passes through the G-segment from outside to inside the loop, as it is shown in Fig. 1. The data presented in Fig. 4 *A* reflect this feature of the gyrase model. In the model shown in Fig. 7 the T-segment passes through the break from inside to outside the loop. Thus, the minor mode of the enzyme action has to reduce the steady-state fraction of knots below equilibrium level. The fact that formation of knots by DNA gyrase has been never observed experimentally gives additional support to the suggested model. Unfortunately, the very low level of activity of the enzyme in the minor mode makes it difficult to study the issue quantitatively.

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