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RECOGNITION OF SUPERCOILED DNA BY
DROSOPHILA TOPOISOMERASE II¹

Neil Osheroff² and Douglas L. Brutlag

Department of Biochemistry, Stanford University
School of Medicine, Stanford, California 94305

The type II topoisomerase from the fly, *Drosophila melanogaster*, is able to distinguish the topological structure of DNA and interacts preferentially with negatively supercoiled over relaxed molecules. Furthermore, this study indicates that the enzyme recognizes points of helix-helix proximity found in supercoiled DNA and upon binding, condenses its nucleic acid substrate.

INTRODUCTION

Topoisomerases from prokaryotes are able to distinguish different topological isomers of DNA and interact preferentially with the form of DNA which serves as the substrate for their supercoiling-relaxation activities. Thus, *Escherichia coli* DNA gyrase, a type II topoisomerase that induces negative superhelical twists in nucleic acid molecules, displays a 4-fold higher kinetic affinity for substrates which are relaxed or topologically unconstrained over those which are supercoiled (1). Moreover, preformed complexes between gyrase and DNA dissociate more rapidly as superhelical density increases (2). In contrast, *E. coli* ω protein, a type I topoisomerase that relaxes nucleic acid molecules, binds more readily to negatively supercoiled

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²During the course of this study, N.O. was a post-doctoral fellow of the Helen Hay Whitney Foundation. Present address: Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

DNA (3).

In order to better elucidate the specificity of eukaryotic topoisomerases, the interaction between the type II enzyme from the fly, *Drosophila melanogaster*, and DNA was examined. Like the prokaryotic enzymes, the *Drosophila* enzyme can discern the topology of DNA. It shows an increased affinity for supercoiled molecules, apparently by recognizing points of helix-helix proximity which are induced by superhelical twisting. In addition, when bound to supercoiled molecules, topoisomerase II condenses the DNA structure. Based on these findings, novel conformational models for the complexing of DNA by eukaryotic topoisomerase II and implications for the biological function of the enzyme are presented.

METHODS

All interactions between purified *D. melanogaster* topoisomerase II (4) and DNA were at 30°C in 20 μ l of 10 mM Tris (Cl) pH 7.9, 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, and 15 μ g/ml BSA. Detailed procedures for the relaxation and ATPase assays are given in Osheroff et al. (5).

RESULTS

Specificity of Topoisomerase II for Supercoiled DNA.

Three lines of evidence indicate that the type II topoisomerase from *D. melanogaster* interacts preferentially with supercoiled DNA over relaxed molecules.

Kinetics of relaxation of supercoiled DNA. The time course for the relaxation of negatively supercoiled closed circular DNA by the *Drosophila* enzyme shows an unusually long linear phase, with as much as 75% of the DNA being relaxed before the velocity of the reaction begins to decrease (figure 1). This is a strong indication that the kinetic affinity of the enzyme is several fold higher for supercoiled molecules than it is for relaxed DNA. Interestingly, the rate of relaxation of positively supercoiled molecules is comparable to that found for negatively supercoiled DNA (5). Thus, while the *Drosophila* protein can discriminate supercoiled from relaxed molecules, it cannot distinguish between DNAs with negative or positive superhelical twists.

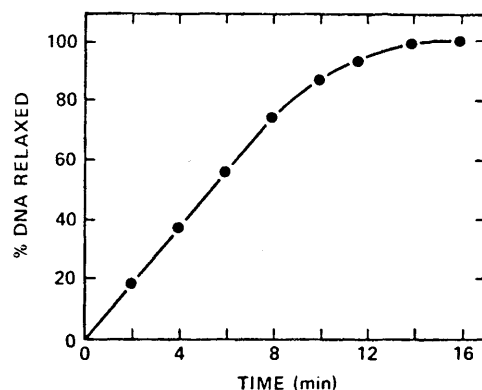


FIGURE 1. Time course for the relaxation of negatively supercoiled closed circular DNA by *Drosophila* topoisomerase II. Reaction mixtures contained 15 fmol of enzyme and 0.3 μg (0.1 pmol) of pBR322 plasmid DNA. Samples were resolved by electrophoresis on agarose gels in 100 mM Tris-borate, 2 mM EDTA pH 8.3 and quantitated by scanning densitometry, as previously described (5).

ATPase activity. Although ATP binding to the *Drosophila* topoisomerase II is sufficient to induce a double-strand DNA passage event, hydrolysis is required for enzyme turnover (5,6). When the relaxation of DNA is monitored under optimal conditions, the reaction is processive and approximately 4 molecules of ATP are converted to ADP and Pi for every superhelical twist removed (5). As can be seen in figure 2, the ATPase activity of topoisomerase II is DNA dependent, being stimulated 17-fold by the presence of negatively supercoiled DNA. Moreover, the enzyme is sensitive to the topological state of the nucleic acid substrate, as supercoiled molecules yield a reaction rate which is 4-fold higher than generated with relaxed or linear DNA. Despite the fact that the enzyme is able to accommodate single-stranded DNA in its active site (5), such structures are clearly not intermediates in the relaxation process, as single-stranded DNA is a very poor activator of the protein's ATPase (figure 2), even at concentrations which are nearly 2500-fold higher than its K_i value ($\sim 10^{-6}$ M) for relaxation.

Competitive binding. In the absence of its ATP co-factor, *Drosophila* topoisomerase II and DNA form a non-covalent complex. Although stable to electrophoresis in agarose gels, this interaction can be dissociated by the addition of SDS at 0°C. When incubated with an equimolar

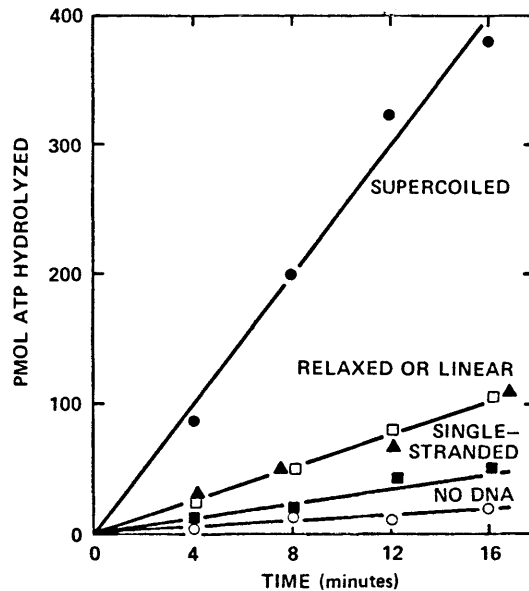


FIGURE 2. ATPase activity of topoisomerase II. Reaction mixtures contained 75 fmol of enzyme, 15 μ g (5 pmol) of pBR322 plasmid DNA, and 1 mM ATP. Single-stranded DNA was made by heating linear pBR322 DNA to 70°C for 2 minutes. Activity was followed by monitoring the release of $^{32}\text{P}_i$ from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, as previously described by Osheroff et al. (5).

mixture of negatively supercoiled, relaxed, and nicked circular molecules, under conditions ranging from no addition of detergent to nearly complete disruption with 0.1% SDS, the enzyme always binds 4 to 12-fold more supercoiled than relaxed or nicked DNA (figure 3). Thus, the recognition of topological structures by the enzyme does not require the presence of a nucleotide cofactor.

Catenation of Supercoiled DNA with Stoichiometric Levels of Topoisomerase II.

The catenation of closed circular duplex molecules of DNA by catalytic levels of topoisomerase II requires the presence of a DNA condensing agent such as spermidine (7,8) or histone H1 (6) (figure 4, lane B). However, catenanes can also be produced by incubating the DNA with high levels of *Drosophila* topoisomerase II in the presence of APP(NH)P (adenyl-5'-yl-imidodiphosphate), an ATP analogue which is

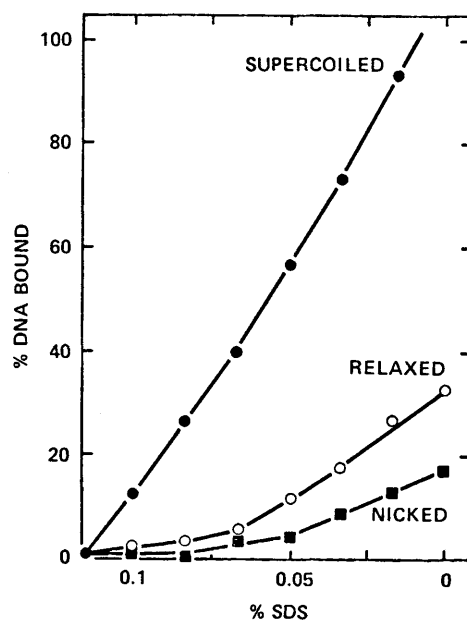


FIGURE 3. Competitive binding of DNA to topoisomerase II. Reaction mixtures contained 2 pmol of enzyme and 0.2 μ g (70 fmol) each of supercoiled, relaxed, and nicked pBR322 DNA. Resolution and quantitation of samples are as in figure 1.

sterically similar to ATP (10), but contains no hydrolyzable β - γ phosphonate bond (figure 4, lane D). A comparable result has been found by Hsieh (9), who employed nicked circular molecules, a high ratio of enzyme to DNA, and ATP. These findings strongly suggest that the enzyme itself can condense the DNA. This idea is further supported by the fact that the *Drosophila* protein, like the type II topoisomerase from bacteriophage T4 (11), can tie knots in closed circular DNA (unpublished results) (9).

The mechanism of the topoisomerase II induced condensation of supercoiled DNA is not known. Although the enzyme may act via charge neutralization, as is the case for the polycationic agents (6-8), other processes are clearly feasible. As a singular example of another mechanism, polyvinyl alcohol, which presumably compacts DNA by the process of 'volume exclusion' (12,13) has been successfully employed to promote the enzymic catenation of closed circular DNA (figure 4, lane C).

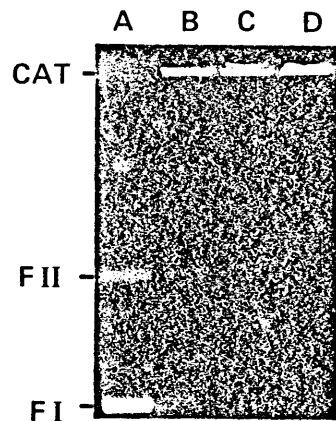


FIGURE 4. Catenation of DNA by topoisomerase II. All reactions contained 0.3 μg (0.1 pmol) of pBR322 closed circular supercoiled DNA. Lanes B and C employed ATP (1 mM) while lane D employed APP(NH)P (1 mM). Lane A, no enzyme; Lane B, enzyme (15 fmol) + histone H1 (0.12 μg); Lane C, enzyme (15 fmol) + polyvinyl alcohol (8%); Lane D, enzyme (2.5 pmol). Prior to electrophoresis on agarose gels (see figure 1), samples were treated with 0.1% SDS - 10 mM EDTA and digested with 1.25 μg of Proteinase K for 20 minutes at 37°C to completely disrupt the binding between protein and DNA. FI, supercoiled DNA; FII, nicked DNA; CAT, catenated networks.

DISCUSSION

The type II topoisomerase from *Drosophila melanogaster* can discern the topological structure of DNA and interacts preferentially with supercoiled molecules, even in the absence of its ATP cofactor. This specificity for supercoiled DNA may also extend to the type I enzyme from *Drosophila*, as the addition of relaxed molecules has virtually no effect on the time course for the catalytic removal of negative superhelical twists (unpublished results).

Recognition of Supercoiled DNA by *D. melanogaster* Topoisomerase II.

As negative superhelical density increases, the structure of closed circular double-stranded DNA changes in many respects: regions with single-stranded character are formed

in the underwound double helix (14); the conversion of right-handed B-DNA to left-handed Z-DNA is facilitated (15); cruciform structures are stabilized (16); increased writhing leads to points of helix-helix proximity (17,18). Since the *Drosophila* topoisomerase II recognizes supercoiled molecules, it is logical that the enzyme can discern one or more of these changes in the helical structure of DNA. Recognition of regions with single-stranded character may be eliminated because 1) single-stranded DNA is a very poor activator of the protein's ATPase (figure 2) and 2) the rate of relaxation of positively supercoiled DNA, which is overwound, is comparable to that found for underwound negatively supercoiled molecules (5). For this second reason, interaction of the enzyme with Z-DNA sequences or cruciform structures may also be excluded, as positive superhelical twisting would destabilize both conformations. However, both negatively and positively supercoiled DNAs contain juxtaposed helices. Therefore, it is likely that topoisomerase II displays a higher affinity for supercoiled over relaxed molecules because it can recognize points of helical proximity in DNA. This conclusion is also consistent with the finding that the *Drosophila* enzyme can condense DNA (figure 4). As described below, the protein may compact nucleic acids by bridging adjacent helices (see figure 5).

Condensation of Supercoiled DNA by Topoisomerase II.

Negatively supercoiled circular DNA can exist in a number of conformations, ranging from a right-handed interwound structure to a left-handed solenoid (figure 5, A and C). Although these are homeomorphic conformers and have identical linking numbers, the solenoid is clearly the more compact of the two structures. The interwound form, which predominates in nature, is energetically more favorable because it minimizes twist deformation (18). Considering these facts, we suggest that the condensation of supercoiled DNA by stoichiometric levels of the *Drosophila* topoisomerase II involves the following mechanism. When bound to the interwound form, the enzyme induces a localized conformational transition in the DNA as shown in figure 5B. After several protein molecules are bound, the conformational equilibrium is shifted from the interwound to the solenoid form, thereby condensing the structure of the DNA. This model, which has also been proposed for the interaction

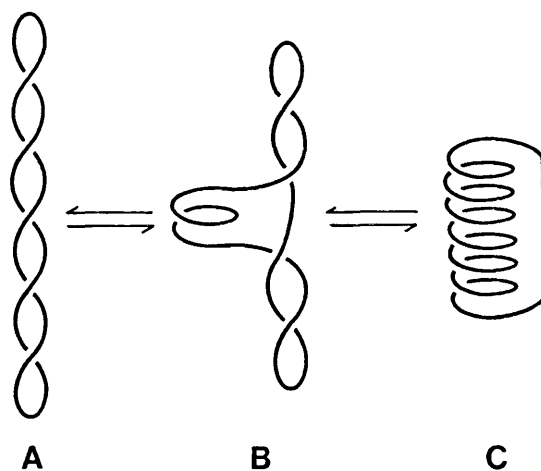


FIGURE 5. Conformation of negatively supercoiled DNA. The right-handed interwound (A) and left-handed solenoid (C) forms are shown. The structure in B, which contains a localized solenoid conformation, depicts a transitional intermediate between these two forms.

of the catabolite activator protein with supercoiled DNA (19), is currently being tested by electron microscopy.

Implications for Biological Activity.

Two major conclusions concerning the *in vivo* function of eukaryotic type II topoisomerases may be drawn from this study. First, the fact that the *Drosophila* protein can discern the topological state of DNA is of consequence to its biological activity, because it allows the enzyme to interact specifically with its substrates, yet still turn over rapidly. Second, by recognizing points of helix-helix proximity, topoisomerase II is able to distinguish both supercoiled DNA and interlocked DNA complexes as substrates. Teleologically, this concept is very important. By employing the double-strand DNA passage reaction to resolve interlocked multimolecular structures which are the products of DNA replication, eukaryotic type II topoisomerases may function in such fundamental cellular processes as chromosome segregation (20-22).

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