Published in final edited form as:
Biochemistry. 2006 September 26; 45(38): 11674–11680.

ABILITY OF VIRAL TOPOISOMERASE II TO DISCERN THE HANDEDNESS OF SUPERCOILED DNA: BIMODAL RECOGNITION OF DNA GEOMETRY BY TYPE II ENZYMES†

A. Kathleen McClendon[‡,§], **Jennifer S. Dickey**[‡,§], and **Neil Osheroff**[‡,‖,*]
Departments of Biochemistry and Medicine (Hematology/Oncology), Vanderbilt University School of Medicine, Nashville, Tennessee 37232−0146

Abstract

Previous studies with human and bacterial topoisomerases suggest that the type II enzyme utilizes two distinct mechanisms to recognize the handedness of DNA supercoils. It has been proposed that the ability of some type II enzymes, such as human topoisomerase IIα and *Eschericia coli* topoisomerase IV, to distinguish supercoil geometry during DNA relaxation is mediated by elements in the variable C-terminal domain of the protein. In contrast, the ability of human topoisomerase IIα and β to discern the handedness of supercoils during DNA cleavage suggests that residues in the conserved N-terminal or central domain of the protein are involved in this process. To test this hypothesis, the ability of *Paramecium bursaria* chlorella virus-1 (PBCV-1) and chlorella virus Marburg-1 (CVM-1) topoisomerase II to relax and cleave negatively and positively supercoiled plasmids was assessed. These enzymes display a high degree of sequence identity with the N-terminal and central domains of eukaryotic topoisomerase II, but naturally lack the C-terminal domain. While PBCV-1 and CVM-1 topoisomerase II relaxed under- and overwound substrates at similar rates, they were able to discern the handedness of supercoils during the cleavage reaction and preferentially cut negatively supercoiled DNA. Preferential cleavage was not due to a change in site specificity, DNA binding, or religation. These findings are consistent with a bimodal recognition of DNA geometry in which topoisomerase II uses elements in the C-terminal domain to sense the handedness of supercoils during DNA relaxation and elements in the conserved N-terminal or central domains during DNA cleavage.

Although the classic structure of the Watson-Crick double helix is free from either torsional or axial stress, DNA in living systems is subject to these topological challenges. Globally, the DNA of eukaryotes and eubacteria is maintained in an underwound (*i.e.*, negatively supercoiled) state (1-4). This underwinding makes it easier to separate the two strands of the genetic material, and thereby facilitates critical processes such as DNA replication and transcription. In contrast, the actions of DNA tracking systems overwind (*i.e*, positively supercoil) the genetic material immediately preceding replication forks and transcription complexes (1,3-5). If this overwinding is not alleviated, the ensuing torsional stress rapidly halts the movement of tracking systems along the double helix (1,3,5-7).

---

†This work was supported by National Institutes of Health research grant GM33944. AKM and JSD were trainees under grants 5 T32 HD07043 and 5 T32 CA09582 from the National Institutes of Health, respectively.

* To whom correspondence should be addressed. Tel: 615−322−4338. Fax: 615−343−1166. E-mail: neil.osheroff@vanderbilt.edu..
§These authors contributed equally to this study.
‡Department of Biochemistry.
‖Department of Medicine (Hematology/Oncology).

In vivo, the topological state of DNA is modulated by enzymes known as topoisomerases (3, 5,8-12). There are two classes of topoisomerases that are distinguished by their catalytic mechanisms. Type II enzymes act by passing an intact double helix through a transient double-stranded break that they generate in a separate segment of DNA (9,10,12-14). As a result of this double-stranded DNA passage reaction, topoisomerase II is able to alleviate torsional stress (*i.e.*, remove superhelical twists) in duplex DNA, remove knots from the genetic material, and untangle chromosomes (3,5,8-12).

It has long been known that topoisomerase II can distinguish negatively supercoiled DNA from relaxed molecules (*i.e.*, molecules that are not under torsional stress) (15-17). The enzyme preferentially binds and cleaves negatively supercoiled plasmids (16,17). The basis for this recognition appears to reflect the ability of topoisomerase II to bind at DNA crossovers (or nodes), which are more likely to form on supercoiled molecules (17,18).

Recently, it was demonstrated that some type II enzymes are capable of discerning the geometry of DNA supercoils. Human topoisomerase IIα (19) and *Eschericia coli* topoisomerase IV (20,21) both relax positive superhelical twists more than an order of magnitude faster than they do negative superhelical twists. In contrast, yeast and *Drosophila* topoisomerase II, and human topoisomerase IIβ relax negatively and positively supercoiled plasmids at similar rates (19, 22). Although type II topoisomerases possess a high degree of amino acid sequence identity in their N-terminal and central homology domains (GyrB and GyrA domains, respectively), these enzymes diverge considerably in their C-terminal domains (9,10,12,23,24). Based on these amino acid sequence differences, it was suggested that the ability of human topoisomerase IIα to preferentially relax positive DNA supercoils resides in the C-terminal domain of the protein (19). Structural and modeling studies of topoisomerase IV and DNA gyrase, coupled with nucleic acid binding experiments, have led to similar conclusions for the bacterial type II enzymes (25-27).

In addition to their ability to distinguish supercoil geometry during DNA relaxation, type II topoisomerases also discern the handedness of supercoils during the DNA cleavage event (28). Both topoisomerase IIα and β maintain lower levels (2– to 4–fold) of cleavage intermediates when positively supercoiled substrates are employed (28). Because the human isoforms display different results in DNA relaxation and cleavage assays, the type II enzyme must utilize distinct mechanisms to distinguish DNA geometry during these two processes. It is not known which domain of topoisomerase II is involved in the recognition of DNA topology during cleavage. However, since supercoil geometry affects DNA cleavage by topoisomerase IIα and β in a similar fashion, it is likely that this ability resides in the conserved N-terminal or central domain of the enzyme.

To more fully assess the recognition of DNA geometry by type II topoisomerases, the ability of *Paramecium bursaria* chlorella virus-1 (PBCV-1)[1] and chlorella virus Marburg-1 (CVM-1)[1] (29-32) to relax and cleave negatively and positively supercoiled plasmids was examined. These enzymes generate high levels of DNA cleavage, but in other respects are similar to eukaryotic topoisomerase II (29-32). Furthermore, the N-terminal and central domains of the chlorella virus enzymes display a high degree of amino acid sequence identity with the corresponding domains of eukaryotic topoisomerase II, but naturally lack the C-terminal domain (29,32). Therefore, the viral type II topoisomerases represent intriguing models with which to address the bimodal ability of the type II enzyme to recognize the geometry of DNA supercoils.

[1]Abbreviations: PBCV-1, *Paramecium bursaria* chlorella virus-1; CVM-1, chlorella virus Marburg-1.

Results indicate that PBCV-1 and CVM-1 topoisomerase II relaxed positively and negatively supercoiled plasmids at similar rates. These findings are consistent with the hypothesis that the C-terminal domain of topoisomerase II plays an instrumental role in sensing the geometry of superhelical twists during the relaxation reaction. In contrast, the viral enzymes were able to discern DNA geometry during the cleavage reaction and, like the human enzymes, generated higher levels of strand breaks with negatively supercoiled substrates. This latter finding demonstrates that the ability to discern DNA geometry during the cleavage event must reside within the N-terminal or central domain of the type II enzyme.

EXPERIMENTAL PROCEDURES

Enzymes and Materials

PBCV-1 topoisomerase II and CVM-1 topoisomerase II were expressed in *Saccharomyces cerevisiae* JEL-1Δ*top1* and purified by a modification (32) of the procedure of Lavrukhin *et al*. (29). All other chemicals were analytical reagent grade.

Negatively supercoiled DNA was prepared using a Plasmid Mega Kit (Qiagen) as described by the manufacturer. Positively supercoiled pBR322 DNA was prepared by treating negatively supercoiled molecules with recombinant *Archaeoglobus fulgidus* reverse gyrase (19,33). The average number of superhelical twists present in DNA substrates and the resulting σ values were determined by electrophoretic band counting relative to fully relaxed molecules (19). For negatively supercoiled substrates, time courses for the relaxation of pBR322 by topoisomerase I were resolved by electrophoresis in 1% agarose gels containing 1−2 μg/ml chloroquine (Sigma) in the running buffer. The initial plasmid contained ~15 to 17 negative superhelical twists per molecule (σ ≈ −0.035 to −0.039). This superhelical density is typical of plasmids isolated from *E. coli*. For positively supercoiled substrates, time courses for the generation of positive superhelical twists by reverse gyrase were resolved by electrophoresis as above in running buffer containing 5−15 μg/ml netropsin B (Boehringer Mannheim). These plasmids contained ~15 to 17 positive superhelical twists per molecule (σ ≈ +0.035 to +0.039). The handedness of positively supercoiled DNA was confirmed by two-dimensional gel electrophoresis (19). Thus, the supercoiled substrates employed for this study contained equivalent numbers of superhelical twists, but were of opposite handedness.

DNA Relaxation

DNA relaxation assays were based on the protocols of Fortune and Osheroff (34) and Dickey et al. (32), and represent the optimal conditions for relaxation by each of the respective chlorella virus enzymes. Reaction mixtures contained 1 nM PBCV-1 topoisomerase II or CVM-1 topoisomerase II, 1 mM ATP, and 5 nM negatively or positively supercoiled pBR322 DNA in a total of 20 μL of relaxation buffer (10 mM Tris-HCl, pH 8.5, 62.5 mM NaCl, 62.5 mM KCl, 0.1 mM NaEDTA, 2.5 mM $MgCl_2$, and 2.5% glycerol). Reaction mixtures were incubated at 25 °C (PBCV-1) or 30 °C (CVM-1) for 0−30 min and relaxation was stopped by the addition of 3 μL of 0.5% SDS and 77 mM EDTA. Samples were mixed with agarose gel loading buffer (60% sucrose in 10 mM Tris-HCl, pH 7.9) and subjected to electrophoresis in 1% agarose gels in 100 mM Tris-borate, pH 8.3, 2 mM EDTA. Gels were stained for 30 min with 0.5 μg/mL ethidium bromide. DNA bands were visualized by UV light and were quantified using an Alpha Innotech digital imaging system (San Leandro, CA). DNA relaxation was monitored by quantifying either the formation of fully relaxed DNA product or the loss of initial supercoiled substrate.

DNA Cleavage

Plasmid DNA cleavage reactions were based on the procedures of Fortune and Osheroff (34) and Dickey et al. (32), and represent the optimal conditions for cleavage by each of the

respective enzymes. Reaction mixtures contained 0−20 nM PBCV-1 or CVM-1 topoisomerase II, and 10 nM negatively or positively supercoiled pBR322 DNA in a total of 20 μL of DNA cleavage buffer (PBCV-1 topoisomerase II: 10 mM Tris-HCl, pH 8.5, 62.5 mM NaCl, 62.5 mM KCl, 0.1 mM NaEDTA, 2.5 mM $MgCl_2$, and 2.5% glycerol; CVM-1 topoisomerase II: 10 mM Tris-HCl, pH 8.5, 120 mM KCl, 0.1 mM NaEDTA, 15 mM $MgCl_2$, and 2.5% glycerol). Reaction mixtures were incubated for 6 min at 25 °C (PBCV-1) or 30 °C (CVM-1) to establish cleavage/religation equilibria. DNA cleavage intermediates were trapped by the addition of 2 μL of 1% SDS and 2 μL of 115 mM NaEDTA, pH 8.0. Proteinase K was added (2 μL of 0.8 mg/mL) and mixtures were incubated for 30 min at 45 °C to digest the type II enzyme. Samples were mixed with 2 μL of agarose gel loading buffer, heated for 2 min at 45 °C, and subjected to electrophoresis in 1% agarose gels in 40 mM Tris-acetate, pH 8.3, and 2 mM EDTA containing 0.5 μg/mL ethidium bromide. DNA bands were quantified by digital imaging as described above. DNA cleavage was monitored by the conversion of supercoiled plasmid to linear molecules.

DNA cleavage reactions were performed in the absence of ATP so that the topological state of the DNA would not change during the course of the reaction. It should be noted that the nucleotide cofactor does not influence the mechanism of topoisomerase II-mediated DNA scission (9).

Site-specific DNA Cleavage

DNA sites cleaved by PBCV-1 topoisomerase II or CVM-1 topoisomerase II in negatively and positively supercoiled DNA were mapped using a modification of the procedure of O'Reilly and Kreuzer (35,36). DNA cleavage mixtures contained 120 nM PBCV-1 topoisomerase II or CVM-1 topoisomerase II, 10 nM negatively or positively supercoiled pBR322 DNA in a total of 160 μl of cleavage buffer. Samples were incubated at 25 °C (PBCV-1) or 30 °C (CVM-1) for 6 min and enzyme-DNA cleavage complexes were trapped by the addition of 16 μl of 1% SDS followed by 16 μl of 115 mM EDTA (pH 8.0). Proteinase K (16 μl of 0.8 mg/ml) was added and mixtures were incubated at 45 °C for 30 min to digest the type II enzyme. DNA products were purified by passage through Qiaquick Spin Columns (Qiagen) as described by the manufacturer. DNA cleavage products were linearized by treatment with *Hin*dIII. Terminal 5'-phosphates were removed by treatment with calf intestinal alkaline phosphatase and replaced with [^{32}P]phosphate using T4 polynucleotide kinase and [γ-^{32}P]ATP. Samples were treated with *Eco*RI and the singly-end labeled DNA products were purified by passage through a CHROMA SPIN+TE-10 column (Clontech). Loading buffer (40% formamide, 8.4 mM EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanole FF) was added and samples were subjected to electrophoresis in 6% sequencing gels. Gels were fixed in 10% methanol/10% acetic acid and dried in vacuo. DNA cleavage products were visualized with a BioRad Molecular Imager FX.

DNA Binding

The ability of PBCV-1 and CVM-1 topoisomerase II to bind negatively and positively supercoiled DNA was assessed using a competitive nitrocellulose filter-binding assay. Binding mixtures contained 400 nM PBCV-1 topoisomerase II or CVM-1 topoisomerase II, 5 nM linear pBR322 DNA that was cleaved with *Hind*III and terminally labeled with [^{32}P]phosphate, and 0−20 nM negatively or positively supercoiled DNA in a total of 20 μl of relaxation buffer that lacked $MgCl_2$. Samples were incubated at 25 °C (PBCV-1) or 30 °C (CVM-1) for 6 min. Under the conditions of the assay, a DNA binding equilibrium was established in less than 1 min. Nitrocellulose membranes (0.45 μm HA, Millipore) were prepared by incubation in binding buffer for 10 min. Samples were applied to the membranes and filtered in vacuo. Membranes were washed 3 times with 1 ml of binding buffer, dried, and submerged in 8 ml of scintillation fluid (Econo-Safe, Research Products International). Radioactivity remaining on the

membranes was quantified using a Beckman LS 5000 TD Scintillation Counter. The percent linear DNA bound to topoisomerase II was determined based on the ratio of radioactivity on the membranes vs. that of the input DNA.

It should be noted that DNA binding experiments were performed in the absence of ATP and Mg^{2+}. This was done to prevent the formation of concatenated DNA multimers, which are too large to pass through the filter, or the generation of covalent enzyme-DNA cleavage complexes during the course of the assay.

DNA Religation

Topoisomerase II DNA religation was monitored by two methods. PBCV-1 topoisomerase II DNA religation was carried out by the procedure of Fortune et al. (30). DNA cleavage/ligation equilibria were established in PBCV-1 topoisomerase II cleavage buffer as described above except that $MgCl_2$ in the reaction buffer was replaced by 2.5 mM $CaCl_2$. Topoisomerase II-DNA cleavage complexes were trapped by the addition of EDTA (pH 8.0) to a 10 mM final concentration. NaCl was added to a 250 mM final concentration in order to prevent re-cleavage of the DNA substrate. Ligation was initiated by the addition of $MgCl_2$ at a 1 mM final concentration and terminated at times up to 60 s by the addition of 2 μL of 1% SDS.

Since DNA cleavage by CVM-1 topoisomerase II is not supported by Ca^{2+} (32), DNA religation was monitored using an alternative procedure (32). CVM-1 topoisomerase II–DNA cleavage/religation equilibria were established as above for plasmid DNA cleavage. Religation was initiated by shifting reactions from a 30 °C bath to −5 °C bath. Reactions were stopped at time points following the temperature shift by the addition of 2 μL of 1 % SDS.

All samples were processed and analyzed as described for plasmid DNA cleavage. The percent DNA cleavage at time 0 was set to 100% and the rate of ligation was determined by quantifying the loss of cleaved DNA over time.

RESULTS

Relaxation of Negatively and Positively Supercoiled DNA

Human topoisomerase IIα relaxes positively supercoiled DNA >10-fold faster than it does negatively supercoiled molecules (19). Based on amino acid comparisons with type II topoisomerases that do not preferentially remove positive superhelical twists, as well as structural and enzymological studies with prokaryotic type II enzymes (25-27), it has been proposed that the C-terminal domain of topoisomerase IIα plays an important role in sensing DNA geometry during relaxation (19).

As an initial test of this hypothesis, the ability of two viral enzymes to relax negatively and positively supercoiled DNA was examined (Figures 1 and 2). PBCV-1 and CVM-1 topoisomerase II are from different strains of chlorella virus. These enzymes are the two shortest type II topoisomerases identified to date (1062 and 1059 amino acids in length, respectively) and are naturally truncated at the end of their central domains (29, 32). Thus, they lack the entire C-terminal domain found in eukaryotic topoisomerase II. PBCV-1 and CVM-1 topoisomerase II generate high levels of DNA cleavage intermediates, but in virtually all other respects are remarkably similar to eukaryotic type II enzymes (29-32).

Since type II topoisomerases relax positively supercoiled DNA in a less processive manner than they do negatively supercoiled molecules (19), DNA relaxation was monitored by quantifying either the appearance of relaxed product (left panel) or the loss of supercoiled substrate (right panel).[2] Results of DNA relaxation assays are shown in Figures 1 (PBCV-1) and 2 (CVM-1). Both viral enzymes displayed high rates of DNA relaxation irrespective of

the handedness of the initial plasmid substrates. As determined by the appearance of relaxed product, PBCV-1 and CVM-1 topoisomerase II appeared to relax negatively supercoiled plasmids at faster rates. Alternatively, as determined by the loss of supercoiled substrate, both enzymes appeared to relax positively supercoiled plasmids at rates that were slightly (2– to 3–fold) faster. Compared to human topoisomerase IIα, which relaxed positively supercoiled molecules ~30–fold and 10–fold faster than negatively supercoiled plasmids by the two methods of analysis, respectively (19), the viral type II topoisomerases display little (if any) preference for relaxing either substrate. These findings support the hypothesis that the C-terminal domain of the protein is required for topoisomerase II to sense the handedness of DNA during relaxation.

Cleavage of Negatively and Positively Supercoiled DNA

As a prerequisite to the strand passage event, topoisomerase II generates a transient double-stranded break in the genetic material (9,10,12-14). In contrast to results observed for DNA relaxation, both human topoisomerase IIα and β are able to discern the handedness of supercoils during the DNA cleavage event (28). The two enzyme isoforms maintain a lower level of DNA cleavage intermediates (2– to 4–fold) when positively supercoiled, as opposed to negatively supercoiled, plasmids are employed as substrates (19,28). This finding demonstrates that topoisomerase II employs distinct mechanisms to recognize DNA geometry during different catalytic processes and suggests that the ability to distinguish the handedness of supercoils during the cleavage event resides in the conserved N-terminal or central domains of the enzyme. To address this issue, the effects of DNA under- and overwinding on the cleavage activities of PBCV-1 and CVM-1 topoisomerase II were examined (Figure 3).

Both viral enzymes were able to discern supercoil geometry during the DNA cleavage reaction. As found for the human enzymes, levels of DNA cleavage intermediates generated by PBCV-1 and CVM-1 topoisomerase II were ~2– to 4–fold lower when positively supercoiled plasmids were used as substrates. These results provide strong evidence that the elements that sense the handedness of DNA supercoils during the cleavage reaction of topoisomerase II must be located in the conserved N-terminal or central domain of the protein, rather than the C-terminal domain.

Three additional experiments were carried out to further investigate the recognition of DNA geometry during the cleavage event. First, the effects of DNA handedness on the site specificity of PBCV-1 and CVM-1 topoisomerase II were examined (Figure 4). Each enzyme cut negatively and positively supercoiled substrates at the same sites. However, levels of scission were reduced in the overwound molecules. Thus, the decrease in cleavage observed with positively supercoiled DNA does not result from a geometry-specific alteration in site specificity.

Second, the effects of supercoil geometry on enzyme-DNA binding were characterized (Figure 5). As determined by competitive binding experiments, PBCV-1 and CVM-1 topoisomerase II bound negatively and positively supercoiled plasmids with similar affinity. Therefore, the recognition of DNA handedness during cleavage does not reflect an increased affinity for negatively supercoiled substrates.

Third, the effects of supercoil handedness on enzyme-mediated DNA religation were examined (Figure 6). Similar rates of religation were observed for both viral enzymes with negatively

[2]In order to allow a more direct comparison of reaction rates with negatively and positively supercoiled plasmids, attempts were made to establish low ionic strength DNA relaxation conditions that would increase the processivity of viral topoisomerase II with positively supercoiled molecules. Unfortunately, this was not possible, as enzyme activity with both substrates decreased precipitously at ionic strengths low enough to support processive relaxation of positively supercoiled DNA (data not shown).

and positively supercoiled substrates. Taken together, these results suggest that the effects of supercoil geometry must manifest themselves somewhere between the initial DNA binding and religation events. Most likely, the actual cleavage event is impacted by the handedness of the DNA substrate.

DISCUSSION

Previous studies with human and bacterial topoisomerases (19-21,25-28) suggest that the type II enzyme utilizes two distinct mechanisms to recognize the handedness of DNA supercoils. It has been proposed that the ability of some type II enzymes, such as human topoisomerase IIα and *E. coli* topoisomerase IV, to distinguish supercoil geometry during DNA relaxation is mediated by elements in the variable C-terminal domain (19,25-27). In contrast, the ability of human topoisomerase IIα and β to discern the handedness of DNA during cleavage suggests that residues in the conserved N-terminal or central domain of the protein are involved in this process.

As a first test of this hypothesis, the ability of two viral enzymes that lack the C-terminal domain, PBCV-1 and CVM-1 topoisomerase II (29,32), to relax and cleave negatively and positively supercoiled plasmids was compared. While these enzymes relaxed under- and overwound substrates at similar rates, they were able to discern the handedness of supercoils during the cleavage reaction and preferentially cut negatively supercoiled DNA. These findings are consistent with a role for the C-terminal domain of topoisomerase II in sensing the geometry of superhelical twists during relaxation. Furthermore, they demonstrate that the ability to discern supercoil geometry during DNA cleavage must reside within the conserved N-terminal or central domain of the type II enzyme.

The bimodal recognition of DNA geometry may contribute to the physiological functions of topoisomerase II. By using the variable C-terminal domain to distinguish positive from negative supercoils during relaxation, specific type II topoisomerases are better suited to actively participate in the removal of torsional stress ahead of replication forks or transcription complexes. Conversely, by using the conserved portions of the protein to discern DNA geometry during cleavage, all type II topoisomerases may maintain lower levels of cleavage complexes with positively supercoiled DNA. This latter characteristic lessens the probability of collisions between DNA tracking systems and covalent topoisomerase II-cleaved DNA complexes, thereby decreasing the chance that the enzyme will introduce permanent strand breaks into the genome during its normal physiological activities.

The catalytic function of topoisomerase II requires simultaneous interaction between the enzyme and two distinct segments of DNA, the G-segment (the double helix that is cleaved by the enzyme and is opened as a “gate”) and the T-segment (the double helix that is “transported” through the open DNA gate) (13). These two DNA segments intersect as a crossover in the active site of the enzyme, irrespective of the supercoiled state of the nucleic acid substrate. However, the geometry of the crossover formed by negative or positive supercoils differs significantly. While DNA nodes formed with positively supercoiled molecules are left-handed and have acute angles (~60°), those formed with negatively supercoiled molecules are right-handed and have obtuse angles (~120°) (27,37). As a result, the preferred path of the T-segment into and out of the active site of topoisomerase II should be greatly affected by the handedness of crossover.

It is not clear how topoisomerase II utilizes elements in different protein domains to sense DNA geometry during different catalytic processes. Ultimately, the solution to this problem may require detailed structural analysis of topoisomerase II-DNA complexes. However, based on our studies with human (19,28) and chlorella virus type II topoisomerases, as well as models

described for geometry sensing in bacterial topoisomerase IV (25-27), we propose the following model for the actions of eukaryotic topoisomerase II.

Although the C-terminal domain of the type II enzyme is distal to the active site of the protein, it is believed to have specific interactions with the T-segment during relaxation (25-27). Thus, recognition of DNA geometry during relaxation most likely is related to the path of the T-segment that is imposed by handedness of the DNA crossover.

In contrast, the elements in the conserved domains of the type II enzyme that discern DNA geometry during cleavage are likely to be located within (or proximal to) the active site of the protein. Therefore, they may be in direct contact with both the T- and the G-segments and provide the catalytic core of topoisomerase II with an innate ability to recognize the angle of the DNA crossover. Since the presence of the T-segment has been shown to increase scission of the G-segment (38), it is possible that the geometry of the crossover may also affect the ability of the enzyme to generate breaks in the DNA backbone. Alternatively, changes in DNA twist associated with under- and overwinding profoundly alter the properties of the double helix. Since the catalytic core of topoisomerase II is in intimate contact with the G-segment (13), the enzyme may recognize the twist of the G-segment rather than the angle of the DNA crossover and maintain lower levels of cleavage complexes with overwound substrates.

In conclusion, topoisomerase II appears to recognize the geometry of DNA supercoils in a bimodal fashion that involves elements in the C-terminal domain for relaxation and the N-terminal or central domain for cleavage. This ability has implications for the catalytic function of topoisomerase II and may account for some of the differences in the physiological roles played by distinct type II enzymes.

REFERENCES

1. Cozzarelli, NR.; Wang, JC. DNA topology and its biological effects. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1990.
2. Kanaar R, Cozzarelli NR. Roles of supercoiled DNA structure in DNA transactions. Curr. Opin. Struct. Biol 1992;2:369–379.
3. Wang JC. DNA topoisomerases. Annu. Rev. Biochem 1996;65:635–692. [PubMed: 8811192]
4. Schvartzman JB, Stasiak A. A topological view of the replicon. EMBO Rep 2004;5:256–261. [PubMed: 14993926]
5. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. Nat. Rev. Mol. Cell. Biol 2002;3:430–440. [PubMed: 12042765]
6. Brill SJ, DiNardo S, Voelkel-Meiman K, Sternglanz R. DNA topoisomerase activity is required as a swivel for DNA replication and for ribosomal RNA transcription. Nci. Monographs 1987;4:11–15. [PubMed: 2442627]
7. Kim RA, Wang JC. Function of DNA topoisomerases as replication swivels in Saccharomyces cerevisiae. J. Mol. Biol 1989;208:257–267. [PubMed: 2549254]
8. Nitiss JL. Investigating the biological functions of DNA topoisomerases in eukaryotic cells. Biochim. Biophys. Acta 1998;1400:63–81. [PubMed: 9748506]
9. Fortune JM, Osheroff N. Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice. Prog. Nucleic Acid Res. Mol. Biol 2000;64:221–253. [PubMed: 10697411]
10. Champoux JJ. DNA topisomerases: structure, function, and mechanism. Annu. Rev. Biochem 2001;70:369–413. [PubMed: 11395412]
11. Sabourin, M.; Osheroff, N. Wiley Encyclopedia of Molecular Medicine. John Wiley & Sons, Inc.; 2002. Topoisomerases; p. 3192-3197.
12. Velez-Cruz, R.; Osheroff, N. Encyclopedia of Biological Chemistry. Elsevier Inc.; 2004. DNA topoisomerases: type II; p. 806-811.

13. Wang JC. Moving one DNA double helix through another by a type II DNA topoisomerase: the story of a simple molecular machine. Q. Rev. Biophys 1998;31:107–144. [PubMed: 9794033]
14. Wilstermann AM, Osheroff N. Stabilization of eukaryotic topoisomerase II-DNA cleavage complexes. Curr. Top. Med. Chem 2003;3:1349–1364.
15. Osheroff N, Shelton ER, Brutlag DL. DNA topoisomerase II from Drosophila melanogaster. Relaxation of supercoiled DNA. J. Biol. Chem 1983;258:9536–9543. [PubMed: 6308011]
16. Osheroff N. Eukaryotic topoisomerase II. Characterization of enzyme turnover. J. Biol. Chem 1986;261:9944–9950. [PubMed: 3015913]
17. Zechiedrich EL, Osheroff N. Eukaryotic topoisomerases recognize nucleic acid topology by preferentially interacting with DNA crossovers. EMBO J 1990;9:4555–4562. [PubMed: 2176156]
18. Roca J, Berger JM, Wang JC. On the simultaneous binding of eukaryotic DNA topoisomerase II to a pair of double-stranded DNA helices. J. Biol. Chem 1993;268:14250–14255. [PubMed: 8390987]
19. McClendon AK, Rodriguez AC, Osheroff N. Human topoisomerase IIα rapidly relaxes positively supercoiled DNA: Implications for enzyme action ahead of replication forks. J. Biol. Chem 2005;280:39337–39345. [PubMed: 16188892]
20. Crisona NJ, Strick TR, Bensimon D, Croquette V, Cozzarelli NR. Preferential relaxation of positively supercoiled DNA by E. coli topoisomerase IV in single-molecule and ensemble measurements. Genes Dev 2000;14:2881–2892. [PubMed: 11090135]
21. Stone MD, Bryant Z, Crisona NJ, Smith SB, Vologodskii A, Bustamante C, Cozzarelli NR. Chirality sensing by Escherichia coli topoisomerase IV and the mechanism of type II topoisomerases. Proc. Natl. Acad. Sci. USA 2003;100:8654–8659. [PubMed: 12857958]
22. Charvin G, Bensimon D, Croquette V. Single-molecule study of DNA unlinking by eukaryotic and prokaryotic type-II topoisomerases. Proc. Natl. Acad. Sci. USA 2003;100:9820–9825. [PubMed: 12902541]
23. Lynn R, Giaever G, Swanberg SL, Wang JC. Tandem regions of yeast DNA topoisomerase II share homology with different subunits of bacterial gyrase. Science 1986;233:647–649. [PubMed: 3014661]
24. Wyckoff E, Natalie D, Nolan JM, Lee M, Hsieh T. Structure of the Drosophila DNA topoisomerase II gene. Nucleotide sequence and homology among topoisomerases II. J. Mol. Biol 1989;205:1–13. [PubMed: 2538621]
25. Corbett KD, Shultzaberger RK, Berger JM. The C-terminal domain of DNA gyrase A adopts a DNA-bending beta-pinwheel fold. Proc. Natl. Acad. Sci. USA 2004;101:7293–7298. [PubMed: 15123801]
26. Hsieh TJ, Farh L, Huang WM, Chan NL. Structure of the topoisomerase IV C-terminal domain: a broken beta-propeller implies a role as geometry facilitator in catalysis. J. Biol. Chem 2004;279:55587–55593. [PubMed: 15466871]
27. Corbett KD, Schoeffler AJ, Thomsen ND, Berger JM. The structural basis for substrate specificity in DNA topoisomerase IV. J. Mol. Biol 2005;351:545–561. [PubMed: 16023670]
28. McClendon AK, Osheroff N. The Geometry of DNA Supercoils Modulates Topoisomerase-mediated DNA Cleavage and Enzyme Response to Anticancer Drugs. Biochemistry 2006;45:3040–3050. [PubMed: 16503659]
29. Lavrukhin OV, Fortune JM, Wood TG, Burbank DE, Van Etten JL, Osheroff N, Lloyd RS. Topoisomerase II from Chlorella virus PBCV-1. Characterization of the smallest known type II topoisomerase. J. Biol. Chem 2000;275:6915–6921. [PubMed: 10702252]
30. Fortune JM, Lavrukhin OV, Gurnon JR, Van Etten JL, Lloyd RS, Osheroff N. Topoisomerase II from Chlorella virus PBCV-1 has an exceptionally high DNA cleavage activity. J. Biol. Chem 2001;276:24401–24408. [PubMed: 11323425]
31. Fortune JM, Dickey JS, Lavrukhin OV, Van Etten JL, Lloyd RS, Osheroff N. Site-specific DNA cleavage by Chlorella virus topoisomerase II. Biochemistry 2002;41:11761–11769. [PubMed: 12269818]
32. Dickey JS, Choi TJ, Van Etten JL, Osheroff N. Chlorella virus Marburg topoisomerase II: high DNA cleavage activity as a characteristic of Chlorella virus type II enzymes. Biochemistry 2005;44:3899–3908. [PubMed: 15751965]

33. Rodriguez AC. Studies of a positive supercoiling machine. Nucleotide hydrolysis and a multifunctional “latch” in the mechanism of reverse gyrase. J. Biol. Chem 2002;277:29865–29873. [PubMed: 12048189]

34. Fortune JM, Osheroff N. Merbarone inhibits the catalytic activity of human topoisomerase IIα by blocking DNA cleavage. J. Biol. Chem 1998;273:17643–17650. [PubMed: 9651360]

35. O'Reilly EK, Kreuzer KN. A unique type II topoisomerase mutant that is hypersensitive to a broad range of cleavage-inducing antitumor agents. Biochemistry 2002;41:7989–7997. [PubMed: 12069589]

36. Baldwin EL, Byl JA, Osheroff N. Cobalt enhances DNA cleavage mediated by human topoisomerase IIα in vitro and in cultured cells. Biochemistry 2004;43:728–735. [PubMed: 14730977]

37. Schoeffler AJ, Berger JM. Recent advances in understanding structure-function relationships in the type II topoisomerase mechanism. Biochem. Soc. Trans 2005;33:1465–1470. [PubMed: 16246147]

38. Corbett AH, echiedrich EL, Osheroff N. A role for the passage helix in the DNA cleavage reaction of eukaryotic topoisomerase II. A two-site model for enzyme-mediated DNA cleavage. J. Biol. Chem 1992;267:683–686. [PubMed: 1309770]

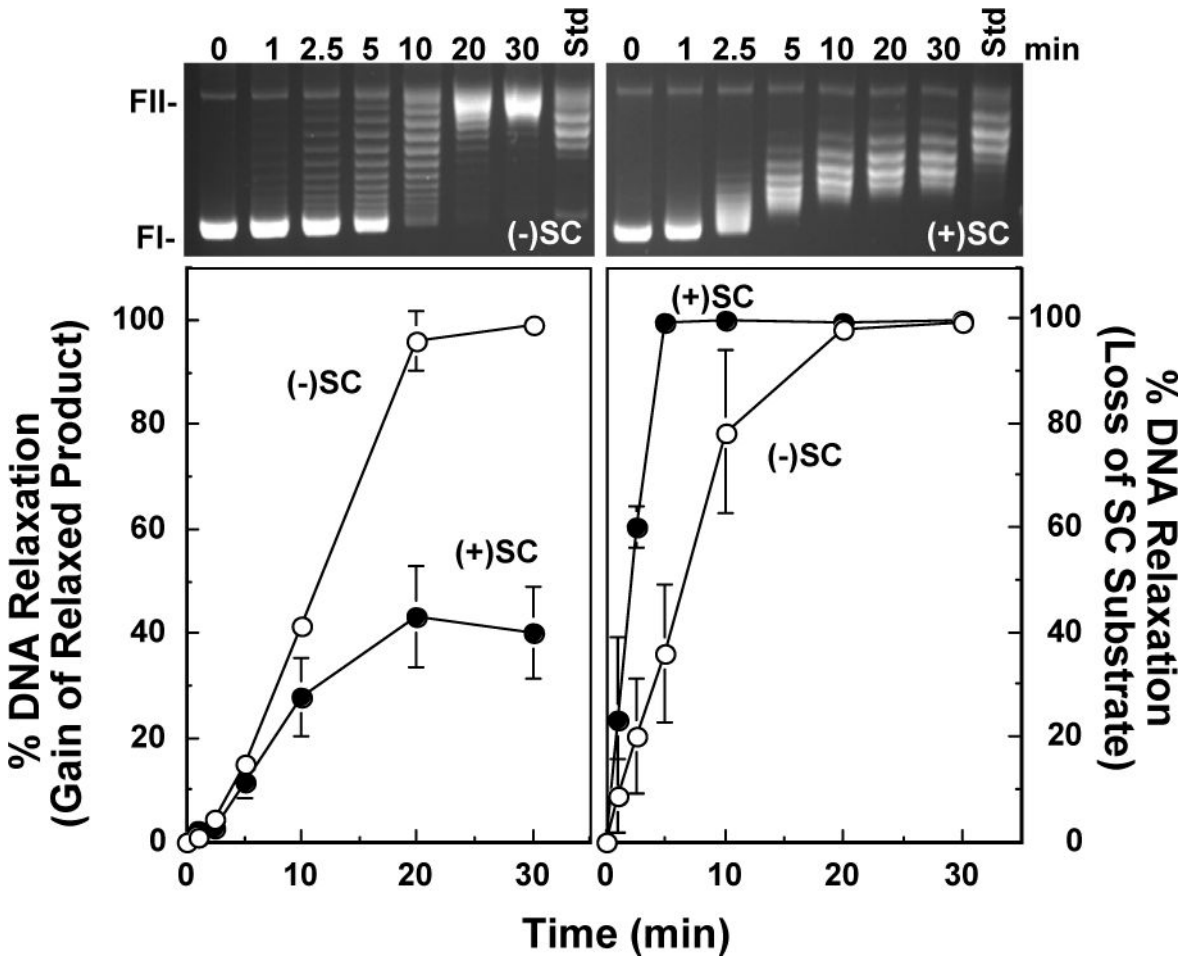

FIGURE 1. PBCV-1 topoisomerase II relaxes negatively and positively supercoiled plasmid DNA at similar rates

Ethidium bromide-stained gels (top) depict a time course for relaxation of negatively [(-)SC] or positively [(+)SC] supercoiled pBR322 plasmid DNA by PBCV-1 topoisomerase II. The positions of the supercoiled DNA (form I, FI) and relaxed DNA (form II, FII) are indicated. DNA relaxation was quantified from either the gain of relaxed product (left panel) or the loss of supercoiled substrate (right panel). Data for negatively supercoiled substrates are represented by open symbols and positively supercoiled substrates by closed symbols. Error bars represent the standard deviation of three to four independent experiments.

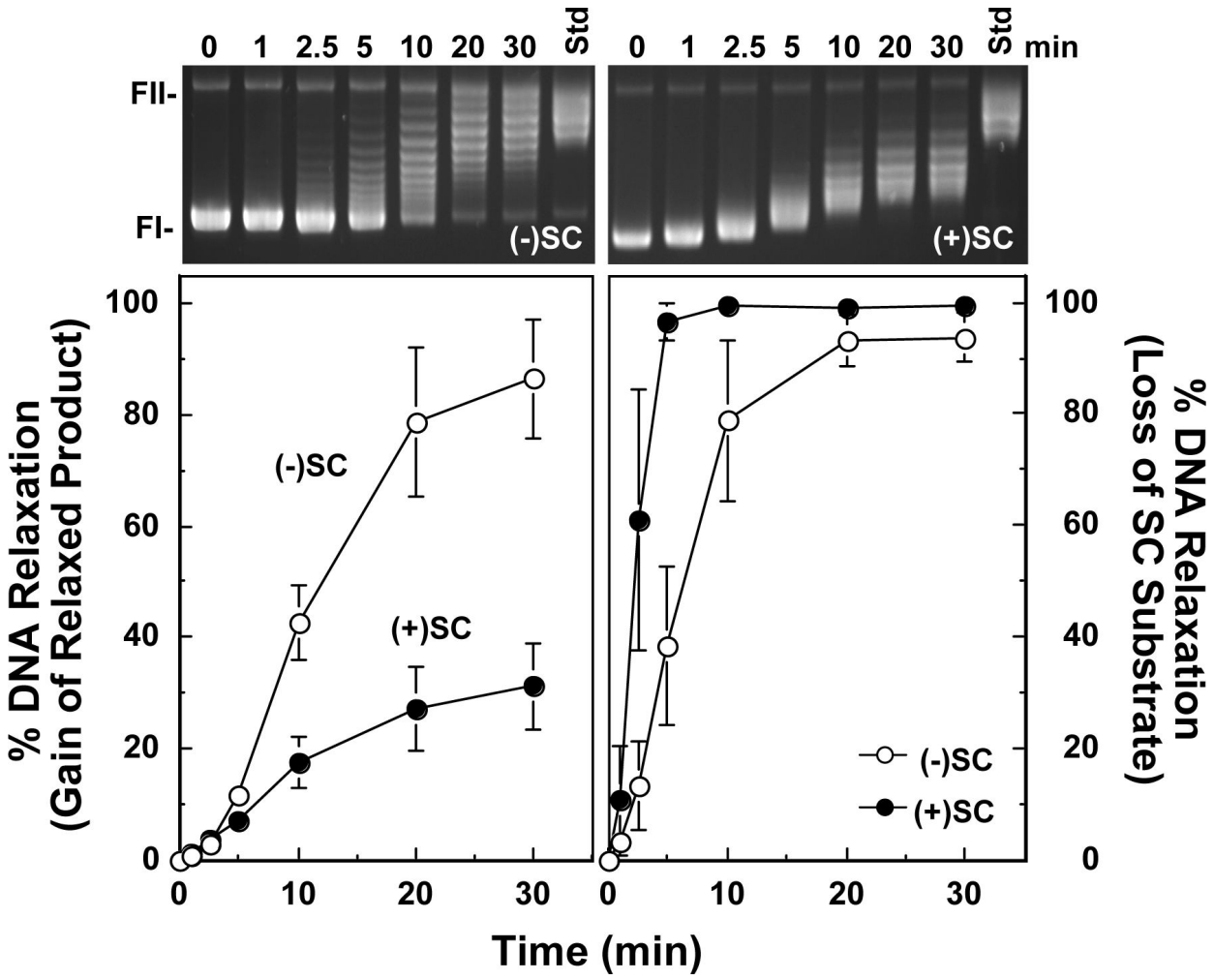

FIGURE 2. CVM-1 topoisomerase II relaxes negatively and positively supercoiled plasmid DNA at similar rates

Ethidium bromide-stained gels (top) depict a time course for relaxation of negatively [(-)SC] or positively [(+)SC] supercoiled pBR322 plasmid DNA by CVM-1 topoisomerase II. The positions of the supercoiled DNA (form I, FI) and relaxed DNA (form II, FII) are indicated. DNA relaxation was quantified from either the gain of relaxed product (left panel) or the loss of supercoiled substrate (right panel). Data for negatively supercoiled substrates are represented by open symbols and positively supercoiled substrates by closed symbols. Error bars represent the standard deviation of three to four independent experiments.

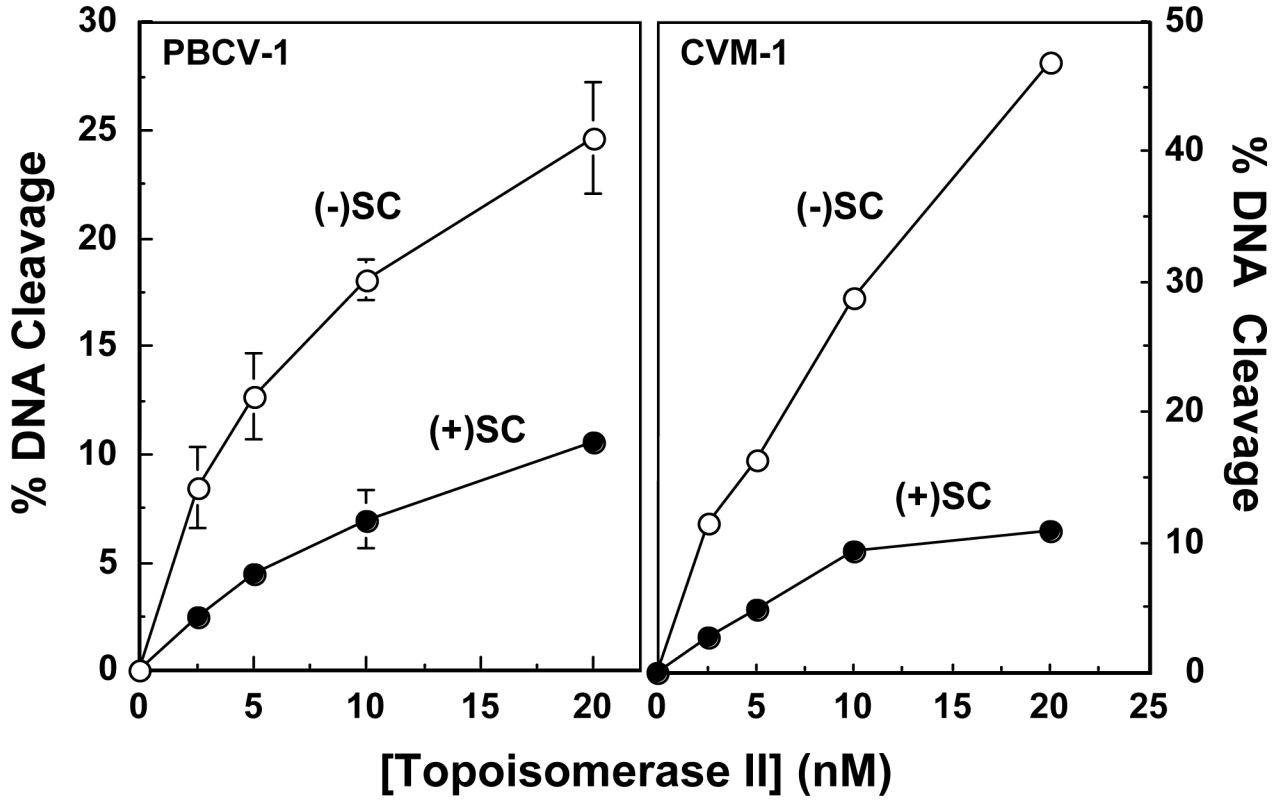

FIGURE 3. Type II topoisomerases from chlorella viruses maintain lower levels of DNA cleavage complexes with positively supercoiled DNA than with negatively supercoiled molecules

The ability of 0−20 nM PBCV-1 (left panel) or CVM-1 (right panel) topoisomerase II to cleave 10 nM negatively [(-)SC, open symbols] or positively [(+)SC, closed symbols] supercoiled pBR322 plasmid DNA is shown. Error bars represent the standard deviation of three to five independent experiments.

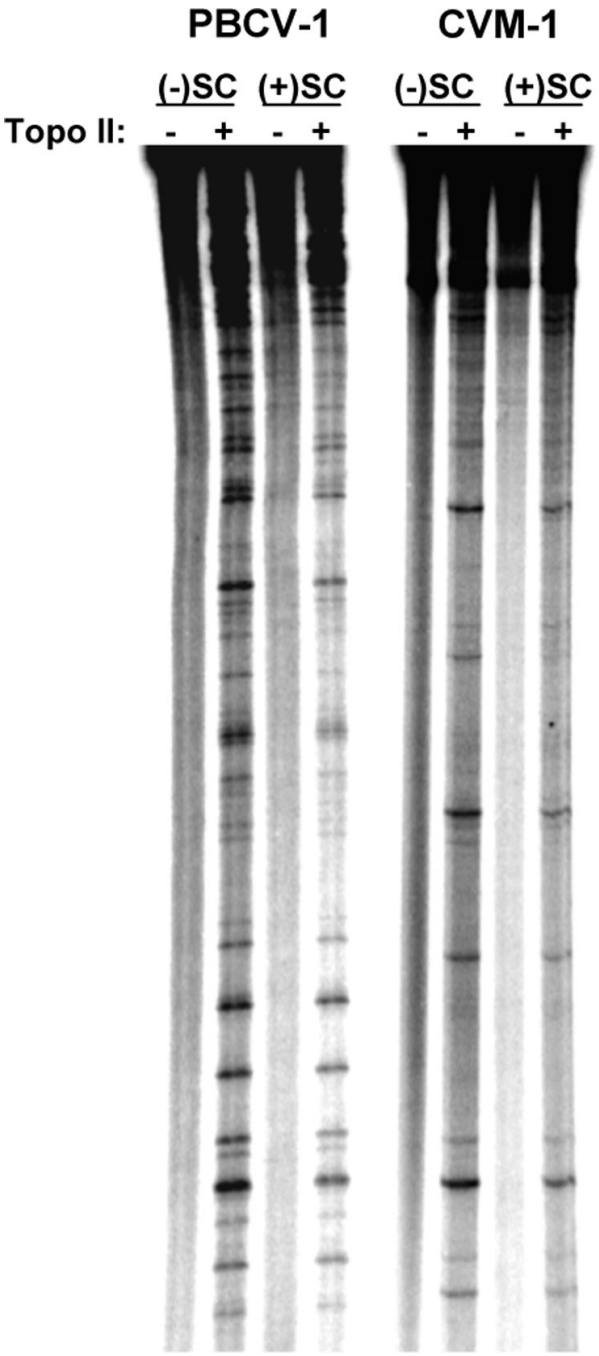

FIGURE 4. The DNA cleavage specificity of chlorella virus type II topoisomerases is not altered by the handedness of the supercoiled substrate

Sites cleaved by PBCV-1 or CVM-1 topoisomerase II were mapped in negatively [(-)SC] or positively [(+)SC] supercoiled pBR322 plasmid DNA. Products of DNA cleavage assays were linearized and singly-end labeled with [^{32}P]phosphate. The autoradiogram is representative of at least two independent assays.

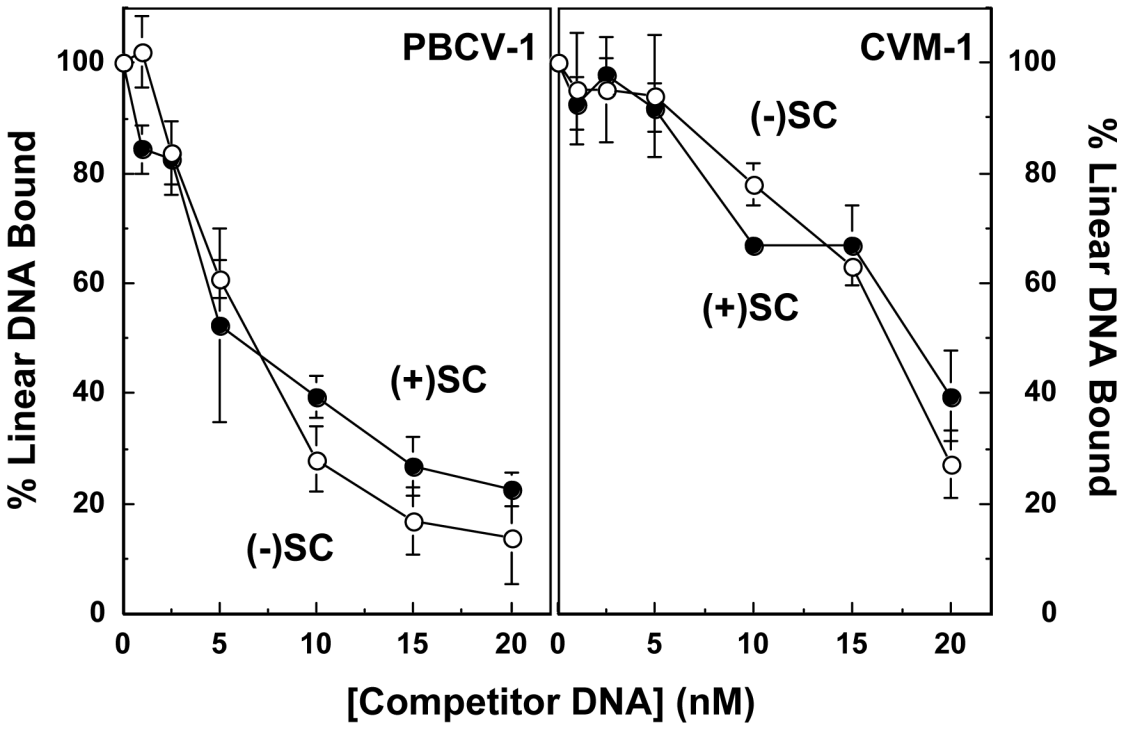

FIGURE 5. Type II topoisomerases from chlorella viruses display similar binding affinities for negatively and positively supercoiled DNA

The ability of 0−20 nM negatively supercoiled [(-)SC, open symbols] or positively [(+)SC, closed symbols] supercoiled pBR322 plasmid DNA to compete with the binding of 5 nM [^{32}P]-labeled linear pBR322 DNA by PBCV-1 (left panel) or CVM-1 (right panel) topoisomerase II is shown. Percent linear DNA bound was determined by the ratio of cpm retained on a nitrocellulose filter *vs.* the input amount of radioactivity. Error bars represent the standard deviation of three independent assays.

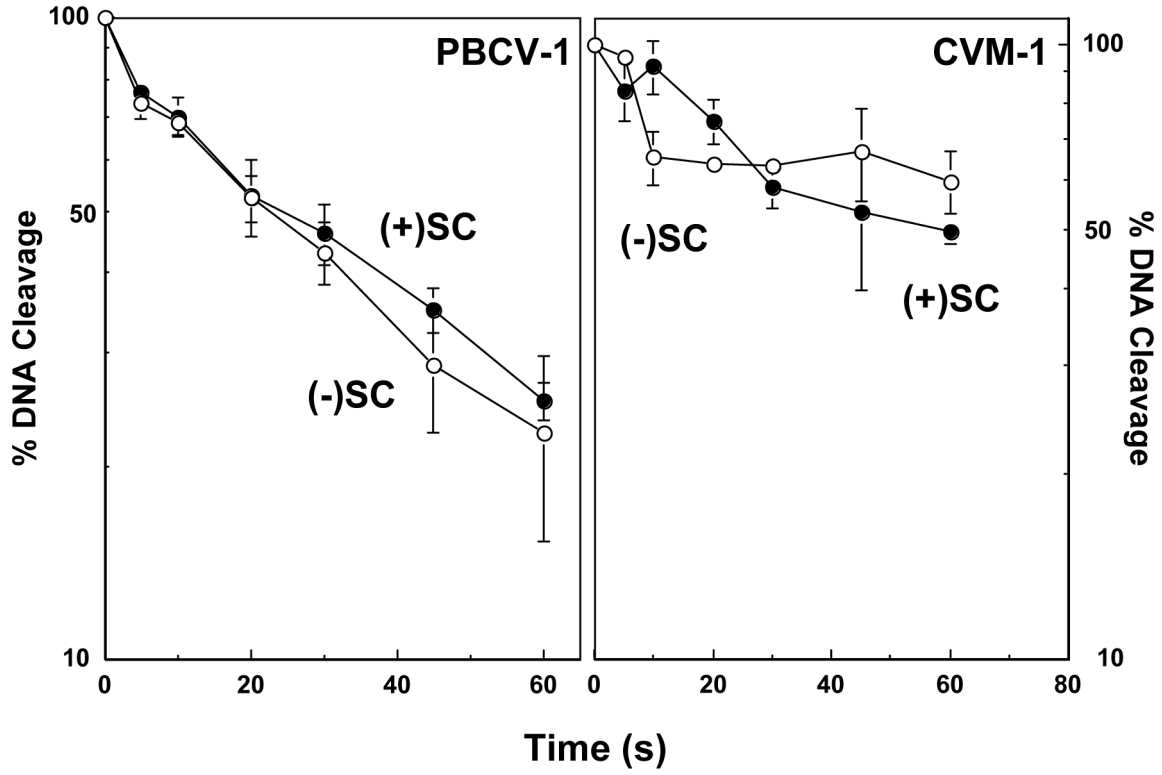

FIGURE 6. Type II topoisomerases from chlorella viruses display similar rates of religation with negatively and positively supercoiled DNA

A time course of religation in the presence of negatively [(-)SC, open symbols] or positively [(+)SC, closed symbols] supercoiled pBR322 plasmid by PBCV-1 (left panel) or CVM-1 (right panel) is shown. DNA religation was initiated by shifting the divalent cation (PBCV-1) or the reaction temperature (CVM-1) as described under Experimental Procedures. The initial level of DNA cleavage was set to 100% and the rate of ligation was determined by quantifying the loss of the cleaved DNA over time. Error bars represent the standard deviation of three independent assays.