Bimodal Recognition of DNA Geometry by Human Topoisomerase IIα: Preferential Relaxation of Positively Supercoiled DNA Requires Elements in the C-Terminal Domain†

A. Kathleen McClendon,‡ Amanda C. Gentry,‡ Jennifer S. Dickey,‡ Marie Brinch,§ Simon Bendsen,§ Anni H. Andersen,§ and Neil Osheroff*,‡,||

Departments of Biochemistry and Medicine (Hematology/Oncology), Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, and Department of Molecular Biology, University of Aarhus, 8000 Århus C, Denmark

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ABSTRACT: Human topoisomerase IIα, but not topoisomerase II*β*, can sense the geometry of DNA during relaxation and removes positive supercoils >10-fold faster than it does negative superhelical twists. In contrast, both isoforms maintain lower levels of DNA cleavage intermediates with positively supercoiled substrates. Since topoisomerase IIα and II*β* differ primarily in their C-terminal domains (CTD), this portion of the protein may play a role in sensing DNA geometry. Therefore, to more fully assess the importance of the topoisomerase IIα CTD in the recognition of DNA topology, hTop2αΔ1175, a mutant human enzyme that lacks its CTD, was examined. The mutant enzyme relaxed negative and positive supercoils at similar rates but still maintained lower levels of cleavage complexes with positively supercoiled DNA. Furthermore, when the CTD of topoisomerase II*β* was replaced with that of the α isoform, the resulting enzyme preferentially relaxed positively supercoiled substrates. In contrast, a chimeric topoisomerase IIα that carried the CTD of the *β* isoform lost its ability to recognize the geometry of DNA supercoils during relaxation. These findings demonstrate that human topoisomerase IIα recognizes DNA geometry in a bimodal fashion, with the ability to preferentially relax positive DNA supercoils residing in the CTD. Finally, results with a series of human topoisomerase IIα mutants suggest that clusters of positively charged amino acid residues in the CTD are required for the enzyme to distinguish supercoil geometry during DNA relaxation and that deletion of even the most C-terminal cluster abrogates this recognition.

Topoisomerases are essential enzymes that modulate the topological state of DNA in the cell (*1*−*7*). 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 (*3, 4, 7*−*9*). As a consequence of their double-stranded DNA passage reactions, type II topoisomerases are able to alleviate torsional stress in duplex DNA and remove knots and tangles from the genetic material (*1*−*7*).

Based on amino acid comparisons to prokaryotic type II enzymes, eukaryotic topoisomerase II can be divided into three domains (*1, 4, 7, 10*−*12*). The N-terminal (or ATPase) domain contains the site for ATP binding and hydrolysis that is required for the DNA strand passage event. The central (or DNA cleavage/ligation) domain contains the active site tyrosyl residue that covalently attaches to the 5′-terminus of DNA during the scission event. These two domains are highly conserved in all eukaryotes. In contrast, the C-terminal domain (CTD)[1]varies from species to species. This portion of the protein is not required for catalytic activity, and its contributions to the enzymatic actions of eukaryotic type II topoisomerases have remained obscure. The CTD appears to be important for the cellular physiology of topoisomerase II and contains nuclear localization signals and sites of phosphorylation (*13*−*20*). Furthermore, recent work suggests that the CTD contributes to the chromosomal localization of topoisomerase II isoforms and the mitotic functions of topoisomerase IIα (*21*). However, it is not known whether this proposed function of the CTD is mediated by direct DNA contacts or by protein−protein interactions.

Vertebrates express two closely related isoforms of topoisomerase II, α and *β* (*1*−*9, 22*−*24*). While these isoforms display similar enzymological characteristics, topoisomerase IIα and II*β* play distinct physiological roles (*2, 5, 20, 22*−*27*). Topoisomerase IIα is believed to be the isoform that functions in growth-dependent processes, including mitosis and DNA replication (*2, 5, 28, 29*). The enzyme works behind replication forks to resolve precatenanes and later in the cell cycle to unlink intertwined daughter chromosomes

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* To whom correspondence should be addressed: tel, 615-343-4338; fax, 615-343-1166; e-mail, neil.osheroff@vanderbilt.edu.

‡ Department of Biochemistry, Vanderbilt University School of Medicine.

§ University of Aarhus.

|| Department of Medicine (Hematology/Oncology), Vanderbilt University School of Medicine.

[1] Abbreviation: CTD, C-terminal domain.

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that were not resolved during replication (*2*−*5*). While this decatenation activity appears to be the essential function of topoisomerase IIα, evidence suggests that the enzyme also may act ahead of the replication machinery to help alleviate the acute overwinding (i.e., positive supercoiling) that is created by the actions of replicative helicases (*3, 30, 31*).

Several lines of evidence support a role for type II topoisomerases, including topoisomerase IIα, ahead of replication forks. First, *Escherichia coli* topoisomerase IV (whose functions in bacteria appear to parallel those of topoisomerase II in eukaryotes) can partially compensate for the loss of DNA gyrase during replication elongation (*32, 33*). Second, topoisomerase II can compensate for the loss of topoisomerase I in *Saccharomyces cerevisiae*, but the loss of both enzymes abruptly halts DNA synthesis (*34, 35*). This finding indicates that the type II enzyme can assume the role of topoisomerase I, relaxing positive DNA supercoils ahead of the replication machinery. A role for topoisomerase II ahead of the replication fork in yeast is further implied by monitoring the influence of enzyme activity on levels of DNA supercoiling in the 2 μM plasmid during S phase (*36*). When the temperature was raised to the nonpermissive level in a strain that harbored human topoisomerase I and a temperature-sensitive yeast topoisomerase II mutant, levels of positive supercoiling increased several fold. This conclusion is consistent with a recent study that mapped interactions between topoisomerase I and topoisomerase II and active origins of replication (*37*). These authors concluded that the type I and II enzymes acted in a coordinated fashion to allow progression of the replication fork in yeast. Third, in mammalian cells, topoisomerase II activity is required as a swivelase in the late stage of SV40 DNA replication (*38*). In addition, topoisomerase IIα, but not topoisomerase IIβ, is associated with DNA replication forks in human cells (*39*).

If type II topoisomerases play a role ahead of replication forks, then positively supercoiled (i.e., overwound) DNA should be the preferred relaxation substrate. To this point, bacterial topoisomerase IV relaxes positive DNA supercoils ~20-fold faster than it does negative supercoils (*40, 41*). In addition, topoisomerase IIα, the isoform that is involved in replicative processes in vertebrates, relaxes positively supercoiled plasmids >10-fold faster than negatively supercoiled (i.e., underwound) molecules (*31*). In contrast, the enzyme maintains lower levels of DNA cleavage complexes with positively supercoiled substrates, reducing the probability that collisions with replication enzymes would generate permanent double-stranded breaks in the genome (*31*). Topoisomerase IIβ, which is not involved in replicative processes in vertebrates, displays no preferential relaxation of positively supercoiled DNA but does maintain lower levels of cleavage complexes with these substrates (*31, 42*).

Although topoisomerase IIα and IIβ possess a high degree of amino acid sequence identity in their N-terminal ATPase and central DNA cleavage/ligation domains (~79% identity), the two enzymes diverge considerably in their CTDs (~31% identity) (*3, 4, 7, 10, 11, 43*). On the basis of amino acid sequence differences between topoisomerase IIα and IIβ, it was suggested that the ability of the α isoform to preferentially relax positive DNA supercoils resides in the variable CTD of the protein (*31*). Furthermore, the ability of type II topoisomerases to discern DNA geometry during cleavage resides within the conserved N-terminal or central domain of the protein (*31*).

The structure of the CTD of the ParC subunit of topoisomerase IV, which is equivalent to the CTD of eukaryotic topoisomerase II, has been solved (*44, 45*). Modeling studies, coupled with nucleic acid binding experiments, led to the proposal that the CTD of topoisomerase IV interacts with the T-segment [i.e., transport segment (*8*)] of DNA and plays an integral role in the recognition of supercoil geometry during strand passage (*44*−*46*). Moreover, deletion of the CTD of ParC results in an enzyme that no longer preferentially relaxes positively supercoiled molecules (*45*). Finally, two related chlorella virus type II enzymes, PBCV-1 and CVM-1 topoisomerase II, which display a high degree of amino acid sequence identity to human topoisomerase IIα but naturally lack a corresponding CTD, relax negatively and positively supercoiled substrates at similar rates. However, both viral enzymes maintain lower levels of cleavage complexes with positively supercoiled molecules (*47*).

To more fully assess the role of the CTD of topoisomerase IIα in the recognition of DNA topology, hTop2αΔ1175 (*48*), a mutant human enzyme that lacks its CTD, was constructed. The mutant enzyme relaxed negatively and positively supercoiled plasmids at similar rates but still maintained lower levels of cleavage complexes with positively supercoiled DNA. Furthermore, when the CTD of topoisomerase IIβ was replaced with that of the α isoform, the resulting enzyme (hTop2βCTDα) preferentially relaxed positively supercoiled substrates. These findings confirm the hypothesis that human topoisomerase IIα recognizes DNA geometry in a bimodal fashion, with the ability to preferentially relax positive DNA supercoils residing in the CTD. Finally, results with a series of human topoisomerase IIα mutants suggest that clusters of positively charged amino acid residues in the CTD are required for the enzyme to distinguish supercoil geometry during DNA relaxation and that deletion of even the most C-terminal cluster abrogates this recognition.

EXPERIMENTAL PROCEDURES

Enzymes and Materials. Human topoisomerase IIα, hTop2αΔ1175, hTop2αCTDβ, and hTop2βCTDα were expressed in *S. cerevisiae* JEL-1Δ*top1* and purified as described by Kingma et al. (*49*). hTop2αΔ1175 was constructed as described previously (*48*). As a prelude to the construction of hTop2αCTDβ and hTop2βCTDα, a *Kpn*I−*Sal*I fragment of hTop2β from YepWob6 plasmid was inserted into puc18 plasmid and subjected to PCR to introduce an *Avr*II site beginning at nt3157 of hTop2β. The following forward and reverse primers were used, respectively: GTTATTACGGTTTACGTAAGGAGTGGCTCCTAGGAATGTTGGG and CCCAACATTCCTAGGAGCCACTCCTTACGTAAACCGTAATAAC. DNA was subjected to 16 cycles of PCR using the following program: denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and primer extension at 68 °C for 8 min. Following the last round of PCR, primers were allowed to extend for an additional 5 min at 68 °C. The new insert was digested with *Kpn*I and *Sal*I and ligated back into the previously digested YepWob6 plasmid. Human topoisomerases IIα and IIβ in YepWob6 plasmid were then digested with *Avr*II and *Xma*I to release C-terminal fragments beginning at nt3111

in topoisomerase IIα (amino acid 1037) and at the corresponding nt3159 (amino acid 1053) in topoisomerase IIβ. The CTDs were gel purified and ligated back into the *Avr*II and *Xma*I digested YepWob6 plasmid containing the opposite isoform. A set of mutant human topoisomerase IIα enzymes that were partially deleted in their CTD or that replaced amino acids starting with residue 1501 with the corresponding portion of human topoisomerase IIβ were constructed, expressed in *S. cerevisiae* JEL-1Δ*top1* under the control of the yeast Gal promoter, and purified as described previously (*50*). All chemicals were of analytical reagent grade.

Negatively supercoiled pBR322 plasmid 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 (*31, 51*). 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 (*31*). 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 ($\sigma \approx -0.035$ 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 ($\sigma \approx +0.035$ to $+0.039$). The handedness of positively supercoiled DNA was confirmed by two-dimensional gel electrophoresis (*31*). 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 protocol of McClendon et al. (*31*). Reaction mixtures contained 1−2 nM wild-type or mutant human topoisomerase IIα enzymes, 1 mM ATP, and 5 nM negatively or positively supercoiled pBR322 DNA in a total of 20 μL of 10 mM Tris-HCl, pH 7.9, 175 mM KCl, 5 mM $MgCl_2$, 0.1 mM NaEDTA, and 2.5% glycerol. Mixtures were incubated at 37 °C for times up to 60 min and 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, and 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 both by the formation of the fully relaxed DNA product and by the loss of the initial supercoiled substrate.

DNA Cleavage. DNA cleavage reactions were based on the procedure of McClendon et al. (*31*). Reaction mixtures contained 0−800 nM hTop2αΔ1175 and 10 nM negatively or positively supercoiled pBR322 DNA in a total of 20 μL of DNA cleavage buffer (10 mM Tris-HCl, pH 7.9, 100 mM KCl, 5 mM $MgCl_2$, 0.1 mM NaEDTA, and 2.5% glycerol). Mixtures were incubated for 6 min at 37 °C to establish cleavage/religation equilibria. DNA cleavage intermediates were trapped by the addition of 2 μL of 5% SDS and 2 μL of 250 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 (*3*).

DNA Binding. The ability of hTop2αΔ1175 to bind negatively and positively supercoiled DNA was assessed using a competitive nitrocellulose filter-binding assay (*31*). Binding mixtures contained 400 nM hTop2αΔ1175, 5 nM linear pBR322 DNA that was cleaved with *Hin*dIII and terminally labeled with [^{32}P]phosphate, and 0−20 nM negatively or positively supercoiled DNA in a total of 20 μL of binding buffer (10 mM Tris-HCl, pH 7.9, 175 mM KCl, 0.1 mM EDTA, and 2.5% glycerol). Samples were incubated at 37 °C 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 three 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 IIα 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 Ligation. The ability of hTop2αΔ1175 to ligate cleaved DNA was monitored by the procedure of Fortune et al. (*52*). DNA cleavage/ligation equilibria were established in DNA cleavage buffer as described above except that $MgCl_2$ in the reaction buffer was replaced by 5 mM $CaCl_2$. Enzyme−DNA cleavage complexes were trapped by the addition of EDTA, pH 8.0, to a 6 mM final concentration. NaCl was added to a 500 mM final concentration in order to prevent recleavage of the DNA following ligation. Ligation was initiated by the addition of $MgCl_2$ at a 0.25 mM final concentration and terminated at times up to 60 s by the addition of 2 μL of 5% SDS. Samples were processed and analyzed as described for DNA cleavage reactions. 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.

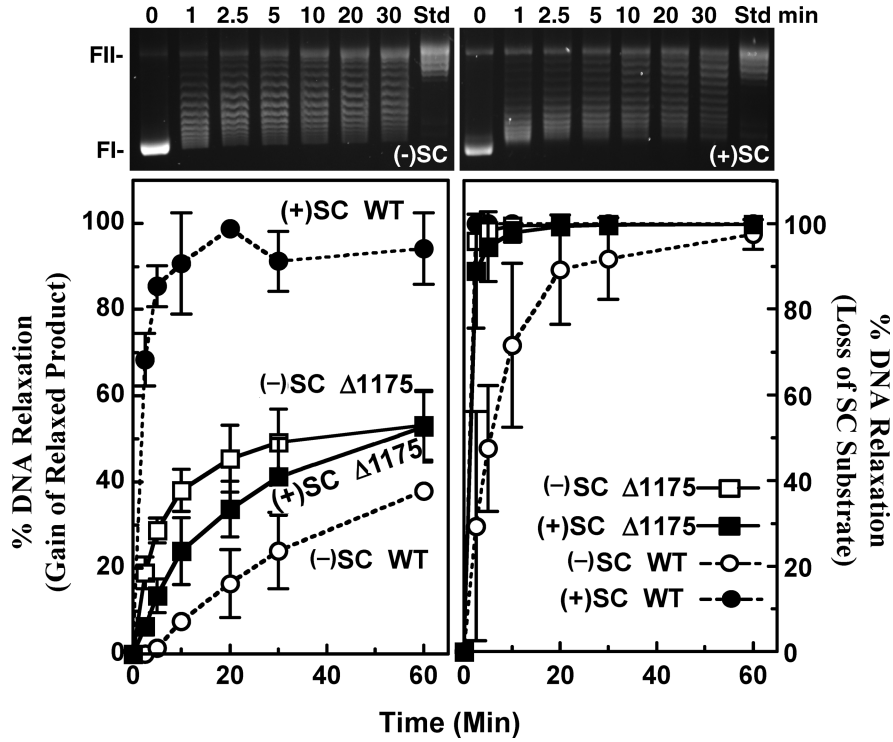

FIGURE 1: Relaxation of negatively and positively supercoiled substrates by hTop2αΔ1175. Ethidium bromide-stained gels (top) depict a time course for relaxation of negatively [(−)SC, left] or positively supercoiled [(+)SC, right] pBR322 plasmid DNA by hTop2αΔ1175. The positions of supercoiled (form I, FI) and nicked 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). Relaxation of negatively (open boxes) and positively (closed boxes) supercoiled substrates by hTop2αΔ1175 (solid lines) is shown. Relaxation by the full-length enzyme (dashed lines) is included for comparison (from ref *31*). Error bars represent the standard deviation of at least three independent experiments.

RESULTS

Recognition of DNA Supercoil Geometry by hTop2αΔ1175. Human topoisomerase IIα senses the geometry of supercoiled DNA during the strand passage reaction and relaxes positively supercoiled DNA >10-fold faster than it does negatively supercoiled molecules (see Figure 1, dashed lines) (*31*). It has been suggested that the CTD of topoisomerase IIα plays an important role in discerning DNA geometry during relaxation (*31*). As an initial test of the hypothesis, the ability of hTop2αΔ1175 to relax negatively and positively supercoiled DNA was characterized. This mutant form of human topoisomerase IIα is truncated following amino acid residue 1175, which corresponds to the C-terminal residue of PBCV-1 topoisomerase II (*48, 53*). hTop2αΔ1175 allows a direct comparison between full-length human topoisomerase IIα and an equivalent enzyme that contains only its N-terminal ATPase and central DNA cleavage/ligation domains.

Since human topoisomerase IIα relaxes positively supercoiled DNA in a less processive manner than it does negatively supercoiled molecules (*31*), DNA relaxation was monitored by quantifying either the gain of relaxed product (Figure 1, left panel) or the loss of supercoiled substrate (Figure 1, right panel). Removal of the CTD of the human enzyme abrogated the ability of topoisomerase IIα to sense DNA geometry during the strand passage reaction. Rates of relaxation of negatively and positively supercoiled plasmids catalyzed by hTop2αΔ1175, determined by either method of analysis, were similar. If anything, negatively supercoiled molecules were relaxed slightly faster than positively supercoiled plasmids. This finding strongly suggests that the ability of human topoisomerase IIα to sense the handedness of its DNA substrate during the strand passage reaction resides in the CTD of the protein.

As a prerequisite to the strand passage event, type II topoisomerases generate a transient double-stranded break in the genetic material (*1, 3, 4, 6*−*8, 30*). A previous study found that human topoisomerase IIα maintained a lower level of DNA cleavage intermediates (3−4-fold) when positively supercoiled, as opposed to negatively supercoiled, plasmids were employed as substrates (*31*). Since topoisomerase IIβ and the chlorella virus enzymes show similar DNA cleavage patterns with underwound and overwound molecules, it has been suggested that the ability to discern DNA geometry during cleavage resides in the N-terminal and/or central domain of the type II enzyme (*42, 47*). As a direct test of this proposal for human topoisomerase IIα, the ability of hTop2αΔ1175 to cleave negatively and positively supercoiled DNA was assessed (Figure 2). Similar to findings with full-length topoisomerase IIα, the truncated human enzyme maintained lower levels (∼2−3-fold) of cleavage complexes with positively supercoiled molecules.

To further characterize the recognition of DNA geometry by the N-terminal and central domains of topoisomerase IIα, the ability of hTop2αΔ1175 to bind and ligate underwound and overwound substrates was determined. A previous study with wild-type human topoisomerase IIα found that the full-length enzyme bound negatively supercoiled plasmids slightly better than positively supercoiled molecules (*31*). In addition, the wild-type enzyme ligated under- and overwound molecules at similar rates (*42*).

Comparable results were observed for hTop2αΔ1175. As determined by competition nitrocellulose filter-binding assays

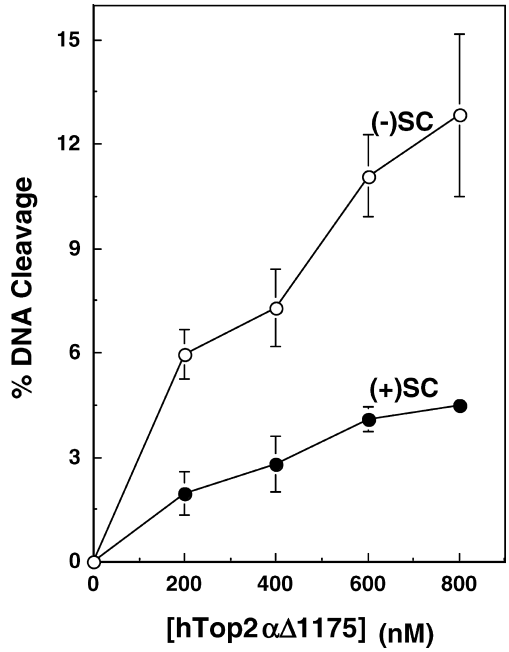

FIGURE 2: Cleavage of negatively and positively supercoiled substrates by hTop2αΔ1175. The ability of hTop2αΔ1175 to cleave negatively [(−)SC, open circles] or positively [(+)SC, closed circles] supercoiled pBR322 plasmid DNA is shown. Error bars represent the standard deviation of three independent experiments.

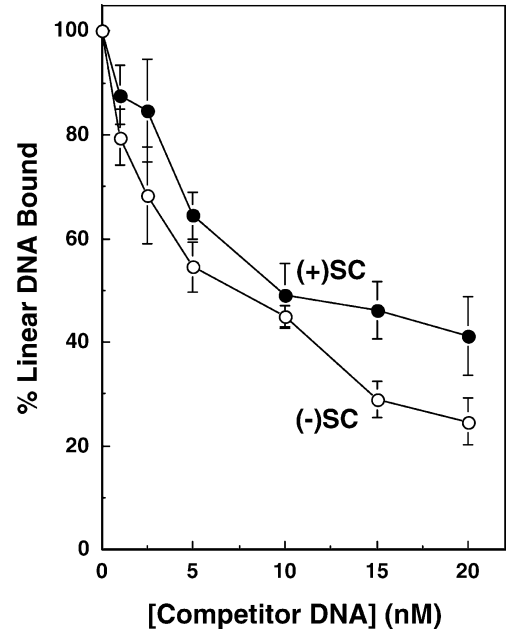

FIGURE 3: Binding of hTop2αΔ1175 to negatively and positively supercoiled DNA. The ability of negatively [(−)SC, open circles] or positively [(+)SC, closed circles] supercoiled pBR322 plasmid DNA to compete with the binding of ^{32}P-labeled linear pBR322 DNA by hTop2αΔ1175 is shown. The percentage of linear DNA bound was determined by the ratio of the cpm retained on a nitrocellulose filter versus the input amount of radioactivity. Error bars represent the standard deviations of three independent experiments.

that monitored the ability of plasmids to displace radioactively labeled DNA from topoisomerase II, the truncated human enzyme bound negatively and positively supercoiled plasmids similarly, with a slight preference for negatively supercoiled DNA (Figure 3). In addition, the enzyme ligated negatively and positively supercoiled molecules at similar rates (Figure 4).

Taken together, the above findings demonstrate that topoisomerase IIα recognizes DNA geometry in a bimodal fashion. While the ability of the human enzyme to sense the handedness of its DNA substrate during strand passage resides in the CTD, the elements that sense DNA geometry during cleavage and ligation must be located in the N-terminal and/or central domain of the protein.

The CTD of Human Topoisomerase IIα Confers the Ability To Recognize Supercoil Geometry during DNA Relaxation. As demonstrated above using hTop2αΔ1175, removal of the CTD of topoisomerase IIα abolishes the ability of the human enzyme to recognize supercoil geometry during DNA relaxation. While this finding implies a direct role for the CTD in discerning the handedness of DNA supercoils during the relaxation process, it is possible that this portion of the enzyme does not interact directly with the DNA substrate. Rather, removing the CTD may alter the structure of topoisomerase IIα and prevent the recognition of DNA topology. Therefore, to more fully examine the role of the CTD of human topoisomerase IIα in the recognition of supercoil geometry during DNA relaxation, two additional mutant enzymes were constructed in which the CTDs of human topoisomerase IIα and IIβ were switched.

First, the ability of topoisomerase IIα that carried the CTD of topoisomerase IIβ (hTop2αCTDβ) to relax negatively and positively supercoiled DNA was determined. As seen in Figure 5, replacement of the CTD of topoisomerase IIα with

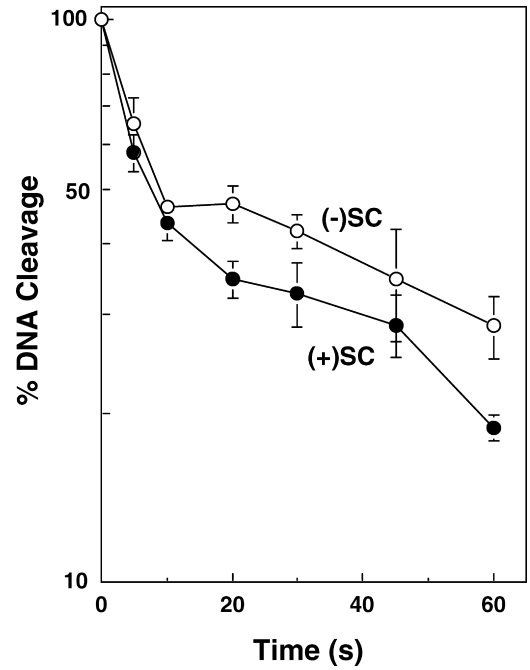

FIGURE 4: Ligation of negatively and positively supercoiled DNA by hTop2αΔ1175. A time course of ligation of negatively [(−)SC, open circles] or positively [(+)SC, closed circles] supercoiled plasmid pBR322 is shown. The initial level of cleavage was set to 100%, and levels of ligation were determined by quantifying the loss of cleaved DNA over time. Error bars represent the standard deviations of three independent experiments.

that of the β isoform resulted in an enzyme that no longer recognized supercoil geometry during relaxation. hTop2α-CTDβ relaxed negatively and positively supercoiled substrates with rates that differed by only 2-fold.

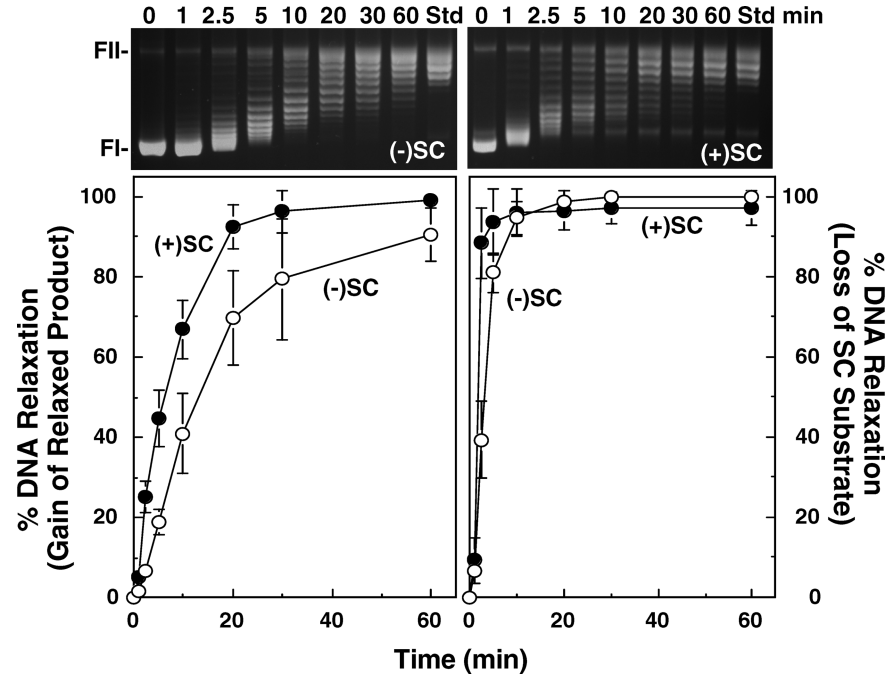

FIGURE 5: Relaxation of negatively and positively supercoiled substrates by hTop2αCTDβ. Ethidium bromide-stained gels (top) depict a time course for relaxation of negatively [(−)SC, left] or positively [(+)SC, right] supercoiled pBR322 plasmid DNA by hTop2αCTDβ. The positions of supercoiled and nicked DNA are as indicated in Figure 1. 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 circles and positively supercoiled substrates by closed circles. Error bars represent the standard deviation of three independent experiments.

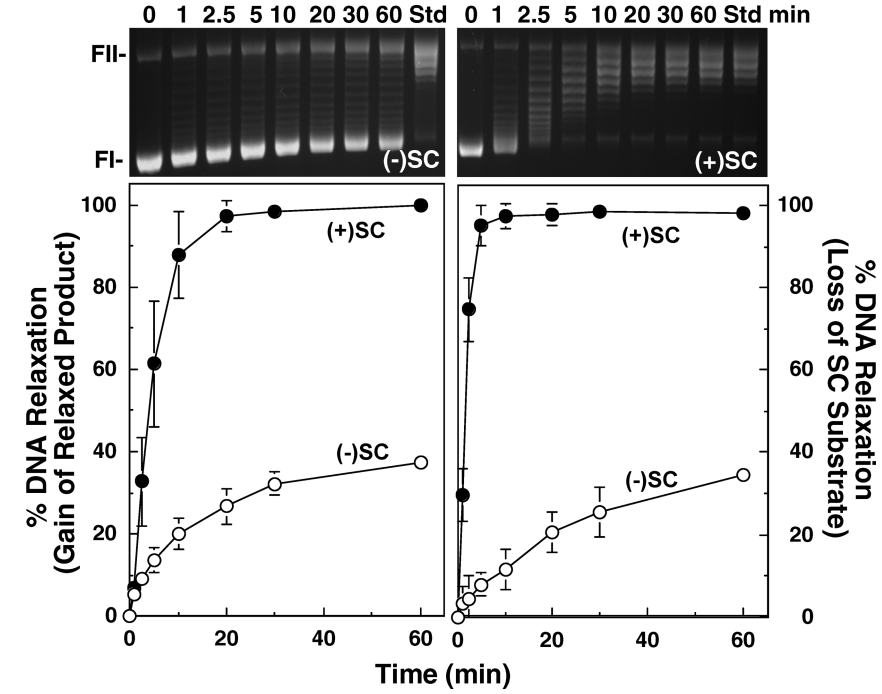

FIGURE 6: Relaxation of negatively and positively supercoiled substrates by hTop2βCTDα. Ethidium bromide-stained gels (top) depict a time course for relaxation of negatively [(−)SC, left] or positively [(+)SC, right] supercoiled pBR322 plasmid DNA by hTop2βCTDα. The positions of supercoiled and nicked DNA are as indicated in Figure 1. 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 circles and positively supercoiled substrates by closed circles. Error bars represent the standard deviation of three independent experiments.

Second, the ability of topoisomerase IIβ that carried the CTD of topoisomerase IIα (hTop2βCTDα) to relax negatively and positively supercoiled DNA was determined. Although wild-type topoisomerase IIβ does not preferentially relax positively supercoiled DNA, the enzyme gained this ability when its CTD was replaced with that of topoisomerase IIα. As seen in Figure 6, hTop2βCTDα relaxed positively supercoiled substrates >10-fold faster than negatively supercoiled molecules.

These results provide strong evidence that the rapid relaxation of positively supercoiled DNA by human topoisomerase IIα is modulated by elements in the CTD.

FIGURE 7: Sequence of the CTD of human topoisomerase IIα. Positively charged amino acid clusters corresponding to those of DNA gyrase and topoIV are demarcated by red bars. Points of C-terminal truncations in topoisomerase IIα mutant enzymes are indicated.

Furthermore, they confirm an active role for the CTD in the recognition of DNA geometry during this process.

Elements in the CTD of Human Topoisomerase IIα That Confer the Ability To Recognize Supercoil Geometry during DNA Relaxation. There is no information available for the three-dimensional structure of the CTD of human topoisomerase IIα. However, crystal structures for the CTDs of the two bacterial type II enzymes, DNA gyrase (GyrA) and topoisomerase IV (ParC), were solved recently (*44*−*46, 54*). The CTD of GyrA adopts a unique fold, called a β-pinwheel, which forms a closed circular structure made up of six "blades" (*46, 54*). The outer rim of each blade contains a cluster of positively charged amino acids that are believed to interact with DNA (*46, 54*). The CTD of ParC adopts a "broken β-propeller" structure that is similar to the β-pinwheel of GyrA but lacks ring closure (*44, 45*). Depending on the bacterial species, the CTD of ParC is predicted to contain as few as three and as many as eight blades. Once again, the outer rims of the blades contain positively charged amino acids. The CTDs of GyrA and ParC both have been shown to bind and bend DNA (*46*).

Extensive efforts were made to model the CTD of human topoisomerase IIα based on the structures of the bacterial type II enzymes. Unfortunately, homology between the CTDs of the different topoisomerase II species was too low to generate a predicted structure with confidence. However, amino acid sequence alignments comparing the CTDs of topoisomerase IIα with those of GyrA and ParC revealed similarities. Like the bacterial enzymes, human topoisomerase IIα contains a series of positively charged amino acid clusters that could potentially interact with DNA. Ten such clusters were identified and are indicated with red bars in Figure 7.

As a first attempt to narrow down the region(s) of the topoisomerase IIα CTD that is responsible for the preferential relaxation of positively supercoiled DNA, a series of C-terminally deleted enzymes was generated. This series contained full-length topoisomerase IIα, a mutant truncated human enzyme that included all of the predicted clusters of positive amino acids (truncated following amino acid 1521, hTop2αΔ1521), and enzymes that lacked one (hTop2αΔ1501), two (hTop2αΔ1476), or six clusters (hTop2αΔ1321), respectively. Time courses for the relaxation of negatively and positively supercoiled plasmids by wild-type topoisomerase IIα, these truncated enzymes, and hTop2αΔ1175 are shown in Figure 8.

The recognition of DNA geometry by hTop2αΔ1521 during relaxation was similar to that of the full-length enzyme. However, removal of even a single cluster of positively charged amino acids from the CTD abolished the ability of topoisomerase IIα to discern the handedness of its DNA substrate. Comparable rates of relaxation of negatively and positively supercoiled plasmids were observed for hTop2αΔ1501, hTop2αΔ1476, and hTop2αΔ1321. These findings imply a role for the positively charged amino acid clusters of the CTD in the recognition of DNA geometry during strand passage. They also suggest that the removal of even the most C-terminal cluster has the potential to impact the ability of topoisomerase IIα to preferentially remove positive superhelical twists.

To further investigate the role of the C-terminal cluster of positive amino acids, a chimeric enzyme was constructed (hTop2αΔ1501/β) in which residues 1502−1531 of human topoisomerase IIα were replaced with the corresponding C-terminal region of topoisomerase IIβ (residues 1585−1621). As seen in Figure 8 (inset, upper right panel), the chimeric enzyme lost its ability to discern the handedness of DNA supercoils during relaxation. These data provide additional evidence that the most C-terminal cluster of positive amino acids in topoisomerase IIα is required to preferentially relax positively supercoiled DNA substrates.

It is notable that the overall rate of relaxation of positively supercoiled DNA dropped (~5−6-fold) as the length of the mutant enzymes decreased. This suggests that the presence

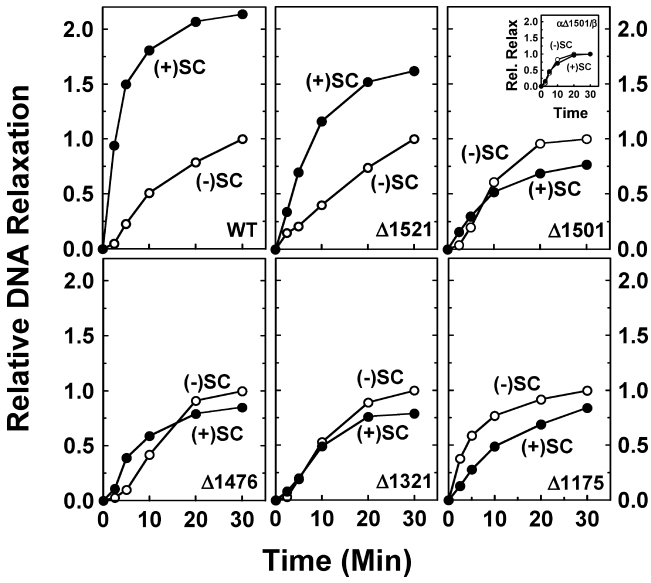

FIGURE 8: Time courses for relaxation of negatively and positively supercoiled substrates by C-terminally truncated topoisomerase IIα mutant enzymes. The ability of hTop2αΔ1521, hTop2αΔ1501, hTop2αΔ1476, and hTop2αΔ1321 (lacking zero, one, two, and six clusters of positive charges, respectively) and hTop2αΔ1501/*β* [(a chimeric enzyme in which residues 1502−1531 of human topoisomerase IIα were replaced with the corresponding C-terminal region of topoisomerase II*β* (residues 1585−1621)] to relax negatively [(−)SC, open circles] and positively supercoiled substrates [(+)SC, closed circles] is shown. Results with full-length human topoisomerase IIα and hTop2αΔ1175 also are included. To aid comparisons, levels of DNA relaxation for negatively supercoiled substrates were normalized to 1.0 at 30 min.

of the CTD enhances the DNA relaxation activity of topoisomerase IIα with positively supercoiled substrates. In some cases (hTop2αΔ1501, hTop2αΔ1476, and hTop2αΔ-1321), however, mutant enzymes also displayed a modest (∼2-fold) increase in the rate of relaxation of negatively supercoiled plasmids or the decatenation of kDNA (not shown). Whether this apparent increase in activity reflects a negative regulatory function of the CTD or a slight variation in the level of active enzyme in the topoisomerase IIα preparations is not known at the present time.

DISCUSSION

Human topoisomerase IIα and II*β* display different abilities to sense the handedness of DNA supercoils (*31, 42*). Whereas the α isoform preferentially removes positive superhelical twists, the *β* isoform relaxes under- and overwound substrates at comparable rates (*31, 42*). Conversely, both enzymes maintain lower levels of DNA cleavage intermediates with positively supercoiled substrates (*31, 42*). These findings suggest that type II topoisomerases discern DNA geometry in a bimodal fashion, using separate portions of the protein to distinguish nucleic acid handedness during different enzymatic processes.

The N-terminal and central domains of topoisomerase IIα and II*β* share a high degree of amino acid sequence identity (*4, 43, 55*). In contrast, the CTDs of the two isoforms diverge considerably. Consequently, it was suggested that the ability of topoisomerase IIα to discern the handedness of DNA supercoils during strand passage resides in the CTD (*31*). This enzyme domain plays important roles in the cellular physiology of topoisomerase II (*13*−*21*). However, since it varies from species to species and is not required for catalytic activity, its role in enzyme action has remained obscure (*1, 4, 7, 10*−*12, 48*).

To investigate the bimodal recognition of DNA geometry by human type II topoisomerases, the present work assessed the role of the CTD in sensing the handedness of DNA supercoils. To this end, the ability of hTop2αΔ1175 (human topoisomerase IIα that lacks the CTD) to relax and cleave negatively and positively supercoiled plasmids was characterized. Whereas hTop2αΔ1175 lost its ability to preferentially relax positively supercoiled plasmids, it still maintained lower levels of cleavage complexes with this substrate. These results provide strong evidence that human topoisomerase IIα requires its CTD to discern the geometry of DNA supercoils during strand passage, while elements in the N-terminal and/or central domains of the enzyme are sufficient to distinguish DNA handedness during cleavage. Further experiments demonstrated that replacing the CTD of topoisomerase II*β* with that of the α isoform conferred the ability to preferentially relax positively supercoiled substrates. This finding confirms a direct role for the CTD of topoisomerase IIα in sensing DNA geometry during strand passage.

There are two possible mechanisms by which the CTD of topoisomerase IIα increases the rate of relaxation of overwound molecules. The first postulates that the catalytic core of the enzyme has a preference for relaxing positive over negative DNA supercoils. This may be due to the difference in the acute vs obtuse crossover angles present in positive (∼60°) and negative (∼120°) DNA nodes, respectively. In this case, interactions between negatively supercoiled molecules and the CTD would alter the geometry of DNA in the active site of the enzyme and impose an acute crossover angle that is similar to that of a positive node (*44, 45*).

The second mechanism postulates that the catalytic core of topoisomerase IIα is indifferent to the handedness of DNA supercoils with regard to relaxation and passes negative and positive supercoils equally and without prejudice. However, positively supercoiled DNA substrates would possess a unique ability to form specific interactions with the CTD of topoisomerase IIα, which would (by a process that has yet to be defined) facilitate and accelerate the strand passage reaction. As part of this mechanism, the CTD could also interact with negatively supercoiled molecules and retard rates of strand passage with this substrate.

If the first mechanism were correct, deletion of the CTD would severely impair the ability of topoisomerase IIα to alter the crossover angle of DNA supercoils. This would result in an enzyme that could still pass positive supercoils at accelerated rates but would be even less competent to pass negatively supercoiled molecules. In contrast, as predicted by the second mechanism, hTop2αΔ1175 passed positive and negative DNA supercoils with a similar ability. Therefore, it appears that the second mechanism more accurately reflects the basis for the preferential relaxation of positive supercoils by human topoisomerase IIα.

The interactions between positively supercoiled DNA and the CTD that enhance rates of strand passage are not known. Unfortunately, there is no structural information available for this region of the protein. Furthermore, modeling studies based on the published structures of the CTDs of bacterial DNA gyrase and topoisomerase IV reveal no global structural

motifs. However, deletion studies with human topoisomerase IIα suggest that clusters of positively charged amino acids in the CTD are important for sensing nucleic acid geometry and that the most C-terminal cluster is required for this process.

Finally, removal of the CTD of topoisomerase IIα had little effect on the ability of the enzyme to recognize the geometry of supercoils during DNA cleavage. This finding demonstrates that the elements in the enzyme that are involved in this process must reside in the N-terminal and/or central domain (*47*). Efforts currently are underway to more specifically identify the portions of topoisomerase IIα that allow it to maintain lower levels of cleavage complexes with positively supercoiled substrates.

In summary, human topoisomerase IIα recognizes the handedness of DNA supercoils in a bimodal fashion. Elements in the CTD of the enzyme are required to discern nucleic acid geometry during DNA relaxation and allow accelerated rates of strand passage with positively supercoiled substrates. Conversely, elements in the conserved N-terminal ATPase and/or central cleavage/ligation domain of the enzyme are sufficient for recognizing the geometry of DNA during cleavage. These elements allow the enzyme to maintain reduced concentrations of cleavage complexes with the overwound substrates that are generated ahead of replication forks and transcription complexes. The use of distinct elements in topoisomerase IIα to recognize DNA geometry in different catalytic processes may allow the enzyme to participate in a variety of critical nuclear events.

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