Use of Divalent Metal Ions in the DNA Cleavage Reaction of Human Type II Topoisomerases[†]

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ABSTRACT: All type II topoisomerases require divalent metal ions to cleave and ligate DNA. To further elucidate the mechanistic basis for these critical enzyme-mediated events, the role of the metal ion in the DNA cleavage reaction of human topoisomerase IIβ was characterized and compared to that of topoisomerase IIα. This study utilized divalent metal ions with varying thiophilicities in conjunction with DNA cleavage substrates that substituted a sulfur atom for the 3′-bridging oxygen or the nonbridging oxygens of the scissile phosphate. On the basis of time courses of DNA cleavage, cation titrations, and metal ion mixing experiments, we propose the following model for the use of divalent metal ions by human type II topoisomerases. First, both enzymes employ a two-metal ion mechanism to support DNA cleavage. Second, an interaction between one divalent metal ion and the 3′-bridging atom of the scissile phosphate greatly enhances enzyme-mediated DNA cleavage, most likely by stabilizing the leaving 3′-oxygen. Third, there is an important interaction between a divalent second metal ion and a nonbridging atom of the scissile phosphate that stimulates DNA cleavage mediated by topoisomerase IIβ. If this interaction exists in topoisomerase IIα, its effects on DNA cleavage are equivocal. This last aspect of the model highlights a difference in metal ion utilization during DNA cleavage mediated by human topoisomerase IIα and IIβ.

A number of essential nuclear processes, such as DNA recombination and replication, generate knots and tangles in the genetic material (*1*−*3*). DNA knots make it impossible to separate the two strands of the double helix, and tangled chromosomes cannot be segregated during mitosis or meiosis (*2*−*5*). Consequently, these detrimental DNA linkages must be resolved to maintain chromosomal integrity. The enzymes that remove knots and tangles from the genome are type II DNA topoisomerases (*2, 4, 6*−*10*).

Type II topoisomerases act by passing an intact double helix through a transient double-stranded break that they generate in a separate segment of DNA (*4, 7, 11*−*13*). Humans encode two isoforms of the enzyme, topoisomerase IIα and IIβ (*4, 7, 9, 10, 13*). These isoforms differ in their protomer molecular masses (170 and 180 kDa, respectively) and are encoded by separate genes (*2, 4, 9, 10*). The physiology of topoisomerase IIα and IIβ differs greatly. Topoisomerase IIα is essential for the survival of proliferating cells (*14*). Enzyme levels are low in quiescent cells and

increase dramatically during periods of growth (*15*−*17*). Furthermore, topoisomerase IIα is regulated over the cell cycle, with protein concentrations peaking in G2/M (*17*−*19*). In contrast, topoisomerase IIβ is dispensable at the cellular level, and its expression is independent of proliferative status and cell cycle (*20, 21*). Finally, while topoisomerase IIα is associated with replication forks and remains tightly bound to chromosomes during mitosis (*6, 14, 22*−*24*), the β isoform dissociates from chromosomes during mitosis (*9, 22, 25*).

Despite the distinct expression patterns and cellular roles of the two isoforms, topoisomerase IIα and IIβ display a high degree (∼70%) of amino acid sequence identity and often exhibit similar enzymological characteristics (*2, 9, 10*). However, numerous studies of enzyme-mediated DNA cleavage and ligation suggest that there are significant (but subtle) differences in the active sites of topoisomerase IIα and IIβ.

First, the DNA cleavage specificities of the two isoforms are similar, but not identical (*26*−*28*). Second, some anticancer drugs that enhance topoisomerase II-mediated DNA cleavage display preferential effects on one isoform or the other (*28*−*30*). Third, covalent topoisomerase IIα−cleaved DNA complexes (i.e., cleavage complexes) that are formed during scission generally persist longer than equivalent complexes with topoisomerase IIβ (*29, 31*). Fourth, topoisomerase IIβ is more sensitive than topoisomerase IIα to alterations in the scissile bond of DNA. For example, topoisomerase IIβ is less able to cleave a DNA substrate that contains a 3′-bridging sulfur atom (i.e.,

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phosphorothiolate) in place of the normal oxygen atom (*32*). It also ligates a nick that has been activated by the addition of a 5′-*p*-nitrophenyl group more slowly (*33*).

All type II topoisomerases require a divalent metal ion to cleave and ligate DNA. Recently, it was shown that human topoisomerase IIα utilizes a two-metal ion mechanism similar to that used by primases, some DNA polymerases, and bacterial DNA gyrase (*34−38*). Given the differences in the active sites of topoisomerase IIα and IIβ, we wanted to determine whether the two isoforms used divalent metal ions in a similar manner. Results indicate that topoisomerase IIβ also utilizes a two-metal ion mechanism for DNA cleavage. Furthermore, they provide the first kinetic evidence of an important interaction between a divalent metal ion and the nonbridging atom of the scissile phosphate in the DNA cleavage reaction of type II topoisomerases.

EXPERIMENTAL PROCEDURES

Enzymes. Human topoisomerase IIα and IIβ were expressed in *Saccharomyces cerevisiae* and purified as described previously (*39−41*).

Preparation of Oligonucleotides. A 50 bp duplex oligonucleotide was designed using a previously identified topoisomerase II cleavage site from pBR322 (*42*). Wild-type oligonucleotide sequences were generated using an Applied Biosystems DNA synthesizer. The 50-mer top and bottom sequences were 5′-TTGGTATCTGCGCTCTGCTGAAG-CC↓AGTTACCTTCGGAAAAAGAGTTGGT-3′ and 5′-ACC-AACTCTTTTTCCGAAGGT↓AACTGGCTTCAGCAGA-GCGCAGATACCAA-3′, respectively. The arrow denotes the point of scission by topoisomerase II. The top strand was composed of two shorter sequences that produced a nick at the location of the scissile bond.

DNA containing a single 3′-bridging phosphorothiolate linkage was synthesized as described previously (*32*). The location of the phosphorothiolate was at the normal scissile bond on the bottom strand. Substrates containing a racemic phosphorothioate in place of the nonbridging scissile bond oxygens of the bottom strand were synthesized by Operon.

Radioactive Labeling of Oligonucleotides. [γ-^{32}P]ATP (~5000 Ci/mmol) was obtained from ICN. Single-stranded oligonucleotides were labeled at their 5′-termini using T4 polynucleotide kinase (New England Biolabs). Following labeling and gel purification, complementary oligonucleotides were annealed by incubation at 70 °C for 10 min and cooling to 25 °C.

DNA Cleavage. DNA cleavage assays were carried out by the procedure of Deweese et al. (*32*). Unless stated otherwise, oligonucleotide substrates were always 5′-end-labeled. DNA cleavage reaction mixtures with human topoisomerase IIα or IIβ contained 200 nM enzyme and 100 nM double-stranded oligonucleotide in a total of 20 μL of 10 mM Tris-HCl (pH 7.9), 135 mM KCl, 0.1 mM EDTA, and 2.5% glycerol. Unless otherwise noted, the concentration of the divalent cation was 5 mM. In some cases, the concentration of divalent cation (MgCl$_2$, MnCl$_2$, or CaCl$_2$) was varied and/or combinations of the cations were used. Experiments that monitored DNA cleavage over a range that included divalent cation concentrations of <1 mM utilized cleavage buffer that lacked EDTA. Reactions were initiated by the addition of enzyme, and mixtures were incubated for 0−30 min at 37 °C. DNA cleavage products were trapped by the addition of 2 μL of 10% SDS followed by 2 μL of 250 mM NaEDTA (pH 8.0). Proteinase K (2 μL of a 0.8 mg/mL solution) was added to digest the enzyme. Cleavage products were resolved by electrophoresis in a 14% denaturing polyacrylamide gel. To inhibit oxidation of cleaved oligonucleotides containing 3′-terminal -SH moieties and the formation of multimers in the gel, 100 mM DTT was added to the sample loading buffer. DNA cleavage products were visualized and quantified using a Bio-Rad molecular imager.

Pre-equilibrium DNA cleavage reactions were monitored for 0.5−3 s using a KinTek (Austin, TX) model RQF-3 chemical quench flow apparatus. Cleavage was initiated by rapidly mixing equal volumes of two independent solutions. The first contained a noncovalent complex formed between human topoisomerase IIβ and ^{32}P-labeled oligonucleotide in cleavage buffer that lacked divalent cation. The second solution contained cleavage buffer in which the divalent cation concentration was 2 times higher than normal (10 mM). The two solutions were mixed at 37 °C, and DNA cleavage was quenched with 1% SDS (v/v, final concentration). Products were processed and analyzed as described above.

RESULTS AND DISCUSSION

Interactions between Divalent Metal Ions and Scissile Phosphate Atoms during DNA Cleavage Mediated by Topoisomerase IIβ. As a first step toward defining the requirement for a divalent metal ion in the DNA cleavage reaction of human topoisomerase IIβ, interactions between the cation and the scissile phosphate atoms were assessed. All DNA cleavage substrates utilized in this study had the same sequence. However, experiments took advantage of three alterations in the scissile bonds of these oligonucleotides. First, substrates contained a nick at the scissile bond on the strand that was not being monitored for cleavage. The presence of this nick on the opposite strand greatly enhances the sensitivity of the DNA cleavage reaction, stimulating both rates and levels of scission ~10-fold (*32*).

Second, a substrate was employed that substituted a sulfur for the 3′-bridging oxygen atom at the scissile phosphate (i.e., S−P scissile bond) (*32*). Topoisomerase IIβ cleaved these phosphorothiolate-containing oligonucleotides with all of the characteristics of wild-type substrates, with the following exception: since the resulting 3′-terminal -SH moiety is a poor nucleophile at phosphorus (*32, 43, 44*), the 3′-bridging phosphorothiolate did not support ligation (data not shown). As a result, S−P substrates isolate the forward DNA scission event from ligation, allowing high levels of cleavage complexes to accumulate (*32*). This is in contrast to wild-type oligonucleotides with 3′-bridging oxygen atoms (i.e., O−P scissile bond), which establish a rapid DNA cleavage−ligation equilibrium and maintain low levels of cleavage complexes (*32*).

Third, a substrate was utilized that substituted a sulfur atom for the nonbridging oxygen at the scissile phosphate. This phosphorothioate substrate was a racemic mixture that replaced either the R_P or S_P nonbridging oxygen atom with a sulfur atom. In contrast to the 3′-bridging phosphorothiolate oligonucleotide, the nonbridging phosphorothioate substrate maintained a DNA cleavage−ligation equilibrium and cleavage complexes did not accumulate over time (*32*).

Interactions between divalent cations and the scissile phosphate atoms were determined by comparing the ability of topoisomerase IIβ to cleave DNA substrates containing an oxygen atom or a sulfur atom at the 3′-bridging or nonbridging position in the presence of metal ions of varying "hardness" (i.e., thiophilicity) (*38*). The ions used for this study were Ca^{2+}, Mg^{2+}, and Mn^{2+}. Within this series, Mn^{2+} is the "softest", or most thiophilic, metal and Mg^{2+} and Ca^{2+} are harder, or less thiophilic (*45−47*). Soft metal ions often prefer sulfur over oxygen as an inner-sphere ligand, while hard metals usually coordinate more readily with oxygen (*35, 45−50*). If there is a direct interaction between the metal ion and a scissile phosphate atom that facilitates catalysis, relative rates (or levels) of scission with substrates containing a sulfur atom in place of the oxygen should increase in the presence of soft (thiophilic) metals (*35, 38, 48−50*). Conversely, less cleavage should be generated in reactions that include hard metals (*35, 38, 48−50*).

The first set of experiments established the ability of divalent metal ions to support cleavage of a wild-type O−P substrate by topoisomerase IIβ. Consistent with previous work with topoisomerase IIα, Mn^{2+} and Ca^{2+} generated higher levels of DNA cleavage than Mg^{2+} (Figure 1, top left panel). Levels of cleavage complexes formed in the presence of Mn^{2+} or Ca^{2+} were ~5 or 2 times higher, respectively than those observed with Mg^{2+}. Despite the low equilibrium levels of cleavage seen with Mg^{2+}, initial rates of scission (determined by rapid chemical quench over the first seconds of the cleavage reaction) with the divalent cation were comparable to those generated in the presence of Mn^{2+} (top right panel).

A previous study demonstrated that there is an important interaction between the metal ion and the 3′-bridging atom of the scissile bond in the active site of human topoisomerase IIα that significantly enhances the ability of the enzyme to cleave DNA (*38*). Therefore, a second set of experiments examined the divalent cation preference for cleavage of the 3′-bridging S−P (phosphorothiolate) oligonucleotide by topoisomerase IIβ (Figure 1, middle panels). Levels and rates of cleavage observed in the presence of the thiophilic metal ion, Mn^{2+}, were considerably higher than those generated with the hard metal, Ca^{2+}. Furthermore, levels and rates of cleavage for reactions that included Ca^{2+} fell significantly below those with Mg^{2+}. Taken together, these results strongly suggest that the divalent cation contacts the 3′-bridging atom of the scissile bond in the active site of human topoisomerase IIβ. Furthermore, like topoisomerase IIα, this metal ion−DNA interaction mediated by topoisomerase IIβ stimulates rates of enzyme-mediated scission.

Models proposed for the active site geometry of *Escherichia coli* DNA gyrase (*36*) and human topoisomerase IIα (*38*) both postulate interactions between divalent metal ions and a nonbridging oxygen atom of the scissile phosphate. However, no direct evidence supporting this interaction has been reported. To this point, replacement of nonbridging oxygen atoms with sulfur had little effect on levels of DNA scission generated by either enzyme (*38, 51*). Thus, it was concluded that if these postulated interactions between metal ions and nonbridging oxygen atoms exist, their effects on DNA cleavage mediated by these enzymes were equivocal.

On the basis of mutagenesis studies, it has been proposed that topoisomerase IIβ utilizes divalent cations in a manner
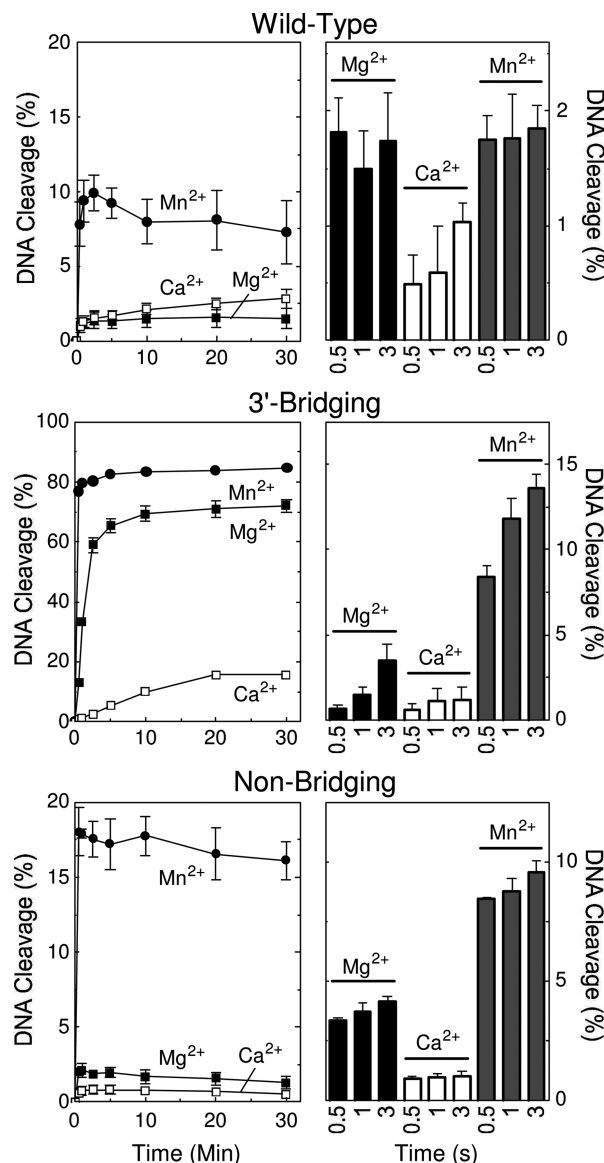
FIGURE 1: Cleavage of nicked oligonucleotide substrates by topoisomerase IIβ in the presence of different divalent metal ions. The nick is located at the scissile bond on the unlabeled strand. Thirty-minute time courses (left panels) and rapid quench time points (right panels) for the wild type (top), 3′-bridging phosphorothiolate (middle), and nonbridging phosphorothioate (bottom) are shown. DNA cleavage was carried out in the presence of 5 mM Mg^{2+} (■), Ca^{2+} (□), or Mn^{2+} (●). Rapid quench results for 0.5, 1, and 3 s are shown in the presence of Mg^{2+} (black bars), Ca^{2+} (white bars), or Mn^{2+} (gray bars). Error bars represent the standard deviation of at least three independent experiments.

similar to that of DNA gyrase (*52, 53*). Nonetheless, a third set of experiments was carried out to determine whether interactions could be observed between the metal ion and a nonbridging atom of the scissile phosphate in the active site of human topoisomerase IIβ.

In marked contrast to the findings with DNA gyrase and topoisomerase IIα (*38, 51*), dramatic differences were seen with a substrate that contained a sulfur atom in the nonbridging position (Figure 1, bottom panels). Levels of cleavage generated by topoisomerase IIβ in the presence of Mn^{2+} were ~8-fold higher than those seen with Mg^{2+}, and rates of scission were 2−3 times faster. Furthermore, levels and rates of cleavage for reactions that included Ca^{2+} dropped below those observed with Mg^{2+}. Finally, as compared to
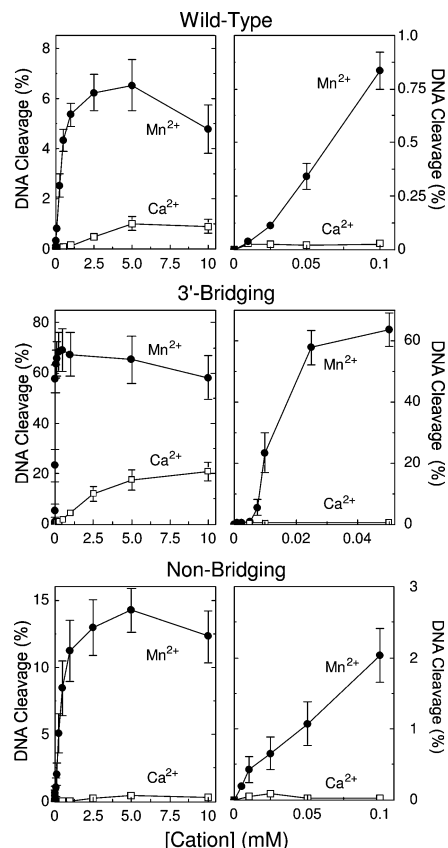
FIGURE 2: Metal ion concentration dependence for DNA cleavage of nicked wild-type, phosphorothiolate, and phosphorothioate substrates by topoisomerase IIβ. Results for the wild type (top), 3′-bridging phosphorothiolate (middle), and nonbridging phosphorothioate (bottom) are shown. In the left panels, Mn^{2+} (●) or Ca^{2+} (□) was titrated from 10 μM to 10 mM for the wild type, from 1 μM to 10 mM for 3′-bridging substrates, or from 5 μM to 10 mM for nonbridging substrates. In the right panels, expanded DNA cleavage at concentrations up to 100 μM for wild-type and nonbridging substrates or 50 μM for the 3′-bridging substrate is shown. All reaction mixtures were incubated for 30 min prior to stopping the reaction. Error bars represent the standard error of the mean of two independent experiments or the standard deviation of at least three independent experiments.

results with the wild-type substrate (top panel), substitution of sulfur at the nonbridging position increased the level of cleavage ~2-fold in the presence of Mn^{2+} and decreased it ~3-fold in the presence of Ca^{2+}. These results provide the first evidence for an interaction between the divalent metal ion and a nonbridging atom of the scissile phosphate for any type II topoisomerase and suggest that this interaction enhances the ability of topoisomerase IIβ to cleave DNA.

A Two-Metal Ion Mechanism for DNA Cleavage Mediated by Human Topoisomerase IIβ. On the basis of metal ion mixing experiments, DNA gyrase and topoisomerase IIα were demonstrated to use a two-metal ion mechanism for DNA cleavage (*36, 38*). Two approaches were utilized to determine whether human topoisomerase IIβ uses a similar mechanism to mediate DNA scission. In the first, DNA cleavage was monitored over a range of divalent cation concentrations. These experiments utilized Mn^{2+} (a soft cation) and Ca^{2+} (a hard cation). As seen in Figure 2, the concentration of Mn^{2+} that was required to promote DNA scission mediated by topoisomerase IIβ was considerably lower than that seen with Ca^{2+}.

When cleavage of the wild-type O−P oligonucleotide was monitored (Figure 2, top panels), both divalent cations displayed a mild biphasic concentration dependence. This finding suggests that topoisomerase IIβ utilizes more than one divalent metal ion to mediate DNA cleavage.

Introduction of a 3′-bridging phosphorothiolate had a significant effect on cation titrations and resulted in a more pronounced initial phase with Mn^{2+} (Figure 2, middle panels). This biphasic metal ion concentration dependence suggests that there are two divalent cation sites in the DNA cleavage ligation domain of topoisomerase IIβ and that both need to be filled to support scission. The finding that the initial (i.e., high-affinity) phase with the 3′-bridging phosphorothiolate substrate became more prominent with the soft metal ion (Mn^{2+}) implies that the first site that is filled by the divalent cation is the one that interacts with the bridging atom of the scissile bond.

Introduction of a sulfur at the nonbridging position resulted in an ~2-fold increase in levels of DNA cleavage in the presence of Mn^{2+} and an ~2-fold decrease in the presence of Ca^{2+} (Figure 2, bottom panels) as compared to the wild-type substrate. Although this finding provides further evidence for an important interaction between a metal ion and the nonbridging atom of the scissile phosphate, no prominent initial phase was observed.

Therefore, a second approach was utilized to confirm that two metal ions are required for DNA cleavage mediated by human topoisomerase IIβ. These studies took advantage of the enhanced scission observed for substrates that contained a sulfur atom in either the 3′-bridging or the nonbridging positions in the presence of Mn^{2+}. Experiments compared levels of DNA cleavage monitored in the presence of Ca^{2+}, Mn^{2+}, or a combination of the two divalent cations. Near-saturating concentrations of Ca^{2+} (5 mM) were paired with subsaturating concentrations of Mn^{2+} (up to 100 μM depending on the cleavage substrate). These limiting concentrations of Mn^{2+} supported levels of enzyme-mediated DNA scission that were <15% of the observed maxima.

Figures 3−5 display results for topoisomerase IIβ-mediated DNA cleavage of the wild-type, 3′-bridging phosphorothiolate, and nonbridging phosphorothioate substrates, respectively. Results of parallel experiments that employed topoisomerase IIα are shown for comparison and are discussed following presentation of all of the data for topoisomerase IIβ.

Combining metal ions in reactions that utilized topoisomerase IIβ and a wild-type substrate had a small but discernible effect (Figure 3, top panel). Levels of DNA cleavage in reaction mixtures that contained both divalent cations were slightly higher (~1.5−2-fold) than predicted by summing the amount of cleavage generated in reaction mixtures that contained either Mn^{2+} or Ca^{2+}. This result supports a two-metal ion mechanism for DNA cleavage mediated by the β-isoform and indicates that minor differences between the two cation sites can be discerned even in the presence of an unmodified substrate.

In contrast to the results described above, a dramatic difference was seen in experiments that utilized the 3′-bridging S−P substrate (Figure 4, top panel). Levels of DNA scission in reaction mixtures that contained both Ca^{2+} and Mn^{2+} were ≥10 times greater than the calculated levels derived from the sum of cleavage observed in the presence
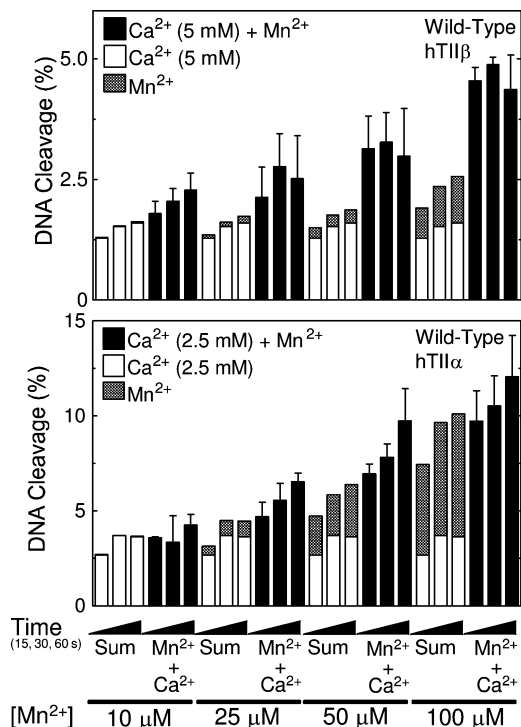
FIGURE 3: Cleavage of nicked wild-type oligonucleotide substrates by topoisomerase IIβ or IIα in the presence of divalent metal ion combinations. DNA cleavage reactions with topoisomerase IIβ (top) or IIα (bottom) were carried out for 15, 30, or 60 s in the presence of 5 mM (IIβ) or 2.5 mM (IIα) Ca^{2+} alone (white bars), 10−100 μM Mn^{2+} alone (stippled bars), or a mixture of 5 mM (IIβ) or 2.5 mM (IIα) Ca^{2+} and 10−100 μM Mn^{2+} (Ca^{2+} + Mn^{2+}, black bars). The calculated sum of the enzyme-mediated DNA cleavage from reaction mixtures containing either Ca^{2+} or Mn^{2+} alone is also shown (Sum, stacked white and stippled bars, respectively). All data represent the average of at least three independent experiments. Error bars for reactions carried out in the presence of Ca^{2+} and Mn^{2+} are shown. Error bars for reactions carried out in the presence of Ca^{2+} or Mn^{2+} alone are not shown for the sake of simplicity.

FIGURE 4: Cleavage of nicked 3′-bridging phosphorothiolate oligo-nucleotide substrates by topoisomerase IIβ or IIα in the presence of divalent metal ion combinations. DNA cleavage reactions with topoisomerase IIβ (top) or IIα (bottom) were carried out for 15, 30, or 60 s in the presence of 5 mM (IIβ) or 2.5 mM (IIα) Ca^{2+} alone (white bars), 1−10 μM Mn^{2+} alone (stippled bars), or a mixture of 5 mM (IIβ) or 2.5 mM (IIα) Ca^{2+} and 1−10 μM Mn^{2+} (Ca^{2+} + Mn^{2+}, black bars). The calculated sum of the enzyme-mediated DNA cleavage from reaction mixtures containing either Ca^{2+} or Mn^{2+} alone is also shown (Sum, stacked white and stippled bars, respectively). Data for topoisomerase IIα are reproduced from ref *38*. All data represent the average of at least three independent experiments. Error bars for reactions carried out in the presence of Ca^{2+} and Mn^{2+} are shown. Error bars for reactions carried out in the presence of Ca^{2+} or Mn^{2+} alone are not shown for the sake of simplicity.

of the individual metal ions. Furthermore, a large enhancement in the rate of DNA scission (compared to calculated sums) was observed when Ca^{2+} and Mn^{2+} were combined. It should be noted that these experiments used Mn^{2+} concentrations (1−10 μM) that partially filled or saturated the high-affinity scissile bond site (see Figure 2, middle panel) without appreciably filling the second site. These data confirm the two-metal ion mechanism as well as the importance of the interaction of the metal ion with the 3′-bridging atom of the scissile phosphate.

As seen in Figure 5 (top panel), the inclusion of a nonbridging sulfur at the scissile phosphate also stimulated DNA cleavage in reaction mixtures that contained a mixture of Ca^{2+} and Mn^{2+}. Levels of cleavage in reaction mixtures that contained both metal ions were ∼5 times higher than predicted by the calculated sums. Once again, these data confirm the two-metal ion mechanism for DNA cleavage mediated by topoisomerase IIβ, and they also validate the significance of the interaction of the metal ion with the nonbridging atom of the scissile phosphate.

Trends similar to those seen with the β-isoform were observed for mixing experiments that employed human topoisomerase IIα and the wild-type (Figure 3, bottom) or 3′-bridging phosphorothiolate substrate (*38*) (Figure 4, bottom). Experiments were identical to those with topoisomerase
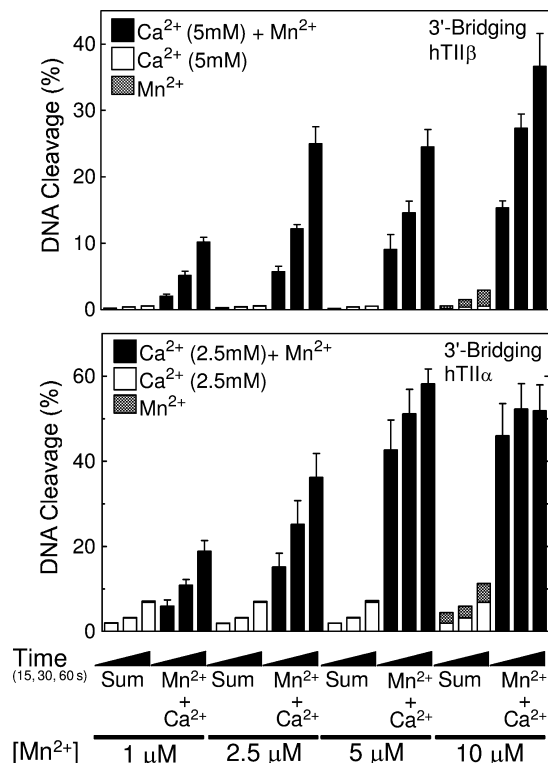
IIβ except that 2.5 mM Ca^{2+} [which is near saturating levels for the α-isoform (*38*)] was used. While differences between predicted and actual results with the wild-type oligonucleotide were less apparent with topoisomerase IIα, there did appear to be a mild enhancement in cleavage when both Ca^{2+} and Mn^{2+} were included in reaction mixtures. As shown with topoisomerase IIβ, the ability of the α-isoform to cleave a substrate with a 3′-bridging sulfur increased dramatically when both metal ions were present (*38*).

In contrast, a marked difference between the two enzyme isoforms was seen in reactions that utilized the nonbridging substrate. Scission levels generated by topoisomerase IIα in the presence of Ca^{2+} and Mn^{2+} were marginally higher compared to those predicted by the calculated sums (Figure 5, bottom) or by those observed in the presence of both divalent cations with the wild-type oligonucleotide (compare with Figure 3, bottom). Similar results were seen when 5 mM Ca^{2+} was used in reaction mixtures (data not shown). These findings indicate that human topoisomerase IIα and IIβ utilize divalent metal ions differently for some aspects of their DNA cleavage reactions. The interaction between a metal ion and the nonbridging atom of the scissile phosphate
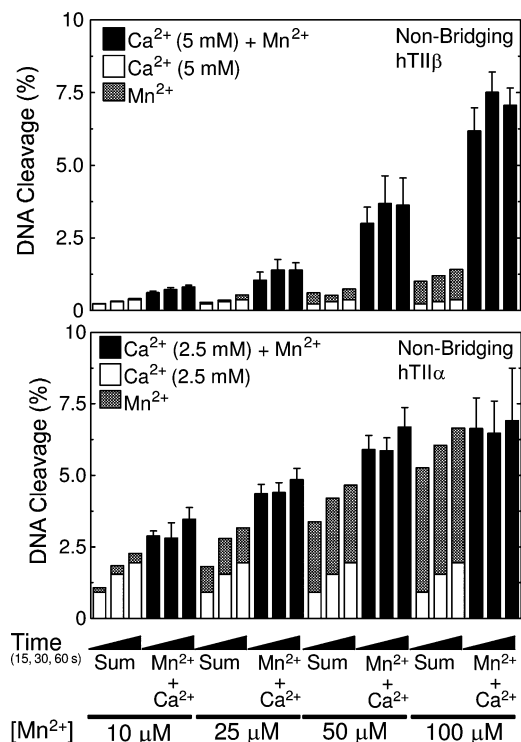
FIGURE 5: Cleavage of nicked nonbridging phosphorothioate oligonucleotide substrates by topoisomerase IIβ or IIα in the presence of divalent metal ion combinations. DNA cleavage reactions with topoisomerase IIβ (top) or IIα (bottom) were carried out for 15, 30, or 60 s in the presence of 5 mM (IIβ) or 2.5 mM (IIα) Ca^{2+} alone (white bars), $10-100$ μM Mn^{2+} alone (stippled bars), or a mixture of 5 mM (IIβ) or 2.5 mM (IIα) Ca^{2+} and $10-100$ μM Mn^{2+} ($Ca^{2+} + Mn^{2+}$, black bars). The calculated sum of the enzyme-mediated DNA cleavage from reaction mixtures containing either Ca^{2+} or Mn^{2+} alone also is shown (Sum, stacked white and stippled bars, respectively). All data represent the average of at least three independent experiments. Error bars for reactions carried out in the presence of Ca^{2+} and Mn^{2+} are shown. Error bars for reactions carried out in the presence of Ca^{2+} or Mn^{2+} alone are not shown for the sake of simplicity.

is important for the DNA cleavage reaction of topoisomerase IIβ and substantially increases levels of scission. However, if the parallel interaction in topoisomerase IIα exists, it has little effect on DNA scission.

A Model for the Use of Divalent Metal Ions for DNA Cleavage Mediated by Human Type II Topoisomerases. A model for the use of divalent metal ions in the DNA cleavage reaction of human type II topoisomerases is proposed in Figure 6. Amino acids that are postulated to interact with the metal ions in the active site of topoisomerase IIα and IIβ are assigned on the basis of previous enzymological studies of mutated *E. coli* DNA gyrase and human topoisomerase IIβ proteins (*36, 52, 53*), as well as structural studies of bacterial topoisomerase III, yeast topoisomerase II, and other DNA enzymes that contain the toprim domain (*54–57*).

Our model has several features. First, topoisomerase IIα and IIβ both employ a two-metal ion mechanism to support DNA cleavage. Second, both enzyme isoforms utilize an interaction between a divalent metal ion (metal ion 1) and the 3'-bridging atom of the scissile phosphate to accelerate rates of enzyme-mediated DNA cleavage, most likely by stabilizing the leaving 3'-oxygen. At present, it is not known whether metal ion 1 also interacts with the nonbridging
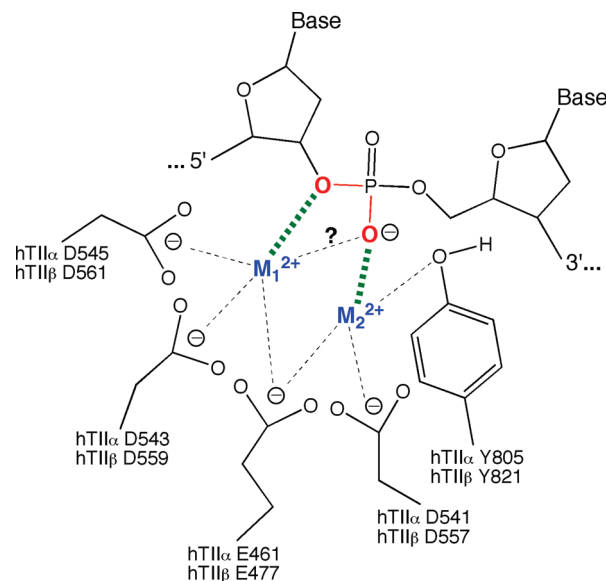

FIGURE 6: Two-metal ion model for DNA cleavage by human topoisomerase IIα and IIβ. Details are described in the text. Amino acids that are postulated to interact with the metal ions in the active site of both isoforms are shown. The model postulates that metal ion 1 (M_1^{2+}, colored blue) makes a critical interaction with the 3'-bridging atom (colored red) of the scissile phosphate (bond colored green), which most likely is needed to stabilize the leaving 3'-oxygen (left, colored red). In addition, metal ion 2 (M_2^{2+}, (colored blue) is required for DNA scission and likely interacts with the nonbridging oxygen (colored red) during scission. This divalent cation may stabilize the DNA transition state and/or help deprotonate the active site tyrosine. While interactions between M_2^{2+} and the nonbridging atom of the scissile phosphate significantly enhance DNA cleavage mediated by topoisomerase IIβ, effects on DNA scission mediated by topoisomerase IIα are equivocal. The model was adapted from ref *36*.

oxygen. Third, a second metal ion (metal ion 2) appears to contact a nonbridging atom of the scissile phosphate in the active site of topoisomerase IIβ. This interaction plays a significant role in DNA cleavage mediated by the β-isoform and greatly stimulates scission. As proposed previously, this metal ion is believed to stabilize the DNA transition state and/or help deprotonate the active site tyrosine (*36, 38, 53*). Although topoisomerase IIα has an absolute requirement for metal ion 2, the role of this divalent cation in its DNA cleavage reaction is unclear. Results of this study cannot rule out an interaction between the metal ion and the nonbridging oxygen in the active site of topoisomerase IIα. However, if the interaction exists, it does not affect rates of DNA cleavage.

In conclusion, topoisomerase IIα and IIβ both employ a two-metal ion mechanism to support DNA cleavage but appear to utilize one of the two metal ions differently. This study provides the first evidence of the interaction between a divalent metal ion and the nonbridging atom of the scissile phosphate and a testable model to further investigate the role of metal ions in topoisomerase II-mediated processes.

ACKNOWLEDGMENT

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