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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 in order to cleave and ligate DNA. In order 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 II β was characterized and compared to that of topoisomerase II α . The present 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 non-bridging oxygens of the scissile phosphate. Based on 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 non-bridging atom of the scissile phosphate that stimulates DNA cleavage mediated by topoisomerase II β . If this interaction exists in topoisomerase II α , 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 II α and II β .

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 II α and II β (4,7,9,10,13). These isoforms differ in their protomer molecular masses (170 vs. 180 kDa, respectively) and are encoded by separate genes (2,4,9,10). The physiology of topoisomerase II α and II β differs greatly. Topoisomerase II α is essential for the survival of proliferating cells (14). Enzyme levels are low in quiescent

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cells and increase dramatically during periods of growth (15–17). Furthermore, topoisomerase II α is regulated over the cell cycle, with protein concentrations peaking in G2/M (17–19). In contrast, topoisomerase II β is dispensable at the cellular level, and its expression is independent of proliferative status and cell cycle (20,21). Finally, while topoisomerase II α 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 II α and II β display a high degree (~70%) of amino acid sequence identity and often show 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 II α and II β .

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 II α -cleaved DNA complexes (*i.e.*, cleavage complexes) that are formed during scission generally persist longer than equivalent complexes with topoisomerase II β (29,31). Fourth, topoisomerase II β is more sensitive than topoisomerase II α to alterations in the scissile bond of DNA. For example, topoisomerase II β is less able to cleave a DNA substrate that contains a 3'-bridging sulfur atom (*i.e.*, 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 in order to cleave and ligate DNA. Recently, it was shown that human topoisomerase II α utilized 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 II α and II β , we wanted to determine whether the two isoforms used divalent metal ions in a similar manner. Results indicate that topoisomerase II β also utilizes a two-metal-ion mechanism for DNA cleavage. Furthermore, they provide the first kinetic evidence for an important interaction between a divalent metal ion and the non-bridging atom of the scissile phosphate in the DNA cleavage reaction of type II topoisomerases.

EXPERIMENTAL PROCEDURES

Enzymes

Human topoisomerase II α and II β 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'-TTGGTATCTGCGCTCTGCTGAAGCC↓AGTTACCTTCGGAAAAAGAGTTGGT-3' and 5'-ACCAACTCTTTTTCCGAAGGT↓AACTGGCTTCAGCAGAGCGCAGATACCAA-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 non-bridging 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 on 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 reactions with human topoisomerase II α or II β 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 below 1 mM utilized cleavage buffer that lacked EDTA. Reactions were initiated by the addition of enzyme and were incubated for 0 to 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 0.8 mg/mL) 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 s to 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 II β 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 II β

As a first step towards defining the requirement for a divalent metal ion in the DNA cleavage reaction of human topoisomerase II β , interactions between the cation and the scissile phosphate atoms were assessed. All DNA cleavage substrates utilized in the present study had the same sequence. However, experiments took advantage of three alterations in the scissile bonds of these oligonucleotides. First, all 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 II β 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 phosphorous (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 non-bridging oxygen at the scissile phosphate. This phosphorothioate substrate was a racemic mixture that replaced either the *R*_P or *S*_P non-bridging oxygen atom with a sulfur atom. In contrast to the 3'-bridging phosphorothioate oligonucleotide, the non-bridging 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 II β to cleave DNA substrates containing an oxygen atom or a sulfur atom at the 3'-bridging or non-bridging position in the presence of metal ions of varying "hardness" (*i.e.*, thiophilicity) (38). The ions used for this study were Ca²⁺, Mg²⁺, and Mn²⁺. Within this series, Mn²⁺ is the "softest," or most thiophilic metal, and Mg²⁺ and Ca²⁺ 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 contain 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 II β . Consistent with previous work with topoisomerase II α , Mn²⁺ and Ca²⁺ generated higher levels of DNA cleavage than Mg²⁺ (Figure 1, top left panel). Levels of cleavage complexes formed in the presence of Mn²⁺ or Ca²⁺ were ~5 or 2 times higher, respectively than those observed with Mg²⁺. Despite the low equilibrium levels of cleavage seen with Mg²⁺, 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 Mn²⁺ (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 II α 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 II β (Figure 1, middle panels). Levels and rates of cleavage observed in the presence of the thiophilic metal ion, Mn²⁺, were considerably higher than those generated with the hard metal, Ca²⁺. Both the levels and rate of cleavage for reactions that contained Ca²⁺ fell significantly below those with Mg²⁺. The drop in Ca²⁺-supported scission of the S-P, as compared to the O-P DNA substrate, together with the relative rise in cleavage levels and rates with Mn²⁺, strongly suggest that the divalent cation contacts the 3'-bridging atom of the scissile bond in the active site of human topoisomerase II β . Furthermore, like topoisomerase II α , this metal ion-DNA interaction mediated by topoisomerase II β stimulates rates of enzyme-mediated scission.

Models proposed for the active site geometry of *Escherichia coli* DNA gyrase (36) and human topoisomerase II α (38) both postulate interactions between divalent metal ions and a non-bridging oxygen atom of the scissile phosphate. However, no direct evidence supporting this interaction has been reported. To this point, replacement of non-bridging 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 non-bridging oxygen atoms exist, their effects on DNA cleavage mediated by these enzymes were equivocal.

Based on mutagenesis studies, it has been proposed that topoisomerase II β utilizes divalent cations in a manner 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 non-bridging atom of the scissile phosphate in the active site of human topoisomerase II β .

In marked contrast to the findings with DNA gyrase and topoisomerase II α (38,51), dramatic differences were seen with a substrate that contained a sulfur atom in the non-bridging position (Figure 1, bottom panels). Levels of cleavage generated by topoisomerase II β in the presence of Mn²⁺ were ~8-fold higher than those seen with Mg²⁺, and rates of scission were 2 to 3 times faster. Furthermore, levels and rates of cleavage for reactions that contained Ca²⁺ dropped below those observed with Mg²⁺. Finally, as compared to results with the wild-type substrate (top panel), substitution of sulfur at the non-bridging position increased cleavage ~2-fold in the presence of Mn²⁺ and decreased it ~3-fold in the presence of Ca²⁺. These results provide the first evidence for an interaction between the divalent metal ion and a non-bridging atom of the scissile phosphate for any type II topoisomerase and suggest that this interaction enhances the ability of topoisomerase II β to cleave DNA.

A Two-Metal-Ion Mechanism for DNA Cleavage Mediated by Human Topoisomerase II β

On the basis of metal ion mixing experiments, DNA gyrase and topoisomerase II α were demonstrated to use a two-metal-ion mechanism for DNA cleavage (36,38). Two approaches were utilized to determine whether human topoisomerase II β 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 Mn²⁺ (a soft cation) and Ca²⁺ (a hard cation). As seen in Figure 2, the concentration of Mn²⁺ that was required to promote DNA scission mediated by topoisomerase II β was considerably lower than that seen with Ca²⁺.

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 II β 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 dramatically more pronounced initial phase with Mn²⁺ (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 II β and that both need to be filled in order 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 (Mn²⁺) 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 non-bridging position resulted in an ~2-fold increase in levels of DNA cleavage in the presence of Mn²⁺ and an ~2-fold decrease in the presence of Ca²⁺ (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 non-bridging 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 II β . These studies took advantage of the enhanced scission observed for substrates that contained a sulfur atom in either the 3'-bridging or the

non-bridging 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 sub-saturating 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 II β -mediated DNA cleavage of the wild-type, 3'-bridging phosphorothiolate, or non-bridging phosphorothioate substrate, respectively. Results of parallel experiments that employed topoisomerase II α are shown for comparison and are discussed following presentation of all of the data for topoisomerase II β .

Combining metal ions in reactions that utilized topoisomerase II β and a wild-type substrate had a small but discernable effect (Figure 3, top panel). Levels of DNA cleavage in reactions that contained both divalent cations were slightly higher (~1.5– to 2-fold) than predicted by summing the amount of cleavage generated in reactions 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 above results, a dramatic difference was seen in experiments that utilized the 3'-bridging S–P substrate (Figure 4, top panel). Levels of DNA scission in reactions 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 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 metal ion interaction with the 3'-bridging atom of the scissile phosphate.

As seen in Figure 5 (top panel), the inclusion of a non-bridging sulfur at the scissile phosphate also stimulated DNA cleavage in reactions that contained a mixture of Ca^{2+} and Mn^{2+} . Levels of cleavage in reactions 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 II β , and they also validate the significance of the metal ion interaction with the non-bridging atom of the scissile phosphate.

Trends similar to those seen with the β isoform were observed for mixing experiments that employed human topoisomerase II α and the wild-type (Figure 3, bottom) or 3'-bridging phosphorothiolate substrate (38) (Figure 4, bottom). Experiments were identical to those with topoisomerase II β 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 II α , there did appear to be a mild enhancement in cleavage when both Ca^{2+} and Mn^{2+} were included in reactions. As shown with topoisomerase II β , 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 non-bridging substrate. Scission levels generated by topoisomerase II α 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 II α and II β utilize divalent metal ions differently for some aspects of their DNA cleavage reactions. The interaction between a metal ion and the non-bridging atom of the scissile phosphate is important for the DNA cleavage reaction of topoisomerase II β and substantially raises levels of scission. However, if the parallel interaction in topoisomerase II α 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 II α and II β are assigned based on previous enzymological studies of mutated *E. coli* DNA gyrase and human topoisomerase II β 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 II α and II β 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 the present time, it is not known whether metal ion 1 also has interactions with the non-bridging oxygen. Third, a second metal ion (metal ion 2) appears to contact a non-bridging atom of the scissile phosphate in the active site of topoisomerase II β . 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 II α has an absolute requirement for metal ion 2, the role of this divalent cation in its DNA cleavage reaction is unclear. Results of the present study cannot rule out an interaction between the metal ion and the non-bridging oxygen in the active site of topoisomerase II α . However, if the interaction exists, it does not affect rates of DNA cleavage.

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

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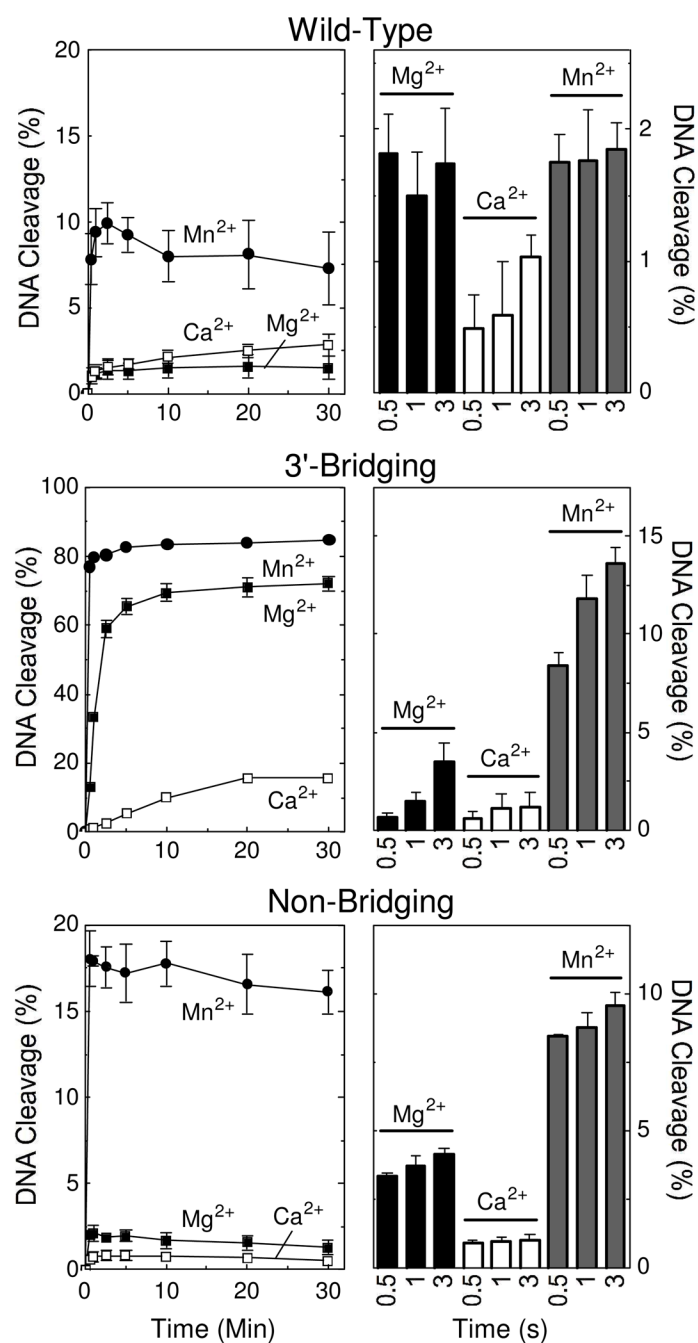
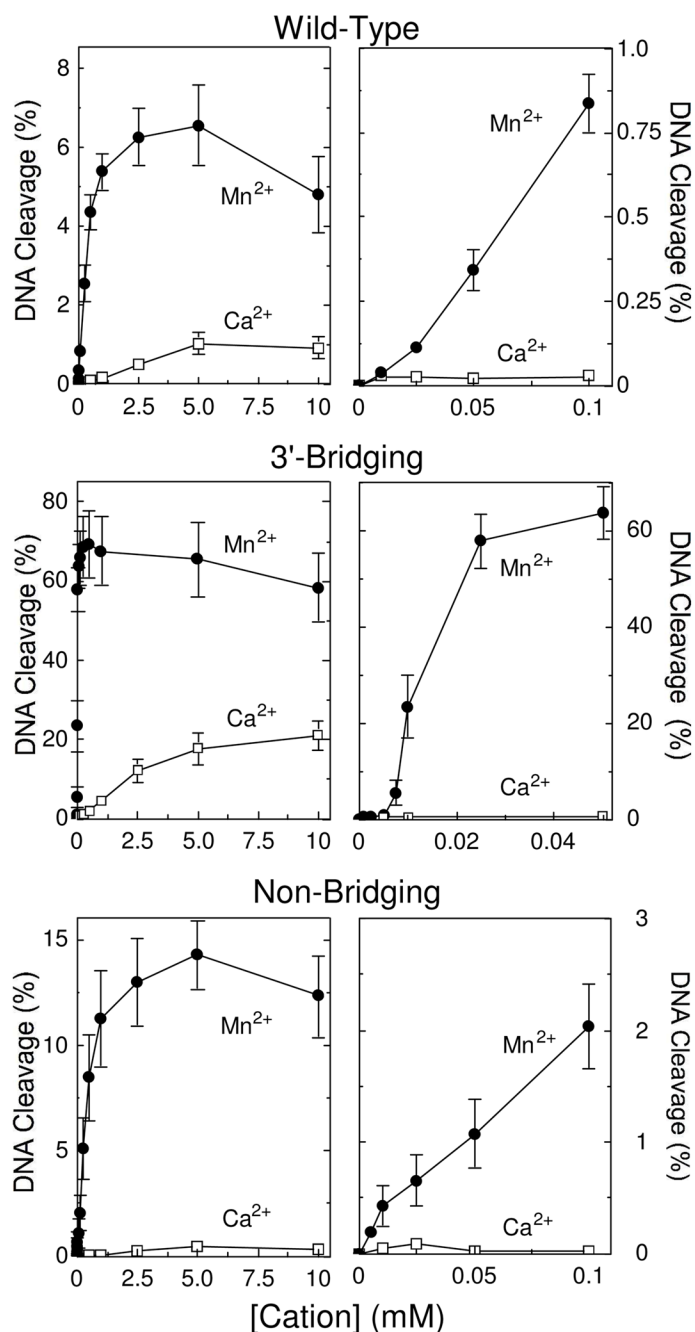


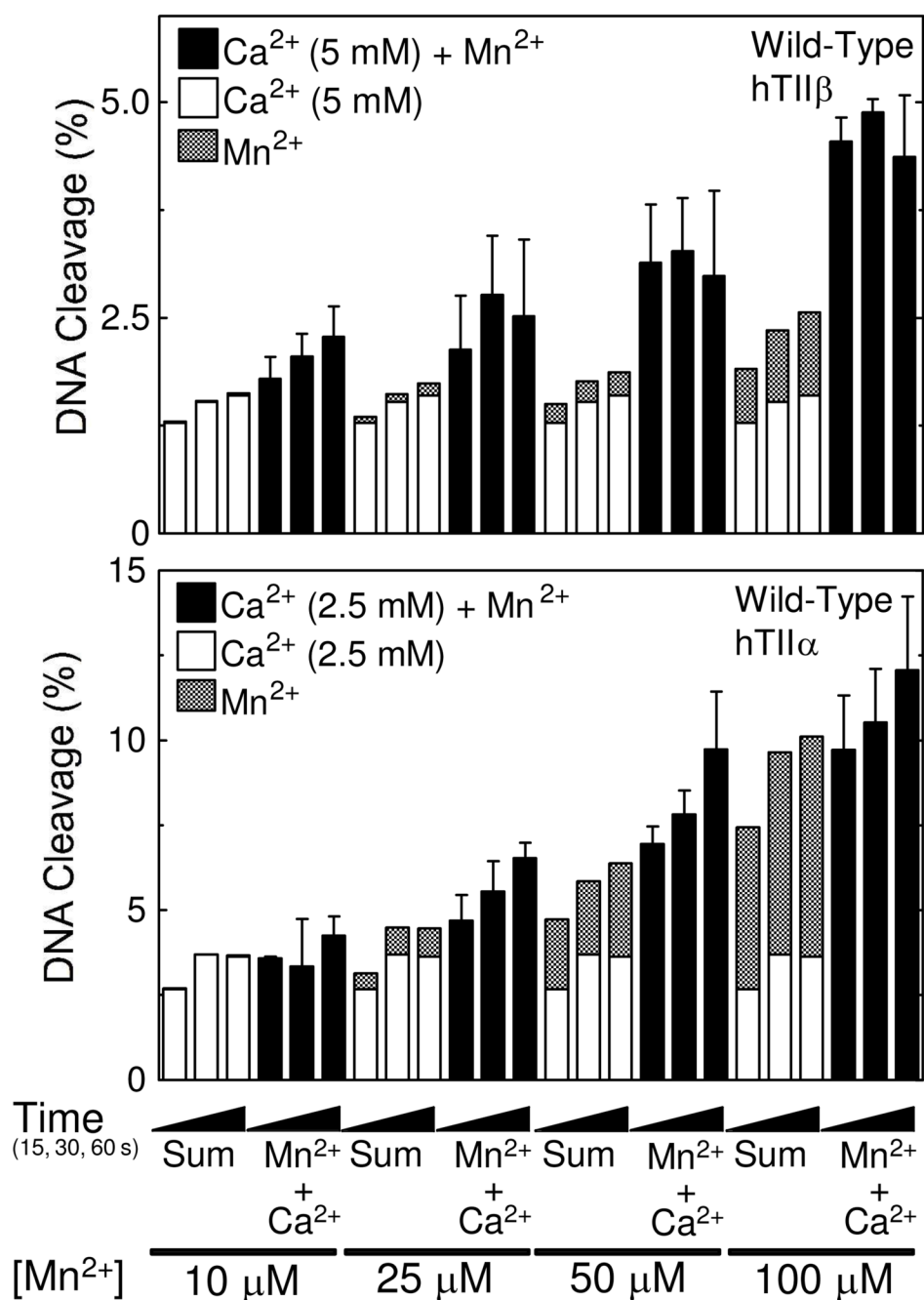
Figure 1.

Cleavage of nicked oligonucleotide substrates by topoisomerase II β 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 non-bridging phosphorothiolate (bottom) are shown. DNA cleavage was carried out in the presence of 5 mM Mg²⁺ (closed square), Ca²⁺ (open square), and Mn²⁺ (closed circle). Rapid quench results for 0.5, 1, and 3 seconds are shown in the presence of Mg²⁺ (black bars), Ca²⁺ (open bars), and Mn²⁺ (gray bars). Error bars represent the standard deviation of at least three independent experiments.

**Figure 2.**

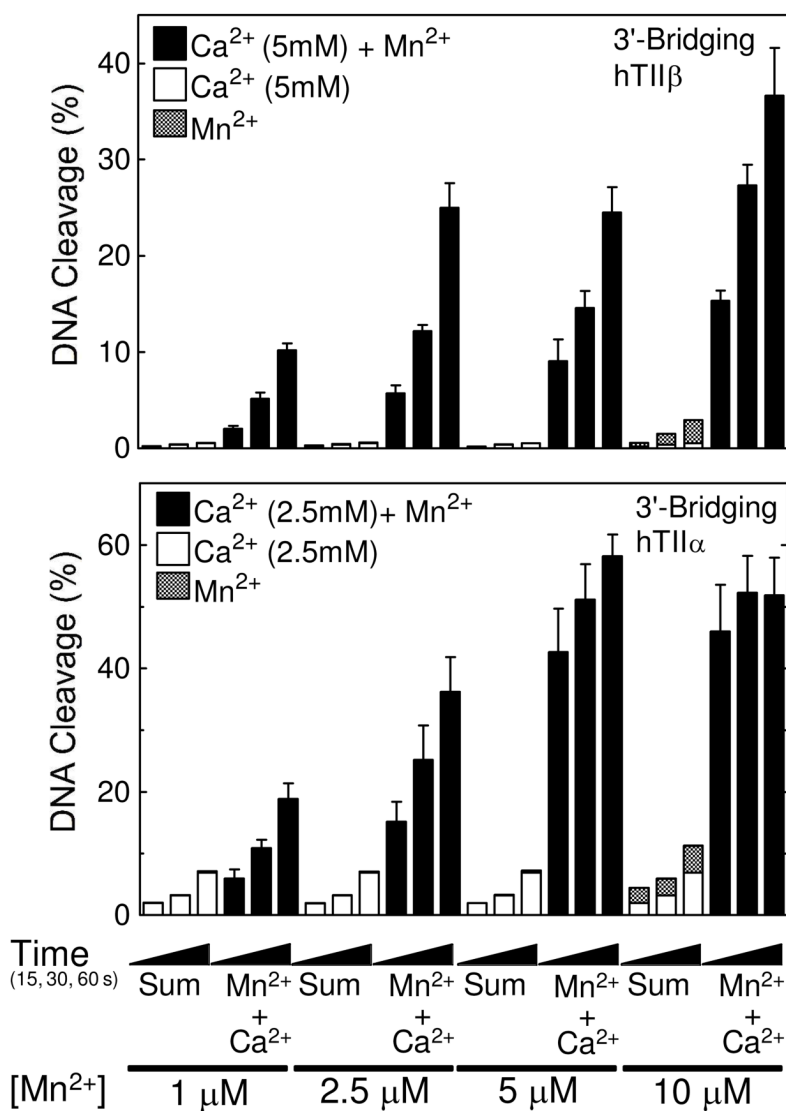
Metal ion concentration dependence for DNA cleavage of nicked wild-type, phosphorothiolate, and phosphorothioate substrates by topoisomerase II β . Results for the wild-type (top), 3'-bridging phosphorothiolate (middle), and non-bridging phosphorothioate (bottom) are shown. Left panels, Mn²⁺ (closed circles) or Ca²⁺ (open squares) were titrated from 10 μ M to 10 mM for wild-type, 1 μ M to 10 mM for 3'-bridging substrates, or 5 μ M to 10 mM for non-bridging. Right panels, expanded DNA cleavage at concentrations up to 100 μ M for wild-type and non-bridging substrates or 50 μ M for the 3'-bridging substrate are shown. All reactions 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.

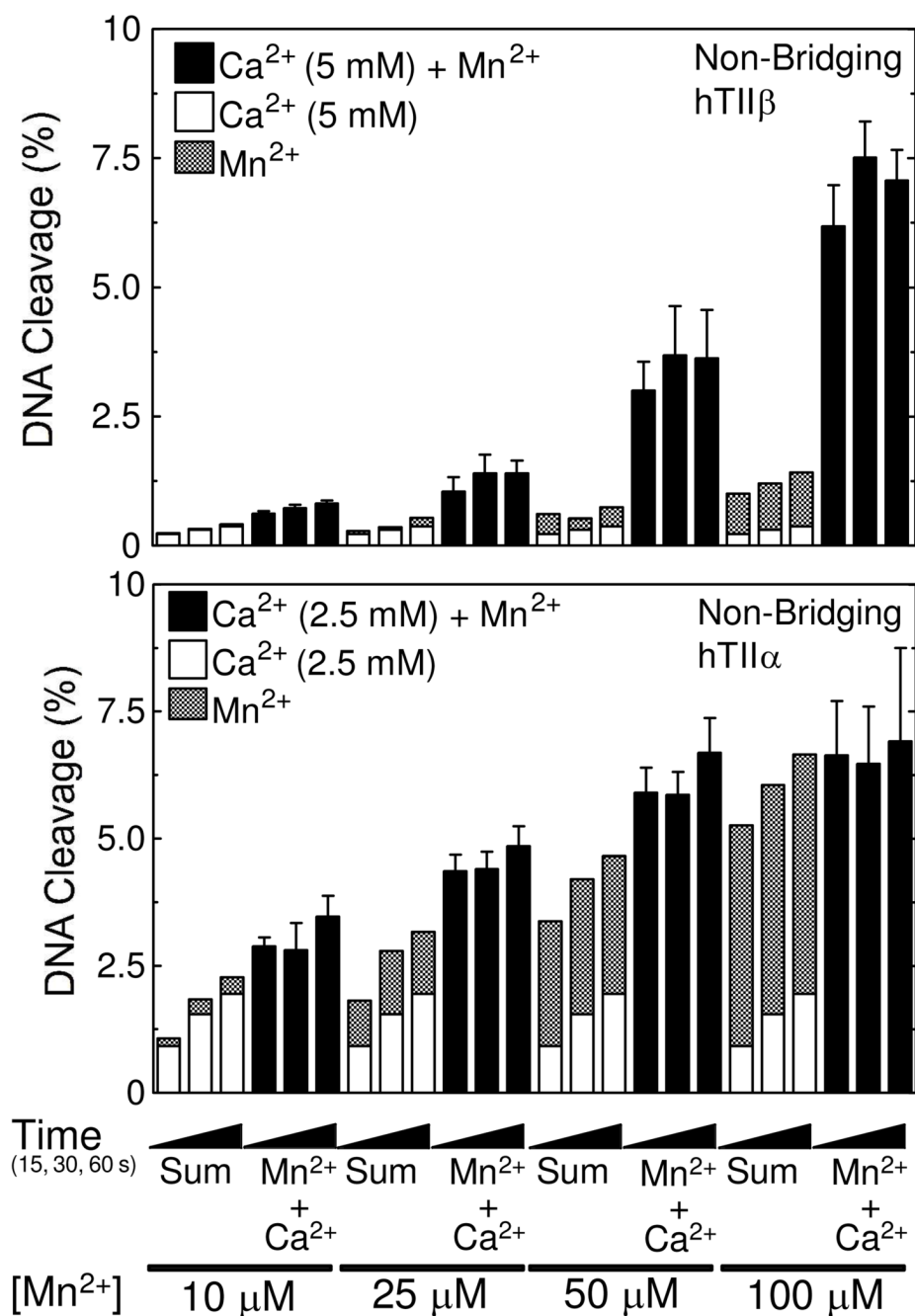
**Figure 3.**

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) Ca²⁺ alone (open bars), 10–100 μM Mn²⁺ alone (stippled bars), or a mixture of 5 mM (IIβ) or 2.5 mM (IIα) Ca²⁺ and 10–100 μM Mn²⁺ (Ca²⁺ + Mn²⁺, closed bars). The calculated sum of the enzyme-mediated DNA cleavage from reactions containing either Ca²⁺ or Mn²⁺ alone is also shown (Sum, stacked open 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 $\text{Ca}^{2+} + \text{Mn}^{2+}$ are shown. Error bars for reactions carried out in the presence of Ca^{2+} or Mn^{2+} alone are not shown for simplicity.

**Figure 4.**

Cleavage of nicked 3'-bridging phosphorothiolate oligonucleotide substrates by topoisomerase II β or II α in the presence of divalent metal ion combinations. DNA cleavage reactions with topoisomerase II β (top) or II α (bottom) were carried out for 15, 30 or 60 s in the presence of 5 mM (II β) or 2.5 mM (II α) Ca²⁺ alone (open bars), 1–10 μ M Mn²⁺ alone (stippled bars), or a mixture of 5 mM (II β) or 2.5 mM (II α) Ca²⁺ and 1–10 μ M Mn²⁺ (Ca²⁺ + Mn²⁺, closed bars). The calculated sum of the enzyme-mediated DNA cleavage from reactions containing either Ca²⁺ or Mn²⁺ alone is also shown (Sum, stacked open and stippled bars, respectively). Data for topoisomerase II α 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 Ca²⁺ + Mn²⁺ are shown. Error bars for reactions carried out in the presence of Ca²⁺ or Mn²⁺ alone are not shown for simplicity.

**Figure 5.**

Cleavage of nicked non-bridging 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) Ca²⁺ alone (open bars), 10–100 μM Mn²⁺ alone (stippled bars), or a mixture of 5 mM (IIβ) or 2.5 mM (IIα) Ca²⁺ and 10–100 μM Mn²⁺ (Ca²⁺ + Mn²⁺, closed bars). The calculated sum of the enzyme-mediated DNA cleavage from reactions containing either Ca²⁺ or Mn²⁺ alone is also shown (Sum, stacked open 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 $\text{Ca}^{2+} + \text{Mn}^{2+}$ are shown. Error bars for reactions carried out in the presence of Ca^{2+} or Mn^{2+} alone are not shown for simplicity.

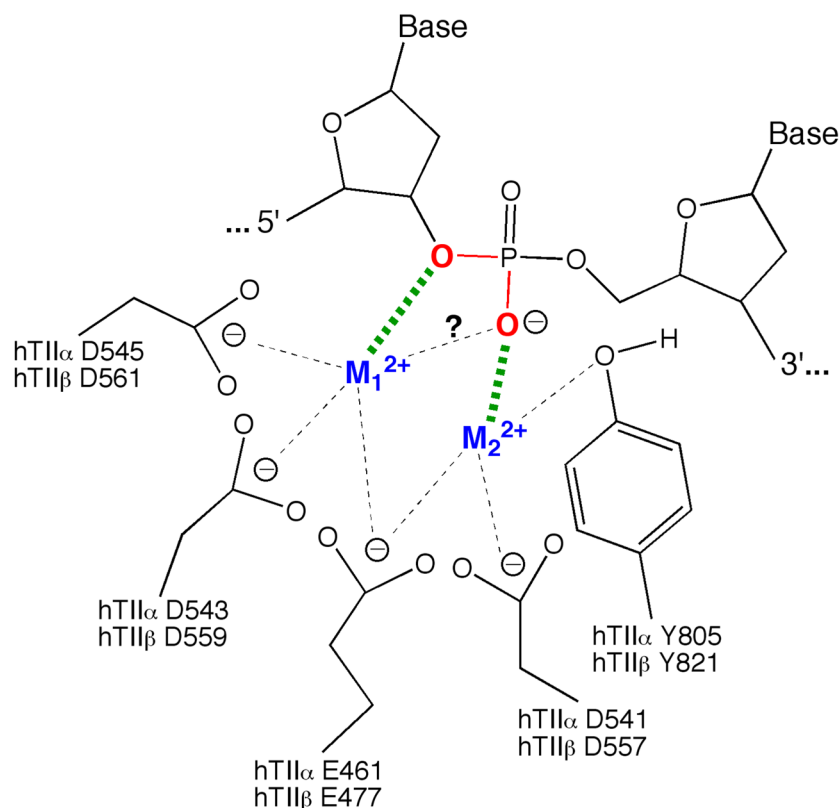


Figure 6.

A two-metal ion model for DNA cleavage by human topoisomerase II α and II β . 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 the metal ions bind to topoisomerase II in an ordered fashion, in which the binding of metal ion 1 (M_1^{2+} , shown in blue) is a prerequisite for the binding of metal ion 2 (M_2^{2+} , shown in blue). M_1^{2+} makes a critical interaction with the 3'-bridging atom (shown in red) of the scissile phosphate (bond shown in green), which most likely is needed to stabilize the leaving 3'-oxygen (left, shown in red). M_2^{2+} is required for DNA scission and likely interacts with the non-bridging oxygen (shown in 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 non-bridging atom of the scissile phosphate significantly enhance DNA cleavage mediated by topoisomerase II β , effects on DNA scission mediated by topoisomerase II α are equivocal. The model was adapted from Ref. (36).