Rapid Reports

Coordinating the Two Protomer Active Sites of Human Topoisomerase IIα: Nicks as Topoisomerase II Poisons†

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ABSTRACT: Topoisomerase II modulates DNA topology by generating double-stranded breaks in DNA. Results of the current study indicate that the presence of a nick at one scissile bond dramatically increases the rate of cleavage by human topoisomerase IIα at the scissile bond on the opposite strand. We propose that this enhanced activity at the second strand coordinates the two protomer subunits of topoisomerase II and allows the enzyme to create double-stranded breaks. Finally, the presence of a nick on one strand induces cleavage on the opposite strand. Thus, nicks are topoisomerase II poisons that generate novel sites of DNA cleavage.

Type II topoisomerases are ubiquitous enzymes that play essential roles in fundamental nuclear processes, such as replication, transcription, recombination, and mitosis (*1*). These enzymes help regulate DNA under- and overwinding and remove knots and tangles from the genome (*1*−*3*). While lower eukaryotes have only a single type II enzyme, topoisomerase II, humans and other vertebrates encode two isoforms, topoisomerase IIα and II*β* (*2, 3*). Topoisomerase IIα is the isoform that is required for cell survival, is expressed at high levels in proliferating cells, and is required for replicative processes and mitosis (*1, 3*).

Type II topoisomerases alter the topological state of DNA by generating transient breaks on both strands of the double helix (*2*−*4*). The two scissile bonds are located across the major groove from one another and are staggered by 4 bp (*5, 6*). Each bond is cleaved by an individual tyrosine residue that resides in a separate protomer subunit of the homodimeric protein (*2*−*4, 7*). During the cleavage event, these active site tyrosine residues form covalent links with the newly generated 5′-DNA termini (*2*−*4*). This linkage preserves the energy of the sugar−phosphate backbone of DNA and maintains genomic integrity.

Beyond their critical cellular roles, topoisomerase IIα and II*β* are the targets for some of the most successful anticancer agents in clinical use (*3, 8, 9*). Topoisomerase II-targeted drugs, such as etoposide and doxorubicin, kill cells by increasing levels of enzyme-associated DNA breaks (*3, 8, 9*). Anticancer drugs and other compounds that enhance enzyme-mediated DNA cleavage are called topoisomerase II poisons to distinguish them from agents that act by inhibiting overall catalytic activity (*3*).

Given the central nature of the DNA cleavage reaction to the physiological and pharmacological functions of topoisomerase II, it is important to understand the mechanistic details of this process. In this regard, it has long been known that type II topoisomerases generate two "coordinated" nicks on the opposite strands of the double helix as opposed to one "unified" double-stranded DNA break. Evidence against a unified mechanism stems from the findings that levels of scission on the two strands of a cleavage site often are nonequal (*6*) and that the enzyme religates the two strands of cleaved molecules in an independent fashion (*7*). At equilibrium, ∼0.5−1% of human topoisomerase IIα in a scission reaction exists as a covalent enzyme-cleaved DNA complex (*10*) (Figure S1 of the Supporting Information). Approximately half of these complexes contain double-stranded DNA breaks with the remainder containing single-stranded breaks. This finding suggests that every time the enzyme cuts one strand of the double helix, it goes on to cut the second strand a high proportion of the time. If the enzyme cut the two strands of DNA in an uncoordinated fashion, virtually no double-stranded breaks would be generated (1% cleavage of the first strand × 1% cleavage of the second strand ≈ 0.01% double-stranded breaks). Thus, there must be a relatively high degree of coordination between the two protomer active sites of topoisomerase IIα, even if they do not act in complete concert. Consistent with this conclusion, once topoisomerase II cleaves the first strand, it cuts the second strand at a rate that is >10-fold faster (*11, 12*). However, the mechanism that underlies the enhanced cleavage of the second strand is not known.

It is notable that the DNA substrates for first and second strand cleavage are not equivalent. Unlike the former substrate, which is an intact duplex, the latter contains a nick at one of the scissile bonds. Consequently, it has been proposed that human topoisomerase IIα generates a high proportion of double-stranded DNA breaks because nicked molecules are a more efficient substrate for the enzyme (*12*). To this point, equilibrium levels of cleavage on one strand of an oligonucleotide that contains a nick at the scissile bond on the opposite strand are

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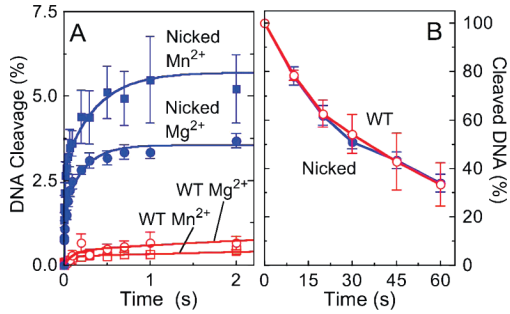

FIGURE 1: A nick at the scissile bond enhances the forward rate of DNA cleavage by topoisomerase IIα. (A) Rapid time courses for cleavage were followed up to 3 s (plots are shown to 2 s) with un-nicked duplex [wild type (WT), red] or scissile bond-nicked (−1/+1 position, blue) substrate in the presence of 5 mM Mg^{2+} or Mn^{2+}. Data were best fitted by double-exponential curves (see the Supporting Information). (B) Topoisomerase IIα-mediated ligation is shown for un-nicked duplex [wild type (WT), red] or scissile bond-nicked (blue) substrate. DNA cleavage was set to 100% at time zero. Error bars are the standard error of the mean (SEM) of two independent experiments or the standard deviation (SD) of at least three independent experiments.

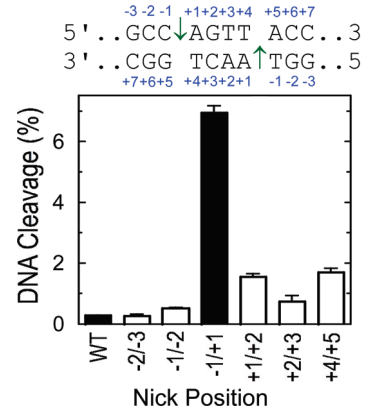

FIGURE 2: A nick at one scissile bond greatly enhances topoisomerase IIα-mediated DNA cleavage at the scissile bond on the opposite strand. The partial sequence of the 50-mer cleavage substrate and the positions of the scissile bonds (arrows) are shown at the top. Levels of DNA cleavage at the native scissile bond on the bottom strand are shown for the un-nicked 50-mer (wild-type, WT) and for substrates containing a nick at the −2/−3, −1/−2, −1/+1, +1/+2, +2/+3, or +4/+5 position on the top strand. Cleavage for the un-nicked and scissile bond-nicked (−1/+1) substrate is in black. Error bars are the SD of at least three independent experiments.

>20-fold higher than those generated by the enzyme with the equivalent intact duplex (see Figure S2 of the Supporting Information) (*12*).

Therefore, to more fully assess the potential of nicks to coordinate the two protomer active sites of topoisomerase IIα during DNA scission, the kinetics of cleavage of an intact substrate and an equivalent oligonucleotide that contained a nick at one of the scissile bonds were studied by rapid chemical quench. Reactions followed cleavage of the strand opposite the nick over a time course from 5 ms to 3 s (Figure 1A). Mg^{2+} or Mn^{2+} was used as the required divalent cation in these experiments. Although Mg^{2+} likely represents the physiological divalent metal ion, higher levels of cleavage are observed in the presence of Mn^{2+} (*13*). Initial velocities for cleavage of the nicked and duplex substrates in the presence of Mg^{2+} were 110 ± 5.6 and 7.8 ± 2.4 s^{-1}, respectively, and in the presence of Mn^{2+} were 230 ± 12 and 9.4 ± 1.3 s^{-1}, respectively. Taken together, these data suggest that the rate of cleavage increases dramatically (∼15−25-fold) in the presence of a nick.

Levels of topoisomerase II-generated DNA strand breaks can be raised by increasing the forward rate of scission or by inhibiting the backward rate of ligation (*3, 9*). Most clinically relevant topoisomerase II-targeted drugs act by the latter mechanism (*3*). However, if DNA nicks are being used to coordinate the two protomer active sites of human topoisomerase IIα during the cleavage reaction, they should act primarily on the forward rate of scission. To determine whether this is the case, the effect of a nicked scissile bond on the rate of enzyme-mediated DNA ligation of the opposite strand was assessed. As seen in Figure 1B, the nick had no effect on the time course for ligation. The apparent first-order rate constants for the duplex and nicked substrates were 0.031 ± 0.0079 and 0.036 ± 0.0074 s^{-1}, respectively. Together with the kinetic data discussed above, these results indicate that the introduction of a nick at one scissile bond greatly stimulates the forward rate of scission of the second strand by topoisomerase IIα.

Why does topoisomerase IIα preferentially cleave nicked DNA? Structural studies provide insight into this important mechanistic question. Recently, the crystal structure of the catalytic core of yeast topoisomerase II complexed with DNA was determined (*14*). The complex was noncovalent in nature, and the substrate contained nicks at both scissile bonds. Surprisingly, DNA in the active site of the enzyme was markedly reshaped, with its trajectory being bent by ∼150° (*14*). Considerable distortion was observed within the double helix, such that the bases between the scissile bonds existed as A-form rather than B-form DNA. Strain was observed over ∼16 bp of the DNA cleavage site, and the highest degree of strain, as determined by the degree of deviation of twist from B-form DNA, occurred at the scissile bonds on each strand of the double helix.

It is reasonable to assume that the acute bending of the double helix seen in the crystal structure approximates the transition state of the DNA during cleavage by topoisomerase II. Given the high degree of distortion and strain required for the transition state, it is difficult for intact duplex DNA to attain this structure. Therefore, we propose that once the enzyme has cut the first strand of the double helix, the increased conformational flexibility that results from the nick greatly facilitates conversion to the cleavage transition state. As a result, topoisomerase II-mediated scission of the second strand occurs with a much faster rate, effectively coordinating the two enzyme protomer subunits.

Consistent with this proposal, DNA lesions (such as abasic sites or alkylated bases) that distort, bend, or increase the flexibility of the double helix are highly effective topoisomerase II poisons when located between the scissile bonds. Lesions often increase enzyme-mediated DNA cleavage >10-fold and stimulate the forward rate of scission without inhibiting ligation (*3, 15, 16*).

If the hypothesis discussed above is correct, the presence of a nick in the vicinity of the scissile bonds should enhance the flexibility of the double helix and promote DNA cleavage by topoisomerase IIα. However, since DNA strain is greatest at the scissile bonds, placement of a nick precisely at the point of cleavage on one strand should have the largest effect on cleavage of the opposite strand. Consequently, the relationship between the position of the nick and levels of DNA scission by topoisomerase IIα was determined. As seen in Figure 2, the

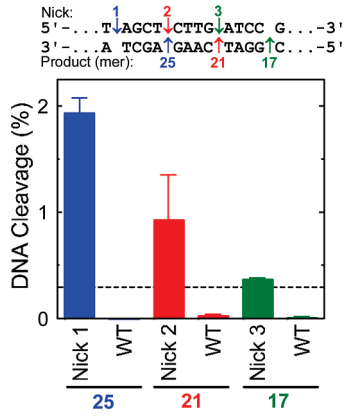

FIGURE 3: Nicks poison topoisomerase IIα. Nicks were incorporated at position 1, 2, or 3 on the top strand of a 50-mer (partially shown at top) that was not cleaved by topoisomerase IIα in the absence of anticancer drugs. Scission was monitored on the bottom strand. Cleavage at the corresponding sites in the un-nicked (wild-type, WT) substrate is shown for comparison. The dashed line represents the cleavage level generated at a typical un-nicked native site [0.29% (see Figure 2)]. Error bars are the SD of at least three independent experiments.

presence of a nick in the vicinity of the scissile bond increased equilibrium levels of cleavage at the opposite scissile bond ~2−6-fold. Moreover, the presence of a nick at the scissile bond (−1/+1 position) increased the level of cleavage at the opposite scissile bond ~25-fold (see Figure S2 of the Supporting Information for DNA cleavage time courses). These findings demonstrate that increased DNA flexibility enhances the ability of topoisomerase IIα to cleave its substrate. The fact that the increase in the level of cleavage was dramatically higher when the nick was placed directly at the scissile bond further supports the proposal that coordination of the two protomer subunits of topoisomerase II results from an enhanced DNA conformational flexibility that follows scission of the first strand.

In addition to stimulating cleavage at the "native" site, the presence of a nick at a position other than that of the scissile bond always generated a novel DNA cleavage band on the opposite strand that was 4 bp from the nick. Cleavage levels at these novel sites were at least as high as that seen at the native site and in some cases were as much as 6-fold higher (see Figure S3 of the Supporting Information).

To further investigate the ability of nicks to induce enzyme-mediated scission at novel DNA sites, a 50-mer substrate was used that was not cleaved by topoisomerase IIα in the absence of anticancer drugs (see the Supporting Information). Nicks were introduced at single sites on one strand of the double helix, and cleavage was characterized on the opposite strand (Figure 3). In all cases, a novel cleavage site located 4 bp from the nick was generated. These novel sites had different sequences, with nicks 1, 2, and 3 inducing cleavage between G and A, T and C, and C and G, respectively. Levels of DNA cleavage were at least comparable to that seen with the native site used in Figure 2 (dashed line) and ranged as much as 6-fold higher.

These findings demonstrate that nicks, like other DNA lesions, are topoisomerase II poisons. However, nicks differ from these lesions in a significant fashion. Abasic sites and modified bases are site-specific poisons that stimulate cleavage only when they are located between the two scissile bonds of a preexisting site of enzyme action (*3, 15, 16*). Furthermore, while they enhance scission at the native site, they do not generate novel sites of cleavage (*3, 15, 16*). In contrast, the actions of nicks are not constrained to preexisting sites of topoisomerase II action. The mere presence of a nick in duplex DNA is sufficient to create a novel cleavage site for human topoisomerase IIα.

In summary, nicks have a profound effect on the DNA cleavage activity of topoisomerase IIα. The presence of a nick at one scissile bond dramatically increases the rate of cleavage at the scissile bond on the opposite strand of the double helix. We propose that nicks trigger faster rates of scission by introducing flexibility in the DNA that allows it to attain the acutely bent transition state that is required for efficient cleavage. This enhanced activity at the second strand coordinates the two protomer subunits of topoisomerase II during the DNA cleavage reaction and allows the enzyme to create double-stranded breaks in the genetic material. Finally, nicks can be added to the list of DNA lesions that poison topoisomerase II. This finding may have implications for the actions of type II enzymes during apoptosis. Moreover, it suggests a mechanism whereby topoisomerase II may impact the physiological effects of endogenous, environmental, or pharmaceutical compounds that damage the genetic material.

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SUPPORTING INFORMATION AVAILABLE

Detailed methods, figures depicting plasmid cleavage, time courses for the cleavage of DNA containing site-specific nicks, and the generation of novel DNA cleavage sites by the presence of nicks. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Wang, J. C. (2002) *Nat. Rev. Mol. Cell Biol. 3*, 430–440.
2. Champoux, J. J. (2001) *Annu. Rev. Biochem. 70*, 369–413.
3. McClendon, A. K., and Osheroff, N. (2007) *Mutat. Res. 623*, 83–97.
4. Schoeffler, A. J., and Berger, J. M. (2005) *Biochem. Soc. Trans. 33*, 1465–1470.
5. Sander, M., and Hsieh, T. (1983) *J. Biol. Chem. 258*, 8421–8428.
6. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., and Chen, G. L. (1983) *J. Biol. Chem. 258*, 15365–15370.
7. Zechiedrich, E. L., Christiansen, K., Andersen, A. H., Westergaard, O., and Osheroff, N. (1989) *Biochemistry 28*, 6229–6236.
8. Pommier, Y., and Marchand, C. (2005) *Curr. Med. Chem. Anticancer Agents 5*, 421–429.
9. Baldwin, E. L., and Osheroff, N. (2005) *Curr. Med. Chem. Anticancer Agents 5*, 363–372.
10. Dickey, J. S., and Osheroff, N. (2005) *Biochemistry 44*, 11546–11554.
11. Mueller-Planitz, F., and Herschlag, D. (2008) *J. Biol. Chem. 283*, 17463–17476.
12. Deweese, J. E., Burgin, A. B., and Osheroff, N. (2008) *Biochemistry 47*, 4129–4140.
13. Deweese, J. E., Burgin, A. B., and Osheroff, N. (2008) *Nucleic Acids Res. 36*, 4883–4893.
14. Dong, K. C., and Berger, J. M. (2007) *Nature 450*, 1201–1205.
15. Sabourin, M., and Osheroff, N. (2000) *Nucleic Acids Res. 28*, 1947–1954.
16. Velez-Cruz, R., Riggins, J. N., Daniels, J. S., Cai, H., Guengerich, F. P., Marnett, L. J., and Osheroff, N. (2005) *Biochemistry 44*, 3972–3981.

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