Spontaneous DNA Damage Stimulates Topoisomerase II-mediated DNA Cleavage

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Apurinic sites are position-specific poisons of topoisomerase II and stimulate DNA scission ~10–18-fold when they are located within the 4-base overhang generated by enzyme-mediated cleavage (Kingma, P. S., and Osheroff, N. (1997) J. Biol. Chem. 272, 1148–1155). To determine whether other major forms of spontaneous DNA damage also act as topoisomerase II poisons, the effects of position-specific apyrimidinic sites and deaminated cytosines (i.e. uracil:guanine mismatches) on the type II enzyme were determined. Both of these lesions stimulated topoisomerase II-mediated DNA scission with the same positional specificity as apurinic sites but were less efficacious. Moreover, apurinic sites dominated the effects of apyrimidinic sites in substrates that contained multiple lesions. The differential ability of spontaneous lesions to enhance DNA cleavage did not correlate with either a decreased stability of the double helix or the size of the gap formed by base loss. Rather, it appears to be due (at least in part) to increased rates of religation for substrates containing apyrimidinic sites or deaminated cytosines. These results suggest that several forms of spontaneous DNA damage are capable of acting as endogenous poisons of topoisomerase II.

The integrity of the genetic material is constantly challenged by events that generate damage in DNA (1–3). An important source of this damage originates from spontaneous alterations within the double helix such as the loss of bases (i.e. generation of apurinic or apyrimidinic sites) and the deamination of cytosine residues (i.e. conversion of cytosine to uracil) (1, 3).

Of all spontaneous DNA lesions, apurinic sites are the most commonly formed (1, 3). It is estimated that ~10,000 apurinic sites are created per mammalian cell on a daily basis (4). Although rates of spontaneous pyrimidine hydrolysis and cytosine deamination in duplex DNA are ~20- and 70-fold lower than that of purine loss (3), respectively, these lesions are frequently induced by exogenous mutagens such as UV irradiation, oxidation, and DNA-reactive chemicals (1, 3, 5–7).

In addition to the reported mutagenic properties of apurinic/apyrimidinic sites and deaminated cytosines (1, 3, 5–7), the induction of spontaneous damage in DNA by heat-acid treatment also enhances the formation of topoisomerase II-cleaved DNA complexes ~6-fold in vitro (8). These cleavage complexes are normally short-lived intermediates in the catalytic cycle of the enzyme and are tolerated by the cell (9–12). However, conditions that shift the cleavage/religation equilibrium of the enzyme toward the cleaved state "poison" topoisomerase II and trigger numerous mutagenic events, such as insertions, deletions, illegitimate recombination, and potentially, cancer-related chromosomal translocations (13–19). When topoisomerase II-mediated DNA breaks are present in sufficient numbers, they initiate a programmed series of events that ultimately culminates in cell death (20–23).

The potentially lethal nature of topoisomerase II has been exploited by several clinically relevant anticancer drugs (13, 14, 17, 22–25). These agents convert this essential enzyme to a cellular toxin by increasing levels of enzyme-mediated DNA breaks. Thus, the same deleterious aspects of topoisomerase II that lead to genomic damage can also be used to cure human disease.

Since the most prevalent DNA lesions induced by heat-acid treatment are apurinic sites (26), a previous study examined the effects of purine loss on DNA cleavage mediated by Drosophila melanogaster topoisomerase II (27). Apurinic sites were found to be position-specific poisons of the type II enzyme, stimulating DNA scission as much as ~10–18-fold, but only when located within the 4-base overhang generated by enzyme-mediated DNA cleavage.

To determine whether other forms of spontaneous DNA damage also act as topoisomerase II poisons, the effects of position-specific apyrimidinic sites and deaminated cytosines (i.e. uracil:guanine mismatches) on the type II enzyme were examined. Both of these lesions stimulated topoisomerase II-mediated DNA cleavage with the same positional specificity as apurinic sites. However, the level of stimulation was lower than that induced by purine loss. Differences in lesion efficacy appear to be due, at least in part, to increased rates of religation for substrates containing apyrimidinic sites and deaminated cytosines.

EXPERIMENTAL PROCEDURES

Topoisomerase II was purified from D. melanogaster embryonic Kc cells as described by Shelton et al. (28). Hepes was obtained from Boehringer Mannheim; proteinase K and SDS were from Merck; bacteriophage T4 poly(nucleotide) kinase and Klenow DNA polymerase were from New England Biolabs, Escherichia coli uracil DNA glycosylase and [γ-32P]ATP (~6000 Ci/mmol) were from Amersham; etoposide was from Sigma (stored at 4 °C, as a 10 mM stock solution in 100% dimethyl sulfoxide); deoxyuridine phosphoramide was from Glen Research; [α-32P]dGTP was from DuPont NEN. All other chemicals were analytical reagent grade.

Preparation of Oligonucleotides—A 40-base single-stranded oligonucleotide that corresponds to residues 67–126 of pBR322 (see Fig. 2) (29) and its complementary oligonucleotide were prepared on an Applied Biosystems DNA synthesizer. The sequence of the top and bottom oligonucleotides were 5′-TGAAATCTAACAATGAGATCAGCCGAGGATGACGATG-3′ and 5′-AGCGTGGCGGAGATGACGATG-3′, respectively. The points of topoisomerase II

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II-mediated DNA cleavage are denoted by the arrows (30, 31). Single-stranded uracil-containing oligonucleotides were prepared in a similar manner utilizing a deoxyuridine phosphorimidate. When appropriate, single-stranded oligonucleotides were radioactively labeled on their 5′ termini in reaction mixtures that contained 10 pmol of oligonucleotide, 5 units of polynucleotide kinase, and 75 pmol of [γ-32P]ATP in a total of 30 μl of kinase buffer (supplied by the manufacturer). Labeled and unlabeled oligonucleotides were isolated by electrophoresis in 7 M urea, 8% polyacrylamide gels. DNA bands were excised from gels and purified using the Qiagen gel extraction protocol. The complementary oligonucleotides were annealed as described by Corbett et al. (31).

Prior to use, uracil bases were removed by incubating 2 pmol (100 nm) of double-stranded oligonucleotide/reaction with 0.2 units (1.7 nm final concentration) of uracil DNA glycosylase in 17 μl of 0.1 M Hepes- HCl, pH 7.9, 0.1 mM EDTA, and 2.5% glycerol for 30 min at 37°C (32, 33). Following treatment with uracil DNA glycosylase, samples were prepared for topoisomerase II assays by the addition of 1 μl of 1 M NaCl, 1 μl KCl and 1 μl of 100 mM MgCl2. Wild type oligonucleotide was treated by an identical procedure to control for the potential effects of this process on topoisomerase II activity. Oligonucleotides that contained a uracil:guanine mismatch were treated in a similar manner in the absence of uracil DNA glycosylase.

**Chemical Hydrolysis of Apyrimidinic Sites**—In order to confirm the presence of apyrimidinic sites in DNA substrates treated with uracil DNA glycosylase, selected oligonucleotides were subjected to hydrolysis in 0.1 N NaOH for 30 min at 37°C. Products were resolved by electrophoresis in denaturing gels as described below. For substrates that contained two apyrimidinic sites, selected oligonucleotides were labeled on their 3′-termini using Klenow DNA polymerase, [α-32P]dGTP, and a template oligonucleotide that contained a 5-base 5′-overhang (5′-GATCC-3′) in addition to the 40-base sequence of the bottom strand. These 3′-labeled oligonucleotides were subjected to hydrolysis as described above. As determined by conversion of full-length substrates to the appropriately sized hydrolysis products, >95% of incorporated uracil residues were converted to apyrimidinic sites by uracil DNA glycosylase.

**Topoisomerase II-mediated DNA Cleavage**—Topoisomerase II-mediated DNA cleavage reactions contained 100 nm wild type oligonucleotide in 1 μl of 10 mM Hepes-HCl, pH 7.9, 0.1 mM EDTA, 50 mM NaCl, 50 mM KCl, 5 mM MgCl2, and 2.5% glycerol and were initiated by the addition of 1 μl of topoisomerase II (100 nm final concentration). Reactions were incubated for 3 min at 30°C and were stopped with 2 μl of 10% SDS following by 1.5 μl of 250 mM EDTA. Cleavage products were digested with proteinase K (2 μl of a 0.8 mg/ml solution) for 20 min at 37°C, precipitated with ethanol twice, and resuspended in 5 μl of 40% formamide, 8.5 mM EDTA, 0.02% bromphenol blue, and 0.02% xylene cyanole FF. Products were resolved by electrophoresis in denaturing 7 M urea, 14% polyacrylamide sequencing gels in 100 mM Tris borate, pH 7.9, and 2 mM NaEDTA. Alternatively, reaction products were resolved under nondenaturing conditions at 10°C in 20% polyacrylamide gels in 100 mM Tris borate, pH 8.3, and 2 mM NaEDTA. Gels were fixed by soaking the wells in 10 min in a 10% methanol and 10% acetic acid solution and dried. Reaction products were visualized and quantified using a Molecular Dynamics PhosphorImager system. Levels of cleavage were calculated from the amount of cleavage product that was produced (i.e. counts in the 15- or 21-mer band) or from the percentage of total substrate that was cleaved (i.e. counts in the 15- or 21-mer band/counts in the total substrate). Similar results were obtained using either method.

**Topoisomerase II-mediated DNA Religation**—Religation assays were carried out by a modification of the procedure of Robinson and Osheroff (34). Cleavage/religation equilibria were established as described under “Topoisomerase II-mediated DNA Cleavage.” Religation was initiated by shifting the reaction temperature from 30 to 4°C and stopped by the addition of 2 μl of 10% SDS at the indicated times. Samples were prepared for electrophoresis and resolved on denaturing gels as described above. The apparent first order rate of religation was determined by measuring the change in the 21-mer cleavage products.

**Spectroscopic Determination of Oligonucleotide Melting Temperatures**—Melting studies utilized a 12-base oligonucleotide that included the central 4-base cleavage overhang and the neighboring 4 residues either side of the topoisomerase II cleavage site (see Fig. 2). After annealing, 150 pmol of double-stranded oligonucleotide was incubated with 2.5 units of uracil DNA glycosylase in 300 μl of 10 mM Hepes-HCl, pH 7.9, 0.1 mM EDTA, and 2.5% glycerol for 30 min at 37°C (32, 33). Following treatment with uracil DNA glycosylase (as appropriate), samples were cooled to 4°C and prepared for assays by the addition of 15 μl of 1 M NaCl, 1 μl KCl and 15 μl of 100 mM MgCl2. Wild type oligonucleotides were treated by an identical procedure to control for the potential effects of this process on stability. Oligonucleotides containing a uracil:guanine mismatch were treated in a similar manner in the absence of uracil DNA glycosylase. Ultraviolet absorbance measurements were recorded using a Cary-14/OLIS spectrophotometer (On-Line Instrument Systems). Reaction temperatures were controlled by a jacketed cell holder connected to a water bath. Oligonucleotide melting was determined by monitoring changes in A260 as a function of temperature over a range of 10–65°C. Melting curves were derivatized incrementally and smoothed with the software provided by the manufacturer. The melting temperature was defined as the temperature at which the first order derivative of the melting curve reached its maximum.

**RESULTS**

Recent evidence indicates that the most prevalent form of spontaneous DNA damage, apurinic sites (1, 3), acts as a position-specific topoisomerase II poison (27). When located within the 4-base stagger generated by enzyme-mediated DNA scission enhance DNA cleavage. Reactions contained wild type double-stranded oligonucleotide (None) or wild type oligonucleotide annealed to a complementary oligonucleotide that contained an apyrimidinic site (−2, +1, +3, +4, +5) at the indicated position on the top strand (the oligonucleotide sequence is shown in Fig. 2). DNA substrates were labeled on the 5′ termini of the bottom (wild type) strand. A control reaction that contained wild type substrate in the absence of topoisomerase II is shown (Std). In addition, a reaction that contained an apurinic site (+2 Apur) at the +2 position on the top strand, which stimulated cleavage ~10-fold, is shown for comparison. The position of the parent 40- and 21-mer cleavage products are indicated.

1 Alternatively, abasic sites were generated in the absence of uracil DNA glycosylase treatment by chemical incorporation of a tetrahydrofuran abasic site analogue during oligonucleotide synthesis. In all cases, similar levels of cleavage were obtained. This indicates that residual uracil DNA glycosylase present in cleavage assays did not affect topoisomerase II activity.
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Cleavage reactions utilized wild type double-stranded oligonucleotide or oligonucleotide that contained an apurinic site (AP(Y)) or uracil located at the indicated positions. Cleavage was stopped by the addition of SDS or reversed by treatment with 15 mM EDTA or 500 mM NaCl prior to the detergent.

### Table I

| Position of DNA lesion | Relative cleavage |
|------------------------|------------------|
|                        | SDS + EDTA + Salt |
| Bottom strand          |                  |
| None                   | 1.0              |
| +3 AP(Y)               | 1.9 ± 0.4        |
| Top strand             |                  |
| None                   | 1.0              |
| +1 AP(Y)               | 3.5 ± 0.3        |
| +3 AP(Y)               | 5.6 ± 0.5        |
| +4 AP(Y)               | 3.2 ± 0.2        |
| +3 Uracil              | 1.7 ± 0.5        |

while those 1 or 2 bases outside these bonds inhibited cleavage ~50%. The effects of deaminated cytosines on DNA cleavage were less pronounced (Fig. 2). When the 3 internal cytosines were converted to uracil residues, cleavage was enhanced at most ~2-fold. Moreover, the deamination of an external cytosine decreased cleavage only 25%.

Since topoisomerase II does not release its DNA cleavage product, scission reactions can be reversed by the addition of high salt or chelation of the required divalent cation (9–12). As shown in Table I, ~90% of cleavage observed with oligonucleotides containing a stimulatory apurinic site or a deaminated cytosine was reversed by either treatment. Furthermore, no oligonucleotide cleavage was observed when topoisomerase II was omitted from reaction mixtures. Taken together, these results indicate that enhanced cleavage of lesion-containing oligonucleotides was mediated by topoisomerase II. In addition, they demonstrate that lesions were not being degraded to nicks during the cleavage assay, since this would have resulted in the formation of nonreversible “suicide” cleavage intermediates (36, 37).

**Apurinic Sites Dominate the Effects of Apurmidinic Sites**—Since apurinic sites are more active topoisomerase II poisons than either apurimic sites or deaminated cytosines, the enzyme may interact preferentially with apurinic sites over other forms of spontaneous DNA damage. To establish the preference of the enzyme for spontaneous DNA lesions, the ability of topoisomerase II to generate double-stranded breaks (as analyzed on nondenaturing polyacrylamide gels) in oligonucleotides that contained both an apurinic site and an apuriminic site was determined (Fig. 3). Comparisons between apurinic sites and deaminated cytosines could not be carried out because treatment of oligonucleotides with uracil DNA glycosylase cannot selectively generate an apurinic site while leaving a second uracil intact.

The underlying premise for the apurinic/apuriminic site preference experiments stems from the finding that DNA lesions located immediately outside the topoisomerase II cleavage stagger diminish the ability of lesions located within the stagger to stimulate cleavage. As seen in Fig. 3, an external apurinic site decreased cleavage stimulation by an internal apurinic site ~6-fold, and an external apurimic site decreased cleavage stimulation by an internal apurimic site ~5-fold. Since the relative reductions in DNA scission for homologous internal/external lesions were similar, a second set of oligonucleotides that contained heterologous lesions was employed to determine whether the enzyme interacts preferential with apurinic over apurimic sites. When an external apurimic site was placed in an oligonucleotide that contained an internal apurinic site, levels of cleavage dropped only
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Cleavage Enhancement Is Not Due to Destabilization of the Double Helix—Previous studies indicate that apurinic sites, apyrimidinic sites, and base mismatches all destabilize the double helix (45–47). Therefore, to further explore the structural basis for enhancement of enzyme-mediated DNA cleavage by spontaneous lesions, their effects on the stability of a topoisomerase II cleavage site were examined. To this end, melting temperatures ($T_m$) were determined for double-stranded 12-mer oligonucleotides containing selected DNA lesions (Table II).

No correlation between $T_m$ values and relative DNA cleavage enhancement (as assessed by levels of DNA cleavage monitored on denaturing polyacrylamide gels) was apparent for internal lesions that stimulated cleavage or for an external apurinic site that abolished cleavage. For example, even though the +3 deaminated cytosine (that enhanced cleavage the least) showed the smallest decrease in $T_m$, the +2 apurinic and +3 apyrimidinic sites (that differed in cleavage enhancement >3-fold) had identical $T_m$ values. Furthermore, the +3/+4 double apyrimidinic site (that enhanced cleavage 7-fold) decreased the $T_m$ of the oligonucleotide by 25 °C, while the single +2 apurinic site (that enhanced cleavage 18-fold) decreased the $T_m$ by only 18 °C.

Finally, the $T_m$ of an oligonucleotide in which the guanine:cytosine base pair at the +2 bottom/+3 top position was inverted was similar to that of the parent DNA substrate. Since cleavage of this latter oligonucleotide is diminished 50% as compared with the parent substrate, it appears that the enzyme does not require local regions of DNA destabilization for cleavage activity.

**Apurinic Sites and Deaminated Cytosines Increase Rates of Topoisomerase II-mediated DNA Religation**—The DNA cleavage/religation equilibrium of topoisomerase II is established by the competing rates of enzyme-DNA cleavage complex formation versus DNA religation (9–12). Thus, levels of DNA cleavage can be profoundly influenced by factors that affect either of these rates (13, 17). For example, anticancer drugs such as etoposide (see Fig. 5, inset) and amascrine enhance

**FIG. 3.** Apurinic sites dominate the effects of apyrimidinic sites. Cleavage reactions contained wild type double-stranded oligonucleotide (None) or oligonucleotide that contained apurinic (R) and/or apyrimidinic (Y) sites at the indicated positions either inside or outside the 4-base cleavage overhang. Substrates were labeled on the bottom strand, and cleavage levels were determined by monitoring production of the double-stranded cleavage product on nondenaturing polyacrylamide gels. Data represent the averages of three independent experiments. Standard deviations are indicated by error bars.

35% (<1.6-fold). In marked contrast, when an external apurinic site was placed in an oligonucleotide that contained an internal apyrimidinic site, levels of cleavage dropped nearly 15-fold. These findings demonstrate that the effects of apurinic sites dominate those of apyrimidinic sites and suggest that the enzyme interacts preferentially with the dominant lesion.

**Cleavage Enhancement by Apurinic/Apyrimidinic Sites Does Not Correlate with the Size of the Gap Formed by Base Loss**—The basis for the increased activity of apurinic sites (versus apyrimidinic sites or deaminated cytosines) against topoisomerase II is unknown. Although structural alterations in lesion-containing DNA are complex (38–43), one simple difference that distinguishes apurinic sites from either apyrimidinic sites or deaminated cytosines is the relatively large gap that is left in the double helix upon removal of a purine base. Therefore, to model larger gap sizes, oligonucleotides containing adjacent abasic sites were constructed (Fig. 4).

Unfortunately, initial attempts to model larger gap sizes that were perpendicular to the DNA axis were not informative, since construction of a duplex oligonucleotide containing abasic sites directly opposite each other (i.e. complementary positions) abolished cleavage. This finding, however, is consistent with structural data that indicates that the double helix collapses at such a double lesion (44).

In contrast, when two adjacent apyrimidinic sites were incorporated parallel to the DNA axis at the +3 and +4 positions on the top strand, levels of topoisomerase II-mediated double-stranded DNA cleavage increased slightly (as compared with either single lesion). However, levels of cleavage did not approach that induced by a single apurinic lesion at the +2 position. A similar enhancement of cleavage was seen in an oligonucleotide containing an adjacent apurinic (+2) and apyrimidinic (+3) site. Thus, relative levels of DNA cleavage do not correlate with the size of the gap formed by base loss.

**FIG. 4.** Cleavage enhancement by adjacent apurinic/apyrimidinic sites. Cleavage reactions contained wild type double-stranded oligonucleotide (None) or oligonucleotide that contained apurinic and/or apyrimidinic sites at the indicated positions on the top and bottom (b) strands. Substrates were labeled on the bottom strand, and cleavage levels were determined by monitoring production of the double-stranded cleavage product on nondenaturing polyacrylamide gels. Data represent the averages of three independent experiments. Standard deviations are indicated by error bars.
topoisomerase II-mediated DNA scission primarily by inhibiting DNA religation (48–50), while other agents such as quinolones and ellipticine act primarily by increasing the rate of cleavage complex formation (51, 52). Finally, cleavage-enhancing apurinic sites have essentially no effect on rates of DNA religation and presumably act by stimulating the rate of cleavage complex formation (27).

To address the mechanistic basis of cleavage enhancement by other spontaneous DNA lesions, rates of topoisomerase II-mediated DNA religation were determined for oligonucleotides that contained either an apyrimidinic site or a deaminated cytosine at the +3 position on the top strand. As seen in Fig. 5, both the apyrimidinic site and the deaminated cytosine increased rates of religation ~3-fold over that of either the wild-type or apurinic (see inset) oligonucleotide. Thus, they probably stimulate DNA cleavage by enhancing cleavage complex formation. In addition, the unexpected increase in religation rates suggests a mechanistic basis that accounts (at least in part) for the relatively modest cleavage enhancement induced by apurinic sites and deaminated cytosines compared with apurinic sites.

**DISCUSSION**

Spontaneous events within the double helix generate thousands of lesions in the mammalian genome on a daily basis (4). Despite their prevalence, the cellular and genetic impact of these damaging lesions has not been fully characterized. Recent evidence indicates that some forms of spontaneous DNA damage, in addition to their effects on replication fidelity (2, 3, 5), enhance double-stranded DNA cleavage mediated by topoisomerase II (8, 27). Thus, by poisoning this enzyme, spontaneous DNA damage can be converted from strand-specific lesions to potentially lethal double-stranded DNA breaks.

Apyrimidinic sites, which are the most frequently induced spontaneous DNA lesions (4), show the most activity against topoisomerase II and stimulate DNA cleavage ~10–18-fold (27). Results of the present study indicate that two other major forms of spontaneous DNA damage, apyrimidinic sites and deaminated cytosines, are also topoisomerase II poisons and enhance DNA cleavage ~2–5-fold. The decreased ability of these lesions to stimulate DNA scission (relative to apurinic sites) may result, at least in part, from increased rates of religation. While the efficacy of apyrimidinic sites and deaminated cytosines is less pronounced than that of apurinic sites, it is notable that a 2000:1 molar excess (i.e. 200 μM) of etoposide is required to achieve a similar level of cleavage enhancement at the DNA site utilized (27), as compared with a 1:1 ratio of these lesions to oligonucleotides.

The structural alterations in DNA that underlie the different ability of spontaneous lesions to enhance topoisomerase II-mediated DNA cleavage are not known. Although apurinic sites generally induce greater distortion in the double helix than do apyrimidinic sites or deaminated cytosines, structural features of spontaneous lesions have not been consistently examined within similar nucleic acid sequences (38–43). However, it is tempting to speculate that a common structural alteration exists in these spontaneous DNA lesions but does so to a greater extent or frequency in apurinic sites. To this point, the base opposite an apurinic site is more likely to adopt an extrahelical “melted” state than the base opposite the other two lesions (39–43). Ultimately, it will be necessary to analyze the structure of these lesions within the context of a topoisomerase II DNA cleavage site to determine the credence of this hypothesis.

Previous studies on apurinic sites (27) and some non-uracil-containing base pair mismatches (similar to those generated by DNA polymerase infidelity) (53) indicate that these lesions stimulate topoisomerase II-mediated DNA scission only when they are located in the 4 base pairs that are internal to the points of DNA cleavage. On the basis of these findings as well as drug specificity and cross-linking studies (22, 35, 54–56), a model, which is called the positional poison model, was proposed to describe the mechanistic basis for topoisomerase II cleavage enhancement (27). This model states that 1) topoisomerase II poisons (both anti-cancer drugs and DNA lesions) induce similar alterations in the structure of DNA and 2) these structural alterations are responsible for enhancing topoisomerase II-mediated DNA scission but must be positioned within the 4-base cleavage overhang in order to stimulate this enzyme activity. The present study indicates that the positional specificity of apyrimidinic sites and deaminated cy-
tosines is identical to that observed for apurinic sites. Therefore, the positional poison model appears to be consistent with the actions of every cleavage-enhancing DNA lesion thus far reported.

The DNA cleavage reaction of topoisomerase II is fundamental to all of the essential catalytic activities of the enzyme (9–12). While low steady-state levels of DNA cleavage are necessary for enzymatic function, considerable circumstantial evidence suggests that increased levels of topoisomerase II-mediated scission cause mutagenic and potentially lethal damage within the genome (13–17, 57). Results of the present study indicate that several different forms of spontaneous DNA damage stimulate topoisomerase II-mediated DNA cleavage and may act as endogenous poisons of the type II enzyme. This ability of spontaneous lesions to induce DNA scission may contribute to their mutagenic properties or may allow topoisomerase II to selectively remove cells with genomic damage from a population.

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