Spontaneous DNA Lesions Poison Human Topoisomerase IIα and Stimulate Cleavage Proximal to Leukemic 11q23 Chromosomal Breakpoints†

Paul S. Kingma,‡ Courtney A. Greider,‡ and Neil Osheroff*,‡,§

Departments of Biochemistry and Medicine (Oncology), Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

Received March 5, 1997; Revised Manuscript Received April 2, 1997⊗

ABSTRACT: Topoisomerase II-targeted drugs, such as etoposide, "poison" this enzyme and kill cells by increasing levels of covalent topoisomerase II-cleaved DNA complexes. In spite of the success of this drug in the treatment of human cancers, a significant proportion of patients treated with etoposide eventually develop secondary leukemias that are characterized by translocations at chromosome band 11q23. Since similar translocations are associated with primary leukemias in previously untreated infants, we questioned whether they could also be triggered by the actions of "endogenous topoisomerase II poisons". Recent studies, which demonstrated that several forms of spontaneous DNA damage stimulate cleavage mediated by *Drosophila* topoisomerase II, suggest that DNA lesions may act as these endogenous poisons. Therefore, to determine whether the ability to recognize spontaneous DNA damage has been conserved from this lower eukaryote to mammalian species, the effects of apurinic sites, apyrimidinic sites, and deaminated cytosine residues on human topoisomerase IIα were assessed. All three lesions were potent poisons of the human enzyme and stimulated cleavage when located within the four-base overhang generated by enzyme-mediated DNA scission. Furthermore, these lesions increased levels of cleavage at five sites proximal to 11q23 translocation breakpoints and did so with an efficacy that was comparable to or greater than that of therapeutic concentrations of etoposide. Although the physiological relevance of these findings has yet to be established, they suggest a potential role for endogenous topoisomerase II poisons in the initiation of leukemic chromosomal breakpoints.

Topoisomerase II is the primary cellular target for some of the most active drugs used in the treatment of human cancers (*1*−*4*). These agents exert their chemotherapeutic actions by increasing levels of covalent topoisomerase II-cleaved DNA complexes (*1*−*4*). Although these complexes are obligatory intermediates in the DNA strand passage reaction catalyzed by the enzyme (*1*, *5*−*7*), when present in high concentrations, they trigger mutagenic and cell death pathways (*4*, *8*−*11*). Thus, agents that enhance topoisomerase II-mediated DNA cleavage are referred to as topoisomerase II "poisons" because they convert this essential enzyme to a potent cellular toxin (*2*, *12*).

Etoposide, which is one such poison, is the most commonly prescribed anticancer drug in clinical use (*4*, *13*, *14*). Unfortunately, despite the success of this agent, between 2 and 12% of patients treated with etoposide-based regimens eventually develop secondary therapy-related leukemias (*13*−*15*). Of these patients, over 50% have genomic translocations involving an 8.3-kilobase breakpoint cluster in the *MLL* oncogene at chromosome band 11q23 (*13*, *14*, *16*−*20*). The nonrandom association of etoposide treatment with these translocations coupled with the fact that etoposide stimulates topoisomerase II-mediated DNA scission at sites proximal to 11q23 breakpoints (*21*) suggests that the type II enzyme may be involved in initiating these translocation events.

Chromosomal translocations involving band 11q23 are also associated with the majority of primary infant leukemias, even though affected infants had no prior exposure to anticancer drugs (*13*, *14*, *22*, *23*). This finding raises the question of how chromosomal breaks that trigger leukemic translocations are initiated in these drug-naive patients. One intriguing possibility is that they are generated by the actions of "endogenous topoisomerase II poisons". Evidence supporting this hypothesis comes from recent work that characterized the effects of DNA lesions on *Drosophila* topoisomerase II (*24*−*26*). These studies demonstrated that several types of spontaneous DNA damage commonly formed in the cell enhance cleavage mediated by the type II enzyme from this lower eukaryote.

Therefore, as a first step toward determining whether endogenous topoisomerase II poisons are capable of initiating chromosomal translocations in mammalian systems, we assessed the ability of spontaneous DNA lesions to stimulate cleavage mediated by human topoisomerase IIα. Results indicated that the three major forms of spontaneous DNA damage, apurinic sites, apyrimidinic sites, and deaminated cytosine residues (i.e., uracil·guanine mismatches) (*27*−*29*), were all potent enhancers of DNA scission mediated by this enzyme. Furthermore, these lesions stimulated DNA cleavage at sites proximal to leukemic 11q23 chromosomal breakpoints with an efficacy that was equal to or greater than that of therapeutic concentrations of etoposide. Although the physiological relevance of these findings has yet to be established, they suggest a potential role for endogenous topoisomerase II poisons in the generation of leukemic chromosomal breakpoints.

† This work was supported by NIH Grant GM53960.
* To whom correspondence should be addressed.
‡ Department of Biochemistry.
§ Department of Medicine (Oncology).
⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

S0006-2960(97)00507-2 CCC: $14.00

EXPERIMENTAL PROCEDURES

Proteinase K was obtained from Merck; bacteriophage T4 polynucleotide kinase and Klenow DNA polymerase were from New England Biolabs. *Escherichia coli* uracil DNA glycosylase and [γ-^{32}P]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 phosphoramidite was from Glen Research; hydroxylapatite (Bio-Gel HTP) was from Bio-Rad. All other chemicals were analytical reagent grade.

*Purification of Human Topoisomerase II*α. Human topoisomerase IIα was purified from ~40 g of *Saccharomyces cerevisiae* containing the human TOP2α gene in the inducible overexpression plasmid YEpWOB6 (*30*). All steps were carried out at 4 °C. Prior to chromatography, the procedure was based on that previously described by Wasserman et al. (*30*). Briefly, cells were lysed, and topoisomerase IIα was extracted from chromosomes that were precipitated with polymin P. Following differential precipitation with $(NH_4)_2SO_4$, the pellet containing topoisomerase IIα was resuspended in buffer I [15 mM $NaPO_4$ (pH 7.7), 1 mM EGTA, 1 mM EDTA, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, 1 mM 2-mercaptoethanol, 0.5 μg of leupeptin/mL, and 1 μg of pepstatin/mL] until it was brought to a conductivity similar to that of HAP-column buffer [15 mM $NaPO_4$ (pH 7.7), 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, 0.5 mM dithiothreitol, and 10 mM $Na_2S_2O_5$] containing 100 mM KPO_4 at pH 7.7.

The protein sample was subsequently applied to a 10 mL hydroxylapatite column that had been equilibrated with HAP-column buffer containing 100 mM KPO_4 at pH 7.7. The column was washed with 30 mL of the same buffer, and protein was eluted with a linear 150 mL gradient of HAP-column buffer containing 100 mM KPO_4 at pH 7.7 to 600 mM KPO_4 at pH 7.7. Fractions containing human topoisomerase IIα (as monitored by gel electrophoresis) were collected and diluted with buffer I until they reached a conductivity similar to that of P-cell column buffer [15 mM $NaPO_4$ (pH 7.7), 1 mM EGTA, 1 mM EDTA, 10% glycerol, 0.5 mM dithiothreitol, and 150 mM KCl]. Protein was concentrated by applying it to a 2.5 mL phosphocellulose collection column (equilibrated in P-cell column buffer), washing with 5 mL of P-cell column buffer, and eluting with 10 mM Tris-HCl (pH 7.7), 0.5 mM dithiothreitol, 0.1 mM EDTA, 40% glycerol, and 750 mM KCl. Protein-containing fractions [as monitored by Bradford assays using the Bio-Rad reagent (Bio-Rad) and bovine serum albumin as a standard] were pooled, aliquoted, and stored in liquid N_2. This protocol typically yields ~0.3 mg of protein that was >90% pure from 1 g of dry packed cells (Figure 1).

Preparation of Oligonucleotides. Experiments utilized a 40-base single-stranded oligonucleotide that corresponds to residues 87−126 of pBR322 (*31*) and its complementary oligonucleotide that were prepared on an Applied Biosystems DNA synthesizer. The sequences of the top and bottom oligonucleotides were 5′-TGAAATCTAACAATG$_i$GCTC-ATCGTCATCCTCGGCACCGT-3′ and 5′-ACGGTGCCGA-GGATGACGATG$_i$GCGCATTGTTAGATTTCA-3′, respectively. Points of topoisomerase II-mediated DNA cleavage are denoted by the arrows (*32*, *33*). Single-stranded uracil-containing oligonucleotides were prepared in a similar manner utilizing a deoxyuridine phosphoramidite. Alternatively, wild type and uracil-containing oligonucleotides that contained 11q23 chromosomal breakpoints (see Figure 4) were obtained from Cruachem, Inc. 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-^{32}P]ATP in a total of 30 μL of kinase buffer (supplied by the manufacturer). Oligonucleotides were purified by electrophoresis in 7 M urea, 8% polyacrylamide gels. DNA bands were excised from gels and purified using the QIAGEN gel extraction protocol. Complementary oligonucleotides were annealed as described by Corbett et al. (*33*).

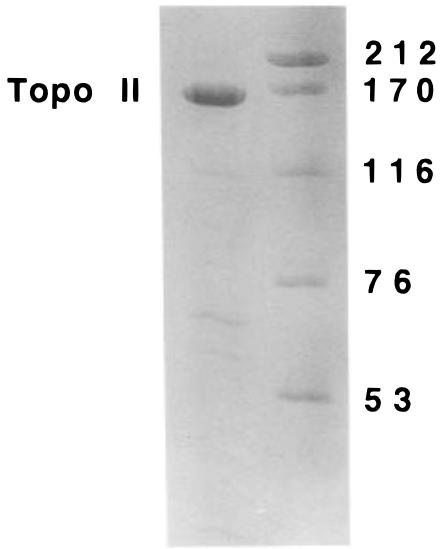

FIGURE 1: Purification of human topoisomerase IIα. A Coomassie-stained 7.5% polyacrylamide gel is shown: lane 1, purified human topoisomerase IIα; lane 2, molecular weight markers.

In order to generate apurinic sites or apyrimidinic sites, uracil bases were removed by incubating 2 pmol (100 nM) of double-stranded oligonucleotide per reaction with 0.2 unit (1.7 nM final concentration) of uracil DNA glycosylase in 17 μL of 10 mM Hepes-HCl (pH 7.9), 0.1 mM EDTA, and 2.5% glycerol for 30 min at 37 °C. Following treatment with uracil DNA glycosylase, samples were prepared for topoisomerase II assays by the addition of 1 μL of 2 M KCl and 1 μL of 100 mM $MgCl_2$. Wild type oligonucleotide was treated by an identical procedure to control for the potential effects of this process on topoisomerase II activity. In order to generate deaminated cytosine residues, oligonucleotides that contained a uracil·guanine mismatch were treated in a similar manner in the absence of uracil DNA glycosylase.

Topoisomerase II-Mediated DNA Cleavage. Topoisomerase II-mediated DNA cleavage reactions were carried out by a protocol similar to that previously described by Kingma et al. (*25*). Reaction mixtures contained 100 nM oligonucleotide in 19 μL of 10 mM Hepes-HCl (pH 7.9), 0.1 mM EDTA, 100 mM KCl, 5 mM $MgCl_2$, and 2.5% glycerol, and reactions were initiated by the addition of 1 μL of human topoisomerase IIα (150 nM final concentration). Reaction mixtures were incubated for 10 min at 37 °C, and reactions were stopped with 2 μL of 10% SDS followed by 1.5 μL of 250 mM EDTA. When appropriate, cleavage reactions were

reversed by the addition of 1.5 μL of 250 mM EDTA or 2 μL of 5 M NaCl for 4 min at 37 °C prior to detergent. 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% bromophenol 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 8.3) and 2 mM NaEDTA. Gels were fixed in 10% methanol and 10% acetic acid for 10 min and dried. Reaction products were visualized and quantified using a Molecular Dynamics PhosphorImager system. Levels of cleavage were calculated relative to the wild type substrate from the amount of cleavage product that was produced or from the percentage of total substrate that was cleaved. Similar results were obtained using either method.

RESULTS AND DISCUSSION

Previous studies demonstrated that several types of spontaneous DNA damage act as position-specific poisons of *Drosophila* topoisomerase II and greatly stimulate DNA cleavage mediated by the enzyme (*24*−*26*). In contrast to *Drosophila* and other lower eukaryotes, vertebrate species contain two distinct isoforms of topoisomerase II, α and β (*1*, *7*, *34*−*36*). Although both of these isoforms will complement yeast topoisomerase II *in vivo* (*37*), they appear to have distinct enzymological, pharmacological, and physiological properties (*7*, *36*, *38*). Therefore, to extend our earlier work with lower eukaryotes to a human system, the effects of apurinic sites, apyrimidinic sites, and deaminated cytosine residues on human topoisomerase IIα were characterized. The α isoform of the human enzyme was employed for this study because it is the isoform that (1) is associated with proliferating tissues (*39*−*41*), (2) has been shown to cleave DNA proximal to translocation breakpoints at chromosomal band 11q23 (*21*), and (3) is most commonly linked to the chemotherapeutic actions of topoisomerase II-targeted anticancer drugs in mammalian cells (*36*, *38*, *42*, *43*).

Spontaneous DNA Lesions Located within the Four-Base Cleavage Overhang Generated by Human Topoisomerase IIα Stimulate DNA Scission. To analyze the effects of spontaneous DNA lesions on cleavage mediated by human topoisomerase IIα, position-specific apurinic sites, apyrimidinic sites, and deaminated cytosine residues were incorporated into a double-stranded 40-mer substrate that contained a single, centrally located topoisomerase II cleavage site. The positions of DNA lesions are designated relative to the point of cleavage on that strand. Bases 5′ to the point of cleavage are denoted by negative numbers, and those 3′ are denoted by positive numbers. The points of scission on both the top and bottom strands are located between the −1 and +1 bases. Cleavage of the top and bottom strands of this substrate by the human type II enzyme results primarily in the formation of 15- and 21-mer products, respectively, so that a four-base cleavage overhang is generated (see Figure 3) (*32*, *33*). To ensure that DNA breaks were generated by the enzyme rather than degradation of lesions, oligonucleotide scission was monitored on the wild type strand complementary to the oligonucleotide containing the damaged residue.

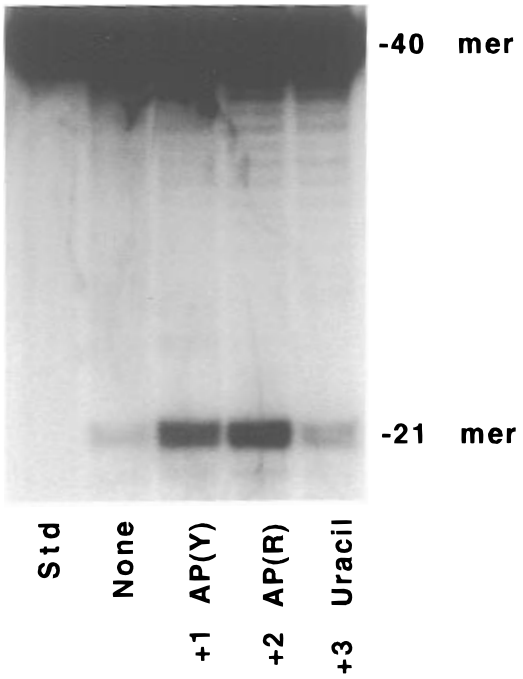

FIGURE 2: Spontaneous DNA lesions stimulate cleavage mediated by human topoisomerase IIα. Reaction mixtures contained human topoisomerase IIα and wild type substrate (None) or oligonucleotides that contained an apurinic site [+2 AP(R)], apyrimidinic site [+1 AP(Y)], or deaminated cytosine residue (+3 Uracil) incorporated at the indicated position on the top strand (see Figure 3). DNA substrates were labeled on the 5′-termini of the bottom strand, and DNA cleavage products were resolved on denaturing polyacrylamide gels. A control reaction mixture that contained wild type substrate in the absence of topoisomerase IIα is shown (Std).

Lesions located within the four-base overhang of the cleavage site stimulated DNA scission mediated by human topoisomerase IIα. As seen in Figures 2 and 3, cleavage was enhanced ∼4−7.5-fold by the presence of a single apurinic site per oligonucleotide. In order to achieve comparable levels of cleavage stimulation by etoposide, a 2000-fold molar excess of drug over 40-mer substrate (i.e., 200 μM etoposide) was required (data not shown). DNA cleavage was also stimulated by apyrimidinic sites and deaminated cytosines, albeit to a lesser extent (∼2.5−4.5-fold by apyrimidinic sites and ∼2-fold by deaminated cytosines) (Figures 2 and 3). These results are similar to those reported for the *Drosophila* type II enzyme (*25*, *26*) and strongly suggest that spontaneous DNA lesions are formidable poisons of human topoisomerase IIα.

Spontaneous DNA lesions are subject to degradation by numerous chemical and cellular factors (*27*−*29*). Since topoisomerase II-mediated cleavage of DNA in the vicinity of a nick can result in the formation of a nonreversible "suicide" cleavage complex (*44*, *45*), two control reactions were carried out to confirm that the cleavage stimulation observed was due to the DNA lesion rather than to a site-specific nick. First, no oligonucleotide cleavage was observed when the human enzyme was omitted from reaction mixtures (not shown). Second, the vast majority of cleavage was reversed by the addition of high salt or chelation of the essential divalent cation with EDTA (Table 1). Therefore, the DNA scission observed with lesion-containing oligonucleotides is mediated by topoisomerase IIα, and cleavage enhancement is not due to the degradation of lesions to nicks during scission reactions.

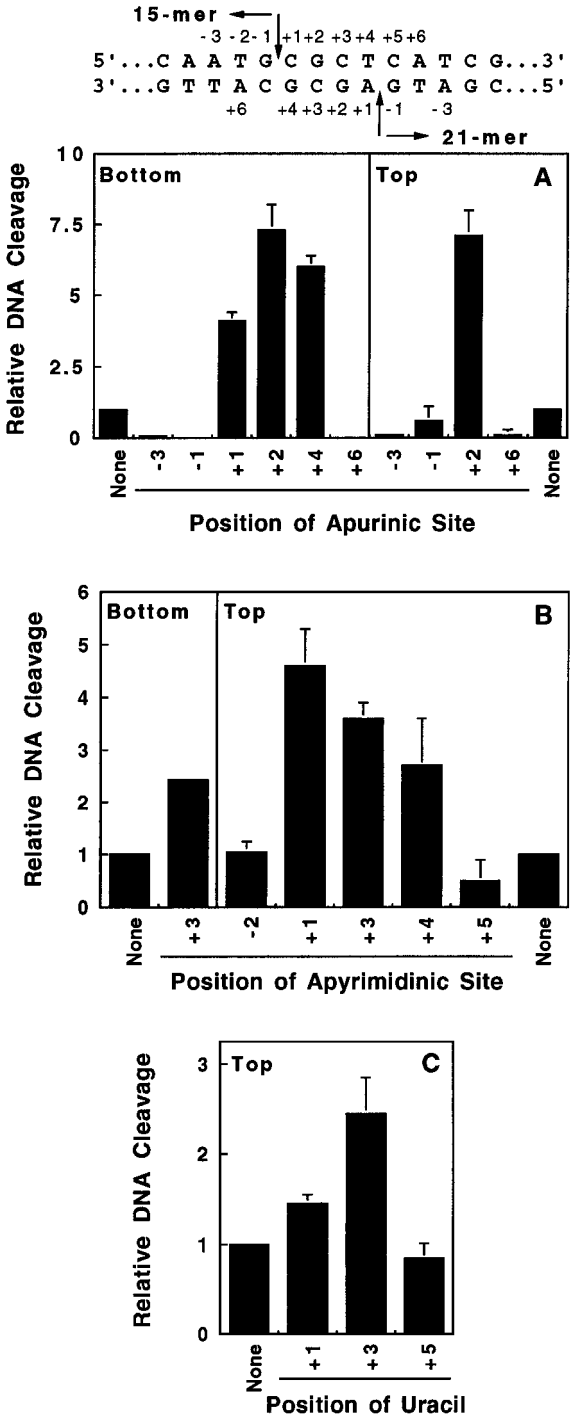

FIGURE 3: Effects of position-specific spontaneous DNA lesions on cleavage mediated by human topoisomerase IIα. The effects of position-specific apurinic sites, apyrimidinic sites, or deaminated cytosine residues on topoisomerase II-mediated DNA cleavage are shown in panels A−C, respectively. DNA lesions were incorporated at the specified positions on the top and bottom strands. Levels of cleavage were determined relative to the wild type (None) substrate for oligonucleotides that contained lesions on the bottom or top strands by monitoring production of labeled 15-mer or 21-mer cleavage products, respectively. Data represent the averages of three independent experiments. Standard deviations are indicated by error bars.

In contrast to the cleavage stimulation observed with internal DNA lesions, apurinic sites located immediately outside the cleavage stagger dramatically inhibited cleavage complex formation (Figure 3). The inhibitory effects of external apyrimidinic sites and deaminated cytosines were less pronounced, as they decreased cleavage complex formation by ~50 and ~25%, respectively.

Table 1: Reversal of DNA Cleavage Mediated by Human Topoisomerase IIα[a]

	relative cleavage		
position of DNA lesion	SDS	EDTA	NaCl
bottom strand			
none	1.0	<0.05	<0.05
+2 AP(R)	7.3 ± 0.9	0.15 ± 0.05	0.3 ± 0.3
top strand			
none	1.0	0.1 ± 0.1	0.1 ± 0.1
+1 AP(Y)	4.6 ± 0.7	0.64 ± 0.05	0.88 ± 0.07
+3 uracil	2.5 ± 0.4	0.25 ± 0.08	0.18 ± 0.05

[a] Cleavage reactions utilized wild type double-stranded oligonucleotide (none), oligonucleotide that contained an apurinic [AP(R)] or apyrimidinic site [AP(Y)], or uracil located at the indicated position. Cleavage was stopped by the addition of SDS or reversed by treatment with 15 mM EDTA or 500 mM NaCl prior to the detergent.

The positional specificity of cleavage-enhancing DNA lesions observed in the present study on human topoisomerase IIα is identical to that previously described for the effects of DNA damage on *Drosophila* topoisomerase II (*25*, *26*) and the effects of base mismatches on the human enzyme (*46*). Thus, it appears that the "positional poison" model (*25*), which was originally proposed to explain the actions of DNA lesions and anticancer drugs against topoisomerase II from lower eukaryotes, is also applicable to human systems. This model postulates that (1) topoisomerase II poisons (both DNA lesions and anticancer drugs) induce similar alterations in the structure of DNA, (2) these structural alterations are responsible for enhancing topoisomerase II-mediated DNA scission, and (3) they must be positioned (through either enzyme binding or covalent linkage to the DNA) within the four-base cleavage overhang in order to stimulate this enzyme activity.

DNA Lesions Stimulate Topoisomerase II-Mediated Scission of Cleavage Sites Proximal to 11q23 Translocation Breakpoints. Translocations involving chromosome band 11q23 are often observed in etoposide-induced secondary leukemias (*13*, *14*, *16*, *17*). Although the events that lead to these chromosomal rearrangements are unknown, it has been suggested that they are triggered by the formation of drug-stabilized topoisomerase II−DNA cleavage complexes proximal to the breakpoints (which are eventually converted to permanent breaks, processed by polymerases or nucleases, and ligated) (*18*, *20*, *21*, *47*, *48*). Since these same chromosomal translocations are present in the majority of primary infant leukemias (*13*, *14*, *22*, *23*), it is possible that they are triggered by the actions of endogenous or environmental topoisomerase II poisons. On the basis of the above results, we questioned whether DNA lesions were capable of stimulating cleavage mediated by human topoisomerase IIα at sites proximal to 11q23 translocation breakpoints.

Two 61-base pair oligonucleotide substrates that spanned nucleotide positions 1050−1110 or positions 1130−1190 of the *MLL* oncogene were utilized for this investigation (*21*, *49*). The first contained two translocation breakpoints at nucleotide positions 1081 and 1082 of the *MLL* gene breakpoint cluster region and two etoposide-enhanced topoisomerase IIα cleavage sites at positions 1067 and 1087. The second contained a single translocation breakpoint at position 1161 and three surrounding cleavage sites at positions 1148,

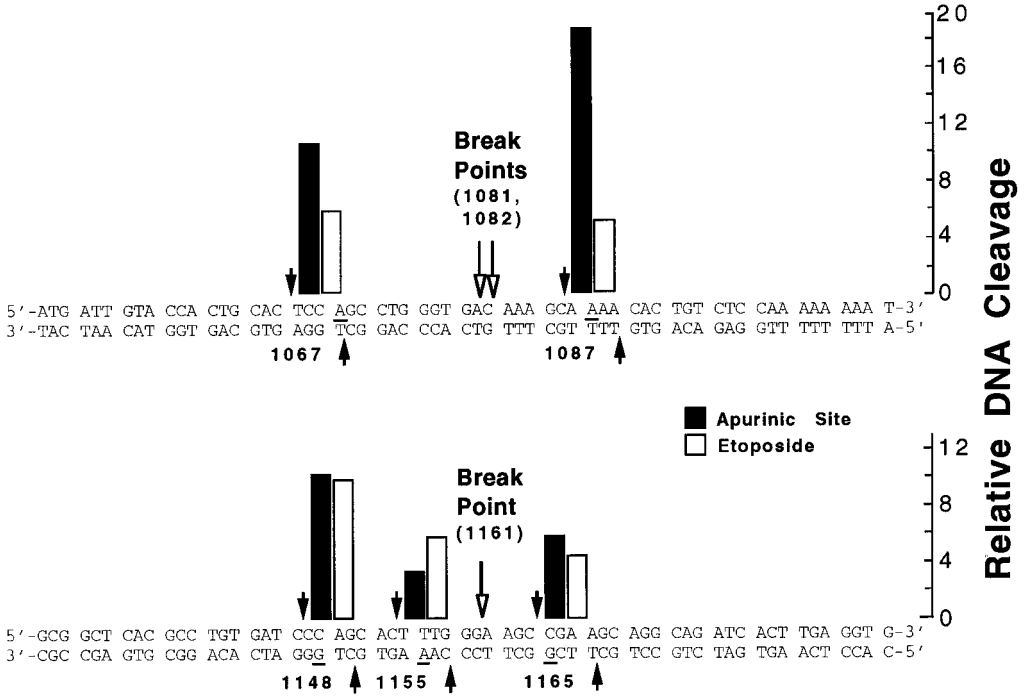

FIGURE 4: Spontaneous DNA lesions stimulate cleavage mediated by human topoisomerase IIα proximal to 11q23 translocation breakpoints. Position-specific apurinic sites were incorporated at the underlined positions. Levels of DNA cleavage (indicated by bars) were determined in the absence of ATP relative to the wild type substrate for both apurinic oligonucleotides (closed bars) and reaction mixtures that contained wild type substrate and 100 μM etoposide (open bars). Data represent the averages of three independent experiments. Points of topoisomerase II-mediated DNA scission are indicated by closed arrows, and the nucleotide positions of the topoisomerase II cleavage sites on the top strand of each oligonucleotide are denoted by numbers previously assigned to the breakpoint cluster (*49*). The locations of chromosome band 11q23 translocation breakpoints are indicated by open arrows.

1155, and 1165. As shown in Figure 4 and Table 2, insertion of a single apurinic site within the 1067 or 1087 cleavage site enhanced enzyme-mediated DNA scission ~10- or 19-fold, respectively. Furthermore, cleavage was stimulated ~11-, 3-, or 6-fold by insertion of this lesion within the 1148, 1155, or 1165 site, respectively.

All five cleavage sites were flanked by a cytosine at the −1 position, which is often predictive of strong etoposide-enhanced DNA scission (*4*). Despite this positive indicator for drug action, the cleavage stimulation induced by a single apurinic lesion at any given site was similar to or greater than that generated by therapeutic concentrations of the drug (i.e., 100 μM) at four of these five sequences (Figure 4).

Topoisomerase II requires ATP to catalyze its double-stranded DNA passage reaction (*5*−*7*). Although the nucleoside triphosphate is not necessary for the enzyme to cleave its DNA substrate (*5*−*7*), it has been suggested that the ability of etoposide to enhance topoisomerase II-mediated DNA scission is strongly dependent on the presence of ATP (*2*, *6*). Since the above experiments were carried out in the absence of a high-energy cofactor, DNA scission was also analyzed in the presence of ATP. As seen in Table 2, ATP had differential effects on the ability of etoposide and apurinic sites to stimulate cleavage at specific sites. In some cases, the nucleoside triphosphate enhanced DNA scission as much as 2.5-fold (relative to wild type controls), while in others, levels of cleavage diminished slightly. These minor alterations notwithstanding, ATP did not preferentially increase drug-induced cleavage over that of the DNA lesion. In fact, in the presence of the nucleoside triphosphate, the level of cleavage observed in every apurinic oligonucleotide was higher than that generated in etoposide-containing reactions.

Table 2: DNA Cleavage Proximal to 11q23 Translocation Breakpoints[a]

cleavage site	poison	relative DNA cleavage	
		without ATP	with ATP
1067	apurinic site	10.4 ± 0.5	17.6 ± 0.4
	etoposide	5.6 ± 0.2	14.3 ± 0.9
	apyrimidinic site	7.2 ± 0.2	6.1 ± 0.1
	uracil	5.0 ± 0.1	3.6 ± 1.0
1087	apurinic site	19.3 ± 3.9	12.3 ± 2.7
	etoposide	5.0 ± 0.6	5.4 ± 0.7
1148	apurinic site	10.6 ± 0.1	18.8 ± 1.6
	etoposide	10.0 ± 0.8	15.8 ± 0.7
1155	apurinic site	3.2 ± 0.9	6.4 ± 0.3
	etoposide	5.9 ± 0.9	5.2 ± 0.4
1165	apurinic site	6.1 ± 0.7	11.2 ± 0.6
	etoposide	4.8 ± 0.3	6.2 ± 0.2

[a] Cleavage reactions utilized wild type double-stranded oligonucleotide with 100 μM etoposide or oligonucleotide that contained an apurinic site located at the underlined positions in Figure 4. Alternatively, reactions utilized a substrate that contained an apyrimidinic site or uracil located at the +3 position of the top strand of site 1067. Levels of cleavage were determined relative to the wild type substrate in the absence of drug.

When placed within the cleavage site at position 1067, an apyrimidinic site or a deaminated cytosine residue also enhanced DNA scission mediated by human topoisomerase IIα (in the presence or absence of ATP) (Table 2). Thus, it appears that a variety of DNA lesions are capable of inducing DNA cleavage proximal to 11q23 translocation breakpoints.

In summary, the effects of DNA lesions on the cleavage activity of human topoisomerase IIα indicate that the recognition of spontaneous DNA damage by type II topoisomerases has been evolutionarily conserved from (at least) invertebrates to mammalian species (*24*−*26*, *46*). Further-

more, apurinic sites, apyrimidinic sites, and deaminated cytosine residues are all capable of stimulating topoisomerase II-mediated cleavage proximal to leukemic breakpoints at chromosome band 11q23. It has been hypothesized that chemotherapy-related translocations in this region are triggered by high levels of etoposide-induced DNA cleavage mediated by topoisomerase II in the *MLL* oncogene (*18*−*21*). If this hypothesis is correct, spontaneous DNA lesions must be considered as candidates for triggering this cancer-associated chomosomal aberration in drug-naive cells.

ACKNOWLEDGMENT

We are grateful to Dr. J. L. Nitiss and A. Rose for supplying yeast cells harboring human topoisomerase IIα, to S. E. Hannah for assistance in purification of the enzyme, and to S. D. Cline and M. Sabourin for critical reading of the manuscript.

REFERENCES

1. Corbett, A. H., and Osheroff, N. (1993) *Chem. Res. Toxicol. 6*, 585−597.
2. Chen, A. Y., and Liu, L. F. (1994) *Annu. Rev. Pharmacol. Toxicol. 34*, 191−218.
3. Froelich-Ammon, S. J., and Osheroff, N. (1995) *J. Biol. Chem. 270*, 21429−21432.
4. Pommier, Y., Fesen, M. R., and Goldwasser, F. (1996) In *Cancer Chemotherapy and Biotherapy: Principles and Practice* (Chabner, B. A., and Longo, D. L., Eds.) pp 435−461, Lippincott-Raven Publishers, Philadelphia.
5. Osheroff, N. (1989) *Pharmacol. Ther. 41*, 223−241.
6. Wang, J. C. (1996) *Annu. Rev. Biochem. 65*, 635−692.
7. Watt, P. M., and Hickson, I. D. (1994) *Biochem. J. 303*, 681−695.
8. Hickman, J. A. (1992) *Cancer Metastasis Rev. 11*, 121−139.
9. Anderson, R. D., and Berger, N. A. (1994) *Mutat. Res. 309*, 109−142.
10. Ferguson, L. R., and Baguley, B. C. (1994) *Environ. Mol. Mutagen. 24*, 245−261.
11. Nitiss, J. L., and Beck, W. T. (1996) *Eur. J. Cancer 32a*, 958−966.
12. Kreuzer, K. N., and Cozzarelli, N. R. (1979) *J. Bacteriol. 140*, 424−435.
13. Smith, M. A., Rubinstein, L., and Ungerleider, R. S. (1994) *Med. Pediatr. Oncol. 23*, 86−98.
14. Smith, M. A., Rubinstein, L., Cazenave, L., Ungerleider, R. S., Maurer, H. M., Heyn, R., Khan, F. M., and Gehan, E. (1993) *J. Natl. Cancer Inst. 85*, 554−558.
15. Pui, C.-H., Ribeiro, R. C., Hancock, M. L., Rivera, G. K., Evans, W. E., Raimondi, S. C., Head, D. R., Behm, F. G., Mahmoud, M. H., Sandlund, J. T., and Crist, W. M. (1991) *N. Engl. J. Med. 325*, 1682−1687.
16. Rowley, J. D. (1995) *Pathol. Biol. 43*, 197−201.
17. Ratain, M. J., and Rowley, J. D. (1992) *Ann. Oncol. 3*, 107−111.
18. Negrini, M., Felix, C. A., Martin, C., Lange, B. J., Nakamura, T., Canaani, E., and Croce, C. M. (1993) *Cancer Res. 53*, 4489−4492.
19. Domer, P. H., Head, D. R., Renganathan, N., Raimondi, S. C., Yang, E., and Atlas, M. (1995) *Leukemia 9*, 1305−1312.
20. Broeker, P. L., Super, H. G., Thirman, M. J., Pomykala, H., Yonebayashi, Y., Tanabe, S., Zeleznik-Le, N., and Rowley, J. D. (1996) *Blood 87*, 1912−1922.
21. Felix, C. A., Lange, B. J., Hosler, M. R., Fertala, J., and Bjornsti, M. (1995) *Cancer Res. 55*, 4287−4292.
22. Pui, C. H. (1992) *Leuk. Lymphoma 7*, 173−179.
23. Ross, J. A., Potter, J. D., and Robison, L. L. (1994) *J. Natl. Cancer Inst. 86*, 1678−1680.
24. Kingma, P. S., Corbett, A. H., Burcham, P. C., Marnett, L. J., and Osheroff, N. (1995) *J. Biol. Chem. 270*, 21441−21444.
25. Kingma, P. S., and Osheroff, N. (1997) *J. Biol. Chem. 272*, 1148−1155.
26. Kingma, P. S., and Osheroff, N. (1997) *J. Biol. Chem. 272*, 7488−7493.
27. Lindahl, T. (1993) *Nature 362*, 709−715.
28. Loeb, L. A., and Preston, B. D. (1986) *Annu. Rev. Genet. 20*, 201−230.
29. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, 2nd ed.; American Society for Microbiology Press, Washington, DC.
30. Wasserman, R. A., Austin, C. A., Fisher, L. M., and Wang, J. C. (1993) *Cancer Res. 53*, 3591−3596.
31. Sutcliffe, J. G. (1978) *Cold Spring Harbor Symp. Quant. Biol. 43*, 77−90.
32. Sander, M., and Hsieh, T. (1983) *J. Biol. Chem. 258*, 8421−8428.
33. Corbett, A. H., Zechiedrich, E. L., and Osheroff, N. (1992) *J. Biol. Chem. 267*, 683−686.
34. Goto, T., and Wang, J. C. (1984) *Cell 36*, 1073−1080.
35. Wyckoff, E., Natalie, D., Nolan, J. M., Lee, M., and Hsieh, T. (1989) *J. Mol. Biol. 205*, 1−13.
36. Drake, F. H., Hofmann, G. A., Bartus, H. F., Mattern, M. R., Crooke, S. T., and Mirabelli, C. K. (1989) *Biochemistry 28*, 8154−8160.
37. Jensen, S., Redwood, C. S., Jenkins, J. R., Andersen, A. H., and Hickson, I. D. (1996) *Mol. Gen. Genet. 252*, 79−86.
38. Withoff, S., De Jong, S., De Vries, E. G. E., and Mulder, N. H. (1996) *Anticancer Res. 16*, 1867−1880.
39. Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson, R. K., and Drake, F. H. (1991) *Cell Growth Differ. 2*, 209−214.
40. Holden, J. A., Rolfson, D. H., and Wittwer, C. T. (1992) *Oncol. Res. 4*, 157−166.
41. Capranico, G., Tinelli, S., Austin, C. A., Fisher, M. L., and Zunino, F. (1992) *Biochim. Biophys. Acta 1132*, 43−48.
42. Woessner, R. D., Chung, T. D., Hofmann, G. A., Mattern, M. R., Mirabelli, C. K., Drake, F. H., and Johnson, R. K. (1990) *Cancer Res. 50*, 2901−2908.
43. Qiu, J., Catapano, C. V., and Fernandes, D. J. (1996) *Biochemistry 35*, 16354−16360.
44. Andersen, A. H., Sørensen, B. S., Christiansen, K., Svejstrup, J. Q., Lund, K., and Westergaard, O. (1991) *J. Biol. Chem. 266*, 9203−9210.
45. Andersen, A. H., Svejstrup, J. Q., and Westergaard, O. (1994) *Adv. Pharmacol. 29A*, 83−101.
46. Bigioni, M., Zunino, F., Tinelli, S., Austin, C. A., Willmore, E., and Capranico, G. (1996) *Biochemistry 35*, 153−159.
47. Ripley, L. S., Dubins, J. S., deBoer, J. G., DeMarini, D. M., Bogerd, A. M., and Kreuzer, K. N. (1988) *J. Mol. Biol. 200*, 665−680.
48. Gu, Y., Cimino, G., Alder, H., Nakamura, T., Prasad, R., Canaani, O., Moir, D. T., Jones, C., Nowell, P. C., Croce, C. M., et al. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 10464−10468.
49. Gu, Y., Alder, H., Nakamura, T., Schichman, S. A., Prasad, R., Canaani, O., Saito, H., Croce, C. M., and Canaani, E. (1994) *Cancer Res. 54*, 2326−2330.

BI970507V