Position-Specific Effects of Base Mismatch on Mammalian Topoisomerase II DNA Cleaving Activity†

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ABSTRACT: To further define the nucleic acid determinants of DNA site recognition by mammalian topoisomerase II, base mismatch effects on the enzyme DNA cleavage activity were determined in a 36-bp synthetic oligonucleotide corresponding to SV40 DNA. DNA cleavage sites induced by topoisomerase II without or with the antitumor drugs teniposide, idarubicin, or amsacrine were mapped using sequencing gels. Selected mismatches were studied, and always one of the two strands had the wild-type sequence. The effects of base mismatches were independent from the studied drugs. Mismatches introduced at the −4, −3, −2, or −1 positions, relative to the enzyme cleavage site, often abolished, or much reduced, DNA cleavage, whereas those at +1 and +2 positions often increased DNA breakage or were without influence. Mismatches at more distant positions, e.g., −7, −8, etc., had no effect. Those at positions −5 and −6 sometimes increased cleavage levels. These effects were always observed at sites already cleaved in the wild-type oligomer; new sites of cleavage were not induced by the studied mismatches. These results were obtained both for the native murine topoisomerase II and for the two recombinant human isozymes. No difference between topoisomerases II α(p170) and β(p180) was seen in their response to mismatches. The results demonstrate that topoisomerase II recognition of the DNA site of cleavage requires fully paired nucleotides at the 3′ terminus. Nevertheless, similarly to other DNA strand transferase enzymes, both topoisomerase II isoforms may have a sequence-specific nicking activity at the 5′ side of unpaired bases.

Eukaryotic type II DNA topoisomerases are essential nuclear enzymes that catalyze the interconversion of topological forms of DNA molecules (Wang, 1985; Osheroff, 1989). The enzyme performs these transformations by coupling the hydrolysis of two ATP molecules to the passage of a DNA duplex through a transient double-stranded cut in another duplex. Biochemical and genetic experiments indicate that type II DNA topoisomerases are essential for the segregation of newly replicated pairs of intertwined chromosomes (Holm et al., 1985, 1989; Downes et al., 1991; Adachi et al., 1991) and are involved in the modulation of the supercoiling state of intracellular DNA (Liu & Wang, 1987). Moreover, topoisomerase II is known to be the target of clinically effective antitumor drugs, including anthracyclines, teniposide, and amsacrine (Zwelling, 1985; Liu, 1989; Pommier & Kohn, 1989; Capranico & Zunino, 1992). These compounds stimulate topoisomerase II-mediated DNA cleavage in purified systems and in living cancer cells by interfering with the breakage−reunion reaction of DNA topoisomerase II and stabilizing a DNA−enzyme complex, termed "cleavable complex", in which the DNA strands are broken and their 5′ termini covalently linked to the protein (Wang, 1985; Osheroff, 1989; Liu, 1989). Topoisomerase II has been implicated in nonhomologous recombination events in the cell (Gale & Osheroff, 1992; Dillehay et al., 1989; Bae et al., 1988; Osheroff et al., 1991). The hypothesis is supported by the findings that *in vivo* sites of recombination often correlate with *in vitro* sites of topoisomerase II DNA cleavage (Han et al., 1993; Sperry et al., 1989), and antitumor topoisomerase II inhibitors promote recombination, mutagenesis, and chromosomal translocations in living cells (Pommier et al., 1985; Chatterjee et al., 1990; Nitiss & Wang, 1988).

Topoisomerase II cleaves the DNA duplex in a site-selective manner; however, the consensus sequence approach has been only partially successful in the prediction of new cutting sites (Sander & Hsieh, 1985; Spitzner & Muller, 1988; Capranico et al. 1990). Topoisomerase II can use short oligonucleotides as DNA substrates, and, since cleavage intensity patterns were the same in oligomers as in larger DNA fragments, oligomers of 30−40 base pairs may contain the same information of larger DNA fragments for enzyme recognition of cleavage sites (Bigioni et al., 1994; Freudenreich & Kreuzer, 1993). Drug stimulation of DNA breakage shows a variable degree of sequence specificity that results from the sum of the site selectivity of the enzyme itself and the sequence specificity of the drug (Capranico et al., 1990; Capranico & Zunino, 1992; Freudenreich & Kreuzer, 1993). Some knowledge of enzyme−drug−DNA interactions was

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recently gained from investigations of base preferences at the site of drug-stimulated DNA cleavage. Topoisomerase II inhibitors may form ternary complexes by binding to nucleotides adjacent to the strand cut and to amino acid residues of the enzyme active site (Capranico et al., 1994a,b; Freudenreich & Kreuzer, 1994).

To gain further information on enzyme−DNA interactions, in the present work we ask the question as to whether local base mispairing might alter DNA cleavage specificity and/or levels produced by mammalian DNA topoisomerase II in the presence or absence of antitumor drugs. We introduced single-base mismatches in a 36-bp oligonucleotide that corresponds to the SV40 sequence and was previously used in a mutational analysis of DNA cleavage (Bigioni et al., 1994). The results demonstrate that topoisomerase II DNA cleavage activity may be enhanced at the 5′ side of a base mismatch. Pairing of DNA strands is instead required at the 3′ termini of the cut for cleaving activity.

EXPERIMENTAL PROCEDURES

Enzymes, Drugs, and Other Materials. Native DNA topoisomerase II was purified from murine leukemic P388 cells as described already (De Isabella et al., 1990). The purified protein was stored at −80 °C in 20 mM KH_2PO_4, pH 7.0, 50% glycerol, 0.5 mM PMSF, 0.1 mM EDTA, and 1 mM β-mercaptoethanol. Recombinant human topoisomerases II α and β were purified from yeast strains carrying an expression vector containing either the human topoisomerase II α or β genes under the control of yeast GAL1 promoter as described previously (Worland & Wang, 1989; Wasserman et al., 1993; Austin et al., 1995). Briefly, a protease-deficient *Saccharomyces cerevisiae* strain containing a plasmid-borne human topoisomerase gene was grown in glucose-free medium to an optical density of 0.4−1, and then induction of topoisomerase expression was achieved by the addition of galactose (2%, final concentration) for 3−16 h. Cells were harvested, resuspended in 50 mM Tris-HCl, pH 7.7, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, 1 mM 2-mercaptoethanol, 1 mM benzamidine, 2 μg/mL leupeptin, and 2 μg/mL pepstatin, and then lysed. Purification of the human proteins was performed by polymin P fractionation, ammonium sulfate precipitation, and phosphocellulose chromatography as reported previously (Worland & Wang, 1989). Human topoisomerase II isoforms eluted from the phosphocellulose column at a KCl concentration around 0.5 M and exhibited single bands in Coomassie-stained gels (Wasserman et al., 1993; Austin et al., 1995). The purified isozymes were stored at −80 °C in 500 mM Tris-HCl, pH 7.7, 200 mM KCl, 10 mM EDTA, and 10 mM EGTA, 10% glycerol. Topoisomerase II strand-passing activity was determined with the P4 unknotting assay for the murine enzyme, as described already (De Isabella et al., 1990). The catalytic activity of recombinant human isozymes was measured with a DNA relaxation assay in 40 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 80 mM KCl, 0.5 mM DTT, and 15 μg/mL bovine serum albumin.

Idarubicin (4-demethoxydaunorubicin), VM-26, and *m*AMSA were obtained from Pharmacia-Farmitalia (Milan), Bristol Italiana (Latina), and the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD), respectively. T4 polynucleotide kinase and polyacrylamide were purchased from GIBCO-BRL, Life Technologies (Basel, Switzerland). [γ-^{32}P]ATP was purchased from Amersham, Milan, Italy.

5′-End-Labeling of DNA Oligonucleotides. DNA oligomers were synthesized with a 380B DNA synthesizer (Applied Biosystems, Milan, Italy), purified with denaturing polyacrylamide gels, and recovered by soaking gel slices in 0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA, pH 8, and 0.1% SDS. Base sequences were confirmed by purine degradation with the Maxam−Gilbert method (Sambrook et al., 1989). DNA oligomers (100 ng) were 5′-end ^{32}P-labeled with T4 kinase (10 units) in 25 μL of 70 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM dithiothreitol, and 50−150 μCi of [γ-^{32}P]ATP for 1 h at 37 °C. Unlabeled complementary oligomers (about 150 ng) were then added to labeled strands in 10 mM Tris-HCl, pH 7.8, 100 mM NaCl, and 1 mM EDTA, heated to 65 °C for 5 min, and then slowly chilled to room temperature. Double-stranded oligomers were then ethanol-precipitated, resuspended in 10 mM Tris-HCl, pH 7.6, and 1 mM EDTA, and stored at −20 °C.

DNA Cleavage Assay. DNA cleavage reactions were performed in 20 μL of 10 mM Tris-HCl, pH 6.0, 10 mM $MgCl_2$, 50 mM KCl, and 1 mM ATP without or with drugs (at 10 μM for VM-26 and *m*AMSA, and 1 μM for idarubicin) at 37 °C for 20 min. In each reaction tube, around 30 units of native murine topoisomerase II, or 2 units of human recombinant isozymes, were mixed with 2×10^4 cpm of 5′-end-labeled SV40 DNA fragments. Reactions were stopped by adding SDS and proteinase K (1% and 0.1 mg/mL, respectively) and incubated at 42 °C for 30 min. DNA was then ethanol-precipitated, resuspended in 2.5 μL of 80% formamide, 10 mM NaOH, 1 mM EDTA, and 0.1% dyes, heated at 95 °C, chilled on ice, and then loaded into a sequencing 17% polyacrylamide gel. Gels were run at 70 W for 2 h, dried, and autoradiographed with Amersham Hyperfilm MP. Topoisomerase II cleavage sites were localized by comparison to purine marker lanes (Bigioni et al., 1994). Enzyme-mediated cleavage generates hydroxyl termini instead of the 3′-P ends generated by Maxam−Gilbert reactions; thus, a shift toward higher molecular weights of 1.5 nucleotides is expected for topoisomerase II cleavage products in short oligomers (Tapper & Clayton, 1981). DNA cleavage levels were determined by Phosphorimager analysis of gels (Molecular Dynamics Phosphorimager Model 425). The percentage of cleaved DNA was determined for each band relative to the total DNA loaded into the lane.

RESULTS

The nucleotide sequence of the studied wild-type oligomer corresponds to the SV40 DNA from genomic positions 4235 to 4270 (Figure 1). Cleavage sites without drugs or with idarubicin have previously been characterized (Bigioni et al., 1994). Murine topoisomerase II produced the strongest cleavage at the 4248 site, while idarubicin stimulated strong cleavage at 4258, 4251, and 4243 sites of the top strand [Figures 1−3, wild-type oligomer; see also Bigioni et al. (1994)]. As expected for chemically-unrelated inhibitors, different patterns of cleavage sites were stimulated by VM-26 and *m*AMSA in the same oligomer (Figure 2). Under our conditions, DNA cleavage was double-stranded at each site and was consistent with a stagger of four bases [not

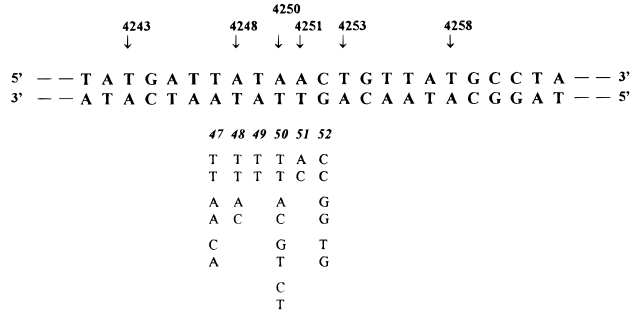

FIGURE 1: DNA sequence of the studied 36-bp wild-type oligonucleotide. Arrows and numbers indicate the bases (position +1) that become covalently linked to topoisomerase II at the cleavage sites selected for quantitative analyses of mispairing effects (see Tables 1−3). Numbers refer to the corresponding sequence in SV40 DNA (GenBank Accession No. V01380). Two-digit numbers in italics below the base sequence indicate the positions where base mismatches (shown below) were introduced. For all the studied mismatches, one of the two strands maintained the wild-type sequence.

Table 1: Levels of VM-26 Stimulated DNA Cleavage Produced by Mammalian DNA Topoisomerases II in the Wild-Type Oligomer

	cleaved DNA (%) at site					
topoisomerase II	4243	4248	4250	4251	4253	4258
murine native	0.8	22	2.6	3.8	6.1	1.3
human α	0.4	13.5	0.7	1.9	2.9	0.2
human β	0.3	7.1	0.15	1.2	0.9	0.7

shown and Bigioni et al. (1994)]. We used the convention to indicate with negative and positive numbers the bases at the 3′ and 5′ termini of each strand break, respectively. Numbers −1, +1, etc., and −1′, +1′, etc., indicate the top and bottom strand sequences, respectively. Single-base mismatches were introduced at the center of the oligomer from 4247 to 4252 (Figure 1). In each case, the mismatch was chosen in such a way to maintain the wild-type sequence of one strand at a time; thus, the comparison with the full-paired oligomer was straightforward.

Mismatches at 4247. T/T, A/A, or C/A mismatches at 4247 had marked effects on the cleavage at 4244, 4246, 4248, 4250, and 4251 sites (Figure 2). VM-26-stimulated DNA cleavage at the 4244 site was slightly increased as compared with the wild-type oligomer, and cleavage without drugs was more intense at the 4244 and 4246 sites (Figure 2). The 4247 base mismatch corresponds to +1′ and +1 positions for these sites, respectively. Opposite effects were observed at other sites: cleavage was much reduced at 4248, 4250, and 4251. For these sites, the mismatch corresponds to −1, −3, and −4 positions, respectively.

We also measured the effects of base mispairing on the extent of DNA cleavage at selected sites (as shown in Figure 1) with Phosphoimager analyses of gels. Percentages of cleaved DNA in the wild-type oligomer were determined in the presence of VM-26 (Table 1). Then, cleavage levels determined in the mismatch-bearing oligomers were compared with and expressed relative to those of the wild-type oligomer (Table 2). Cleavage was undetectable at the 4250 site, and reduced to 9 and 26% of the cleavage in the wild-type oligomer at the 4248 and 4251 sites, respectively (Table 2). We found similar results when performing quantitative analyses in the cases of the other drugs (not shown).

Moreover, the VM-26 stimulation at site 4249 (−2) was abolished, and cleavage levels were higher at the 4252 site for all the studied drugs: in this case, the mismatch was at position −5 (Figure 2). Other sites, such as 4253 and 4258, were only slightly affected by the more distant 4247 mismatches (Table 2). Interestingly, some differences among the studied mismatches were noted: DNA cleavage at 4251 was much less hindered by C/A than by the other mismatches (Figure 2).

Mismatches at 4248. T/T and A/C mismatches at 4248 had marked effects (Figure 2 and Table 2): cleavage was abolished at the 4243, 4250, 4251, and 4252 sites, whereas it was increased at the 4245, 4248, and 4253 sites. In the last case, the effect was more evident without drugs. The 4248 mismatch corresponds to positions −1′, −2, −3, and −4, for the first four sites respectively, and to positions +1′, +1 and −5 for the second set of three sites.

Mismatches at 4249 and 4251. In these cases, unequivocal results were obtained with the most sequence-selective inhibitor studied, the anthracycline derivative idarubicin, and are also particularly convincing comparing directly the cleavage patterns of the wild-type and the two mismatched oligomers (Figure 3 and Table 2). T/T at 4249 and A/C at 4251 were investigated in these experiments. The first mismatch abolished cleavage at 4243, 4244, 4250, 4251, and 4252, whereas it enhanced cleavage at the 4249 site. In the first case, the mismatch was at positions −3′, −2′, −1, −2, and −3, respectively; it was at position +1 relative to the 4249 cleavage site. Completely different effects were observed with the A/C mismatch at 4251 (Figure 3). The intensity pattern with idarubicin was altered only for a higher cleavage level at 4252; however, cleavage without drugs was increased at 4251 and 4252 sites (mismatch at positions +1 and −1, respectively), and it was abolished at sites 4253 and 4255 (mismatch at positions −2 and −4, respectively). Similar data were obtained with VM-26 and *m*AMSA (Table 2 and not shown).

Table 2: Base Mismatch Effects on VM-26 Stimulated DNA Cleavage Levels by Murine Native Topoisomerase II

	relative DNA cleavage (R)[a] at site											
	4243		4248		4250		4251		4253		4258	
mismatch at	post[b]	R	pos	R	pos	R	pos	R	pos	R	pos	R
4247	−1′	un[c]	−1	0.09	−3	un	−4	0.26	−6	0.91	−11	0.80
4248	−2′	un	+1	1.11	−2	un	−3	un	−5	1.80	−10	0.66
4249	−3′	un	+2	4.10	−1	un	−2	un	−4	nd[d]	−9	1.50
4250	−4′	0.90	+2′	0.80	+1	2.53	−1	0.36	−3	0.06	−8	0.41
4251	−5′	1.89	+1′	3.90	+2	1.00	+1	3.40	−2	nd	−7	1.48

[a] DNA cleavage levels were determined with Phosphorimager analyses. The percentage of cleaved DNA was determined for each band relative to the total DNA loaded into the lane. R values between cleaved DNA in the mismatch-bearing oligomers and that in the wild-type oligomer; they are means among the studied base mismatches. [b] pos indicates the mismatch position relative to the indicated cleavage site. [c] un, undetectable. [d] nd, not determined.

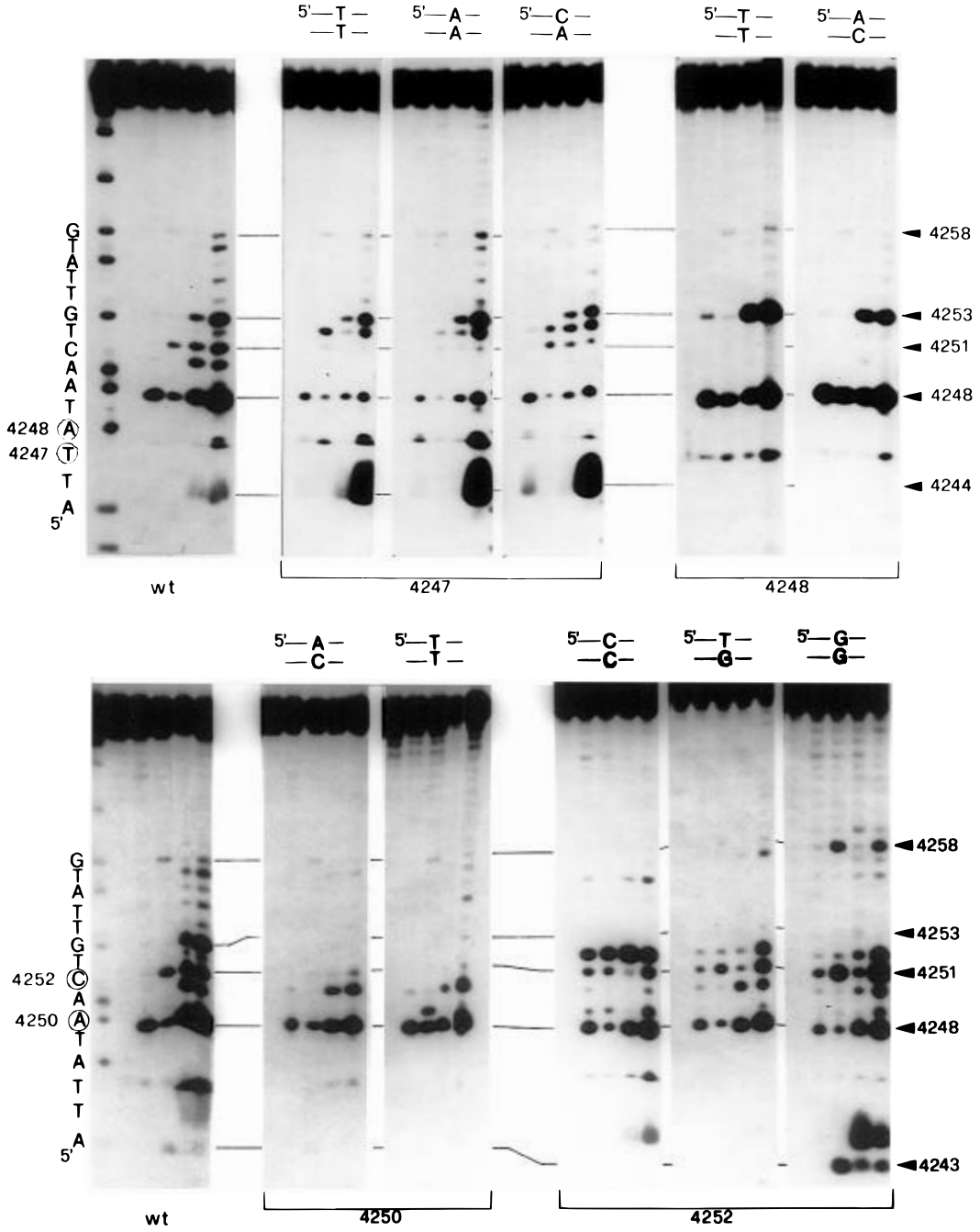

FIGURE 2: Topoisomerase II DNA cleavage in the wild-type and mismatched oligomers. Strands were ^{32}P-5′-end-labeled concurrently to obtain similar specific activities and, then, annealed to unlabeled strands. Oligomers were incubated with topoisomerase II with or without drugs for 20 min at 37 °C in 10 mM Tris-HCl, pH 6.0, 10 mM $MgCl_2$, 50 mM KCl, and 1 mM ATP. DNA cleavage was then analyzed with 17% polyacrylamide denaturing gels. Numbers at the bottom of the gels indicate the position where a mismatch was present. "wt" indicates the wild-type oligomer. Each set of five lanes corresponds to one oligomer bearing the base mismatch shown at the top. In each set, the lanes are DNA control, no drug, 1 μM idarubicin, 10 μM *m*AMSA, and 10 μM VM26, respectively, from left to right. The purine molecular marker lane is shown only for the wild-type oligomer. Mispaired nucleotides are indicated by numbers and circles on the left. Arrows and numbers on the right indicate selected cleavage sites.

Mismatches at 4250. A major influence on DNA cleavage was also in this case at sites immediately 3′ to base mismatches (Figure 2 and Table 2). Cleavage at sites 4243, 4244, and 4252 was abolished in the mismatch-bearing oligomer as compared to the wild-type oligomer. For these sites, the mismatch was at positions −4′, −3′, and −2, respectively. At the same time, cleavage without drugs was about 2.5-fold increased at the 4250 site (Table 2), relative to which the mismatch was at position +1. The T/T mismatch, however, allowed a higher stimulation by idarubicin of DNA cleavage at the 4249 site than the A/C mismatch (Figure 2). This and the above observations may indicate that base mismatches differ somewhat for their effects on topoisomerase II interactions with the double helix in the studied oligomer.

Mismatches at 4252. A much stronger cleavage was observed at the 4252 and 4249 sites when a mismatch was at positions +1 and +1′, respectively, although the C/C mismatch was more effective than T/G and G/G mismatches (Figure 2). At the same time, cleavage was abolished at the 4253, 4254, and 4255 sites relative to which mismatches were at the −1, −2, and −3 positions, respectively, consistent

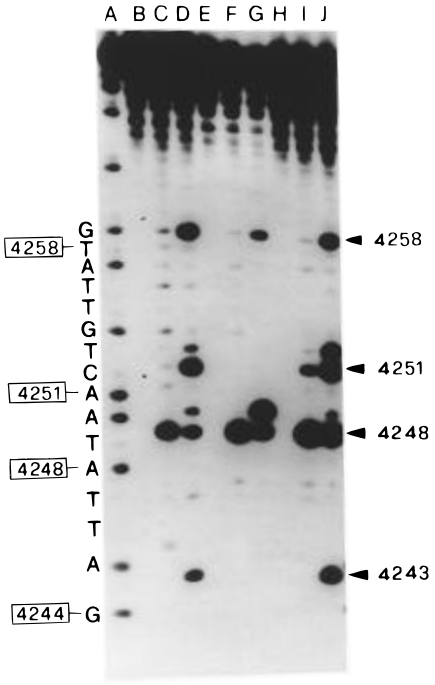

FIGURE 3: Topoisomerase II DNA cleavage in oligomers with a mismatch at 2449 or 4251 without or with idarubicin. Oligomers are (A−D) wild-type, (E−G) with a T/T mismatch at 4249, (H−J) with an A/C mismatch at 4251. Lanes: A, purine molecular markers; B, E, and H, control DNA; C, F, and I, no drug; D, G, and J, 1μM idarubicin. Arrows and numbers on the right indicate selected cleavage sites. On the left, the wild-type base sequence is shown.

with the above findings. Nevertheless, some differences were noted with the mismatch at 4252: the cleavage patterns with and without drugs appeared to be markedly dependent on the type of the mismatch present at 4252; in addition, *m*AMSA did not stimulate cleavage at the 4251 site any more with all the studied mismatches, whereas the other drugs did (Figure 2). These observations suggest that competition among close sites may highly influence the extent of DNA cleavage in these cases. Since the overall cleavage seemed to be stronger with the G/G mismatch than with the other ones (Figure 2), enzyme binding to DNA might also be altered depending on the specific nature of the base mismatch at 4252.

Human Topoisomerase II Isoforms. The studied murine enzyme was purified from leukemic P388 cells and was completely free of DNA topoisomerase I activity (De Isabella et al., 1990; data not shown). Since the native murine enzyme was likely constituted by a mixture of both α and β isoforms of topoisomerase II, we then asked whether base mismatches equally affect DNA cleavage produced by the two isozymes. We thus investigated the recombinant human topoisomerases II α and β, which were expressed in yeast and purified as described previously (Worland & Wang, 1989; Wasserman et al., 1993; Austin et al., 1995). Under our conditions, the two human isozymes similarly stimulated a high cleavage level at the 4248 site of the wild-type oligomer; however, some differences in cleavage intensity patterns could be observed (Figure 4 and Table 1). For instance: cleavages at 4255, 4256, and 4257 sites were stronger with the β than the α isozymes (Figure 4). A thorough analysis of cleavage patterns of the two human isozymes will be reported elsewhere. However, effects of base mismatches on enzyme-mediated DNA cleavage were the same for both isoforms. DNA cleavage was reduced or suppressed at those sites relative to which the mismatch was either at positions −4, −3, −2, or −1, whereas cleavage was somewhat enhanced at sites relative to which the mismatch was at positions +1 or +2 (Figure 4 and Table 3). These results demonstrated that base mismatches had the same position-specific influence on DNA cleavage produced by the two human isozymes.

DISCUSSION

The present results demonstrate that a single base mismatch can affect topoisomerase II DNA cleavage with a strong dependence on its position relative to the cleavage site. It is noteworthy that although competition among sites may be an important factor determining DNA cleavage levels, mismatch effects were consistently observed at the several cleavage sites present in the oligomer. Similar effects were observed either with or without drugs, suggesting therefore that base mispairing may specifically influence enzyme−DNA contacts. A single mismatch abolished or greatly reduced cleavage when at −1, −2, −3, or −4 positions; it may instead increase cleavage when at +1, +2, or −5 positions; it has a minimal effect when at more distant positions (Figure 5).

One exception to this general conclusion was the behavior of site 4252, since cleavage was not reduced by a mismatch at its −1 position, 4251 (Figure 3). The exception was limited to the −1 position, since cleavage at 4252 was indeed reduced when a mismatch was at −2, −3, or −4 positions (Figure 2). Moreover, effects of base mispairing were more dependent on the type of the mismatch studied when at 4252

Table 3: Base Mismatch Effects on VM-26-Stimulated DNA Cleavage Levels by Human Recombinant Topoisomerase II Isoforms

	relative DNA cleavage (R)[a] at site											
	4243		4248		4250		4251		4253		4258	
mismatch at	pos[b]	R	pos	R	pos	R	pos	R	pos	R	pos	R
					Topo II α							
4247	−1′	nd[c]	−1	0.07	−3	0.13	−4	0.16	−6	0.72	−11	0.95
4248	−2′	0.23	+1	2.00	−2	0.24	−3	0.05	−5	2.86	−10	0.85
					Topo II β							
4247	−1′	0.70	−1	0.10	−3	0.73	−4	0.16	−6	1.03	−11	nd
4248	−2′	0.03	+1	3.43	−2	0.65	−3	0.08	−5	1.78	−10	nd

[a] DNA cleavage levels were determined with Phosphorimager analyses. The percentage of cleaved DNA was determined for each band relative to the total DNA loaded into the lane. R values are the ratios between cleaved DNA in the mismatch-bearing oligomers and that in the wild-type oligomer; they are means among the studied base mismatches. [b] pos indicates the mismatch position relative to the indicated cleavage site. [c] nd, not determined.

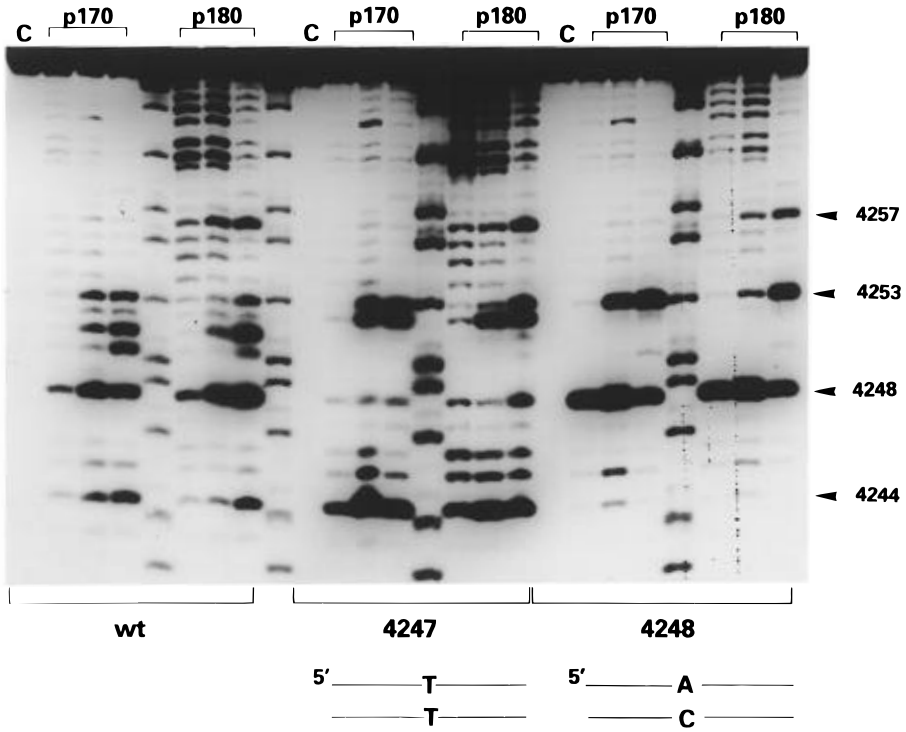

FIGURE 4: Effects of base mismatches on DNA cleavage produced by recombinant human topoisomerase II isoforms, p170 and p180. Numbers at the bottom indicate the nucleotide position of the studied mismatches (shown below). "wt" indicates the wild-type oligomer. At the top of the gel, the samples incubated with the p170 or the p180 isozymes are indicated; C is control DNA. In each set of three lanes, they are from left to right: DNA treated with the isozyme alone, plus 10 μM VM-26, and plus 10 μM *m*AMSA, respectively.

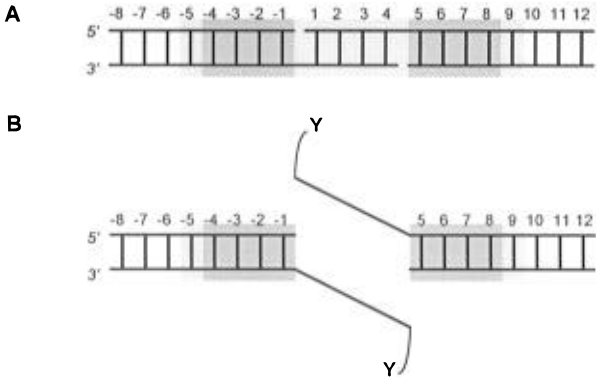

FIGURE 5: Nucleotide positions at the site of topoisomerase II DNA cleavage, which are important for base-mispairing effects. (A) Positions -4 to -1 and 5−8 (dark grey areas) are required to be fully paired for enzyme cleavage activity. At positions 1−4 (light grey areas), a base mismatch may increase cleavage levels. Note that these positions become single strands during the catalytic reaction (B). Mismatches at position +5 may sometimes enhance cleavage. Other more distant positions are without influence.

than at other positions. Interestingly, the 4252 site had a peculiar behavior also in a previous study of base mutation analysis of idarubicin-stimulated topoisomerase II DNA cleavage: the removal of an adenine from its -1 position did not reduce the drug-stimulative effect whereas the (-1)-adenine was critical for drug action at other sites (Bigioni et al., 1994). The cleavage site 4252 corresponds to the 4255 site in the opposite strand, and we suggested that for this double-strand cut the strand recognized by the enzyme is the lower one; thus, mutations in the upper strand have only a marginal influence on topoisomerase II cleavage activity (Bigioni et al., 1994). In agreement with this interpretation, a base mismatch at the -1 position may have a greater influence when changing the DNA−enzyme interaction at the lower strand.

The sequence information that determines the DNA site of cleavage by topoisomerase II is likely contained in few (10−15) base pairs at the cleavage site (Sander & Hsieh, 1985; Spitzner & Muller, 1988; Capranico et al., 1990; Freudenreich & Kreuzer, 1993). Nevertheless, several structural features of the DNA duplex have been shown to influence the enzyme cleavage activity (Liu, 1989; Osheroff et al., 1991; Capranico & Zunino, 1992). Recently, *Drosophila* topoisomerase II has been shown to bind and cleave a DNA hairpin in a single-stranded oligonucleotide, indicating that topoisomerase II may recognize DNA secondary structures (Froelich-Ammon et al., 1994). Interestingly, the enzyme cleavage activity showed strict requirements for the hairpin structure. Our present results now show a marked sensitivity of topoisomerase II to base mismatches close to the site of cleavage (Figure 5), thus indicating that the enzyme may also recognize this particular DNA structural alteration. Similarly, mammalian DNA topoisomerase I has been shown to have a nicking activity at the 5′ side of the mismatch (Yeh et al., 1994). An important difference with the present findings is that topoisomerase I mismatch nicking activity was insensitive to camptothecin, a specific inhibitor of type I enzymes, although the drug stimulated cleavage at the same site in the fully paired DNA substrate (Yeh et al., 1994). λ Int topoisomerase was also shown to recognize and cleave its specific site containing base mismatches (Nash & Robertson, 1989). In agreement with results of the mammalian topoisomerases I and II, Int topoisomerase-induced cleavage was more pronounced at the 5′ side of base mismatches (Nash & Robertson, 1989). These findings indicate that several DNA strand-transferase enzymes are sensitive to position-specific base mismatches. Therefore, it may be suggested that a nicking activity at the 5′ side of base mismtaches might be a universal property of this family

of enzymes, probably due to some common aspects of the cleavage−rejoining reaction. In the case of topoisomerase II, during the strand passage, the +1 to +4 nucleotides must be in a single-stranded form (Figure 5), and thus the subsequent break−rejoining reaction may be conceived as consisting of two steps: first, pairing of DNA bases; and then, religation of the DNA termini. Pairing of the +1 nucleotide to the complementary base may be an important factor for the positioning of the tyrosine-linked 5′ terminus close to the 3′ terminus in order for the DNA strand to be religated. Therefore, mispairing at the +1, and +2 positions might markedly lower the religation rate due to incorrect positioning of the 5′ terminus. A similar mechanism may also explain the enhanced cleavage at the 5′ side of mispaired bases by other topoisomerases (Yeh et al., 1994; Nash & Robertson, 1989).

The observation that rejoining of the broken DNA strands is hindered by the lack of Watson−Crick complementarity at the 5′ terminus may suggest that a base mismatch at +1 or +2 positions might mimic the action of antitumor topoisomerase II inhibitors (Figure 5). A mispairing effect of drug binding to the cleavable complex was previously suggested for intercalating agents (D'Arpa & Liu, 1989). However, both intercalating and nonintercalating agents may share the same or overlapping receptor sites in the DNA−enzyme complex as rationalized by the stacking model of a ternary complex (Capranico et al., 1994a; Capranico & Zunino, 1992). In this model, drugs may act by impeding the correct positioning of the two bases to be religated (those at −1 and +1 positions) (D'Arpa & Liu, 1989; Capranico & Zunino, 1992).

Even though we did not evaluate all possible base mismatches at all the studied nucleotide positions, our results demonstrate that base pairing is instead required at positions adjacent to the 3′ termini for the cleavage reaction to occur (Figure 5). In agreement with the present data, a previous study showed that *Drosophila* topoisomerase II may cleave a "half-site" DNA substrate constituted by a four-base single strand at the 5′ terminus and a double-stranded DNA region at the 3′ terminus (Kroeger et al., 1993). The authors showed that the pairing of DNA bases at the 3′ end was necessary for the enzyme-cleaving activity at their peculiar site. Thus, the present findings may suggest that base pairs from −4 to −1 positions make direct, strong contacts with topoisomerase II subunits, and that enzyme recognition of the site occurs only with fully paired 3′ termini (Figure 5).

Finally, these findings raise the question as to whether the molecular effects of base mismatches on topoisomerase II DNA cleavage have any biological relevance. DNA mispairing may occur due to the action of exogenous compounds or electromagnetic rays, through misincorporation during DNA replication, or during other cellular processes. If these events occur at genomic sites of topoisomerase activity, unrepaired mismatches might constitute hot spots of recombination and chromosomal aberrations due to the inhibition of the religation step of DNA topoisomerase II.

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