Benzo[*a*]pyrene diol epoxide adducts in DNA are potent suppressors of a normal topoisomerase I cleavage site and powerful inducers of other topoisomerase I cleavages

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The catalytic intermediates of DNA topoisomerase I (top1) are cleavage complexes that can relax DNA supercoiling (intramolecular reaction) or mediate recombinations (intermolecular religation). We report here that DNA adducts formed from benzo[*a*]pyrene bay-region diol epoxides can markedly affect top1 activity. Four oligonucleotide 22-mers of the same sequence were synthesized, each of which contained a stereoisomerically unique benzo[*a*]pyrene 7,8-diol 9,10-epoxide adduct at the 2-amino group of a central 2′-deoxyguanosine residue. These four adducts correspond to either cis or trans opening at C-10 of the (+)-(7*R*, 8*S*, 9*S*, 10*R*)- or (−)-(7*S*, 8*R*, 9*R*, 10*S*)-7,8-diol 9,10-epoxides. Their solution conformations in duplex DNA (intercalated and minor-groove bound for the cis and trans opened adducts respectively) can be deduced from previous NMR studies. All four adducts completely suppress top1 cleavage activity at the alkylation site and induce the formation of new top1cleavage complexes on both strands of the DNA 3–6 bases away from the alkylation site. The trans opened adduct from the highly carcinogenic (+)-diol epoxide is the most active in inducing top1 cleavage independently of camptothecin, demonstrating that minor groove alkylation can efficiently poison top1. We also found that this isomer of the diol epoxide induces the formation of top1-DNA complexes in mammalian cells, which suggests a possible relationship between induction of top1 cleavage complexes and carcinogenic activity of benzo[*a*]pyrene diol epoxides.

Polycyclic aromatic hydrocarbons were the first pure chemicals found to possess carcinogenic activity. Their metabolic activation (1) to bay-region diol epoxides (DEs) that *N*-alkylate the exocyclic amino groups of the purine bases in DNA (Fig. 1*A*) (2) is generally believed to account for most if not all of their tumorigenic activity (2, 3). These DEs arise by metabolic epoxidation on either face of a trans dihydrodiol to produce a pair of diastereomers in which the benzylic hydroxyl group is either cis (DE-1) or trans (DE-2) to the epoxide oxygen. For benzo[*a*]pyrene (BaP), the predominant environmental hydrocarbon to which humans are exposed, only DE-2 is highly carcinogenic, and all of this activity is associated with the (+)-enantiomer (Fig. 1*A*) (4).

DNA topoisomerase I (top1) is an essential enzyme in higher eukaryotes (5, 6). The enzyme can relax DNA supercoiling and relieve torsional strain during DNA processing including replication, transcription, and repair. It can also perform intermolecular religation leading to DNA recombinations (7, 8). The catalytic intermediate of top1 is the cleavage complex in which a tyrosine (Tyr723 for human top1) in the enzyme attacks a DNA phosphodiester and forms a covalent bond to phosphorus at the 3′ side of this bond while a 5′-hydroxyl is generated on the other side (see Fig. 1*B* for schematic representation) (5, 6). Such a reaction can be reproduced in short duplex oligodeoxynucleotides (9, 10) such as those used in determining the crystal structure of top1-DNA complexes (11, 12). Top1 is the cellular target of numerous anticancer drugs, including the camptothecins and some DNA intercalators and minor groove binders (for a review, see ref. 6). The anticancer activity of these drugs results from the trapping of top1 cleavage complexes such that the enzyme is inactivated as it cleaves DNA (13, 14). Therefore these agents are often referred to as "top1 poisons." The drug binding sites in the top1 cleavage complexes remain hypothetical (11, 15) and have not been elucidated because the drugs bind reversibly to the complex.

Because the solution conformation of BaP DE adducts bound to the exocyclic amino group of guanine can involve either minor groove binding or intercalation (16) (Fig. 1*B*), depending on the stereochemistry of the adduct, our aims were to synthesize oligonucleotides containing trans-opened (minor groove) and cis-opened (intercalated) DE-2 adducts and to study their effects on top1 DNA cleavage activity. The adducts were incorporated into a sequence from *Tetrahymena* R-chromatin at a previously identified and well characterized top1 cleavage site (7, 10, 17, 18). The oligonucleotides used in the present study are comparable to those used in determining the crystal structure of human top1 fragments complexed with DNA (11, 12). We report that BaP DE adducts have profound effects on top1 activity.

Materials and Methods

Enzymes and Chemicals. Human recombinant top1 was purified from Baculovirus as described (18). Terminal deoxynucleotidyl transferase and T4 polynucleotide kinase were purchased from GIBCO/BRL. [α-^{32}P]-cordycepin 5′-triphosphate and [γ-^{32}P]-ATP were purchased from New England Nuclear; polyacrylamide was from Bio-Rad. Camptothecin was provided by M. C. Wani and M. E. Wall (Research Triangle Institute, Research Triangle Park, NC). Ten millimolar aliquots of camptothecin in dimethyl sulfoxide (DMSO) were stored at −20°C, were thawed, and were diluted in water just before use.

Oligonucleotide Synthesis. The four oligonucleotides 5′-(AAA AAG ACT T**G*G AAA AAT TTT T)-3′ with a modified deoxyguanosine residue (**dG***) corresponding to the adduct formed by either cis or trans opening at C10 of the epoxide ring of (+)- or (−)-BaP DE-2 by the 2-amino group of dG (Fig. 1*A*)

Abbreviations: BaP, benzo[*a*]pyrene; DE, diol epoxide; top1, mammalian DNA topoisomerase I

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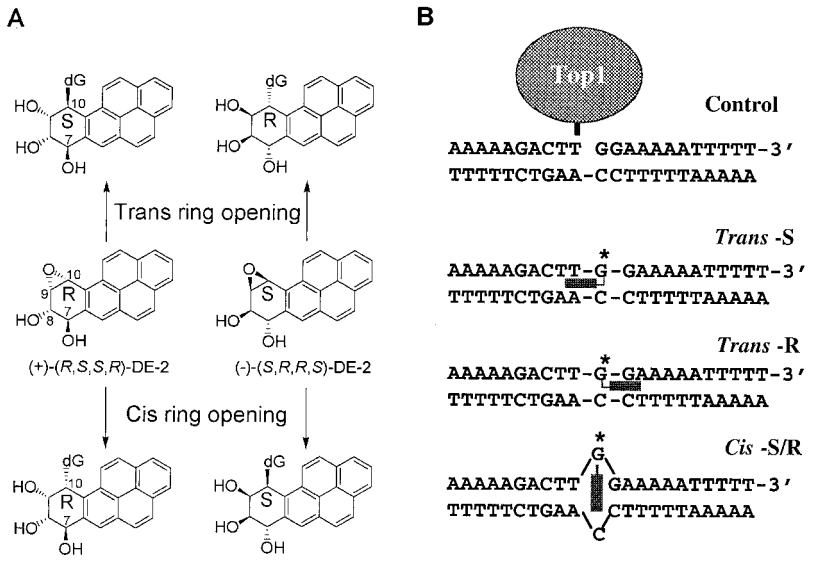

Fig. 1. (*A*) Structures of the BaP DE-2 enantiomers and their adducts at the exocyclic amino group of deoxyguanosine. (*B*) Sequence of the 22-mer oligonucleotide, showing the top1 cleavage site in the control oligonucleotide as well as schematic representations of solution conformations of the BaP DE adducts (shaded rectangles). The trans-*S* and trans-*R* adducts bind in the DNA minor groove and extend toward the 5′- and 3′-ends of the adducted strand (asterisk), respectively, whereas the cis adducts intercalate, with displacement of the adducted base and its complementary partner.

were prepared from diastereomeric mixtures of appropriately blocked phosphoramidites derived from (±)-BaP DE-2 (*trans*-N^2-BaP-dG phosphoramidite and *cis*-N^2-BaPdG phosphoramidite). Syntheses were carried out on a 2-μmol scale by using an automated DNA synthesizer to generate the sequence 3′ to the modified nucleotide followed by a manual step (19, 20) (8–10 μmol of phosphoramidite, 50 μl of 0.5 M 4, 5-dicyanoimidazole in CH_3CN, 20 h at room temperature) for coupling of the diastereomeric *cis*- or *trans*-N^2-BaPdG phosphoramidites (H.K., J.M.S., and D.M.J., unpublished work). The efficiency of the manual coupling step ranged from 10–30% (determined by spectrophotometric assay at 495 nm of the 4, 4′-dimethoxytrityl cation released on detritylation of the coupling product). Manual coupling without end capping (21) was followed by manual oxidation and completion of the remaining sequence on the synthesizer.

The 22-mer oligonucleotides containing the *cis*- or *trans*-N^2-BaPdG adducts were purified by reverse-phase HPLC on a Hamilton PRP-1 column (details are published as supplemental material on the PNAS web site, www.pnas.org), which resolved the oligonucleotides produced from the diastereomeric pairs of *cis*- and pairs of *trans*-N^2-BaPdG phosphoramidites. Retention times for the individual, adducted oligonucleotides are tabulated in the supplemental material. Isolated yields ranged from 6 to 12 A_{260} units of each oligonucleotide. The synthesized oligonucleotides gave the expected mass spectra for $C_{239}H_{287}N_{87}O_{129}P_{21}$ (mass 7,091). Assignment of the absolute configuration for the *cis*-N^2-BaPdG adducts was based on the circular dichroism (CD) spectra of the oligonucleotides (see supplemental material), which showed positive long wavelength bands for 10*R* and negative bands for 10*S* adducted oligonucleotides (22). In contrast, the oligonucleotides containing *trans*-N^2-BaPdG adducts showed only extremely weak long wavelength bands. Therefore, assignment of their absolute configuration was based on the CD spectra of the individual *trans*-N^2-BaPdG adducts (23, 24) obtained after enzymatic digestion to the nucleoside level (25).

Top1 Reactions. Single-stranded oligonucleotides were 5′- or 3′-labeled with phosphate or cordycepin, respectively, as described (8, 18, 26–28). Annealing to the complementary strand was performed in 1× annealing buffer (10 mM Tris·HCl, pH 7.8/100 mM NaCl/1 mM EDTA) by heating the reaction mixture to 95°C and overnight cooling to room temperature.

DNA substrates (≈50 fmol/reaction) were incubated with 5 ng of top1 with or without camptothecin for indicated times at 25°C in 10 μl of reaction buffer (10 mM Tris·HCl, pH 7.5/50 mM KCl/5 mM $MgCl_2$/0.1 mM EDTA/15 μg/ml BSA, final concentrations). Reactions were stopped by adding SDS (final concentration 0.5%). For reversal experiments, the SDS stop was preceded by the addition of NaCl to a final concentration of 0.5 M followed by incubation for 30 min at 25°C. Sequencing of DNA oligonucleotides was performed by using the Maxam Gilbert purine sequencing protocol (29).

Before loading of the electrophoresis, 3.3 volumes of Maxam Gilbert loading buffer (98% formamide/0.01 M EDTA/10 mM NaOH/1 mg/ml xylene cyanol/1 mg/ml bromophenol blue) were added to reaction mixtures. Sixteen percent denaturing polyacrylamide gels (7 M urea) were run at 40 V/cm at 50°C for 2–3 h and were dried on 3MM Whatman paper sheets. Imaging and quantitations were performed by using a PhosphorImager (Molecular Dynamics).

Detection of Covalent Top1-DNA Complexes *in Vivo*. Chinese hamster V79 cells (a gift from S.-J. C. Wei, Rutgers University) (30) were treated with camptothecin or (+)-BaP DE-2 or (−)-BaP DE (1 μM) for 1 h. Top1-DNA complexes were isolated by using the ICE-Bioassay (31–33). In brief, 10^6 cells were pelleted and immediately lysed with 1 ml of 1% sarkosyl. After homogenization with a Dounce, cell lysates were gently layered on step gradients containing four different CsCl solutions (2 ml of each) of the following densities: 1.82, 1.72, 1.50, and 1.45 from bottom to top (see ref. 33). Tubes were centrifuged at 30,700 rpm in a Beckman SW40 rotor for 24 h at 20°C. Fractions of 0.5 ml were collected from the bottom of the tube. Aliquots of each fraction (100 μl) were diluted with an equal volume of 25 mM sodium phosphate buffer (pH 6.5) and were applied to Immobilon-P membranes (Millipore) by using a slot-blot vacuum manifold. Detection of the top1-DNA complexes was performed by Western blotting using the C21 monoclonal top1 antibody (a generous

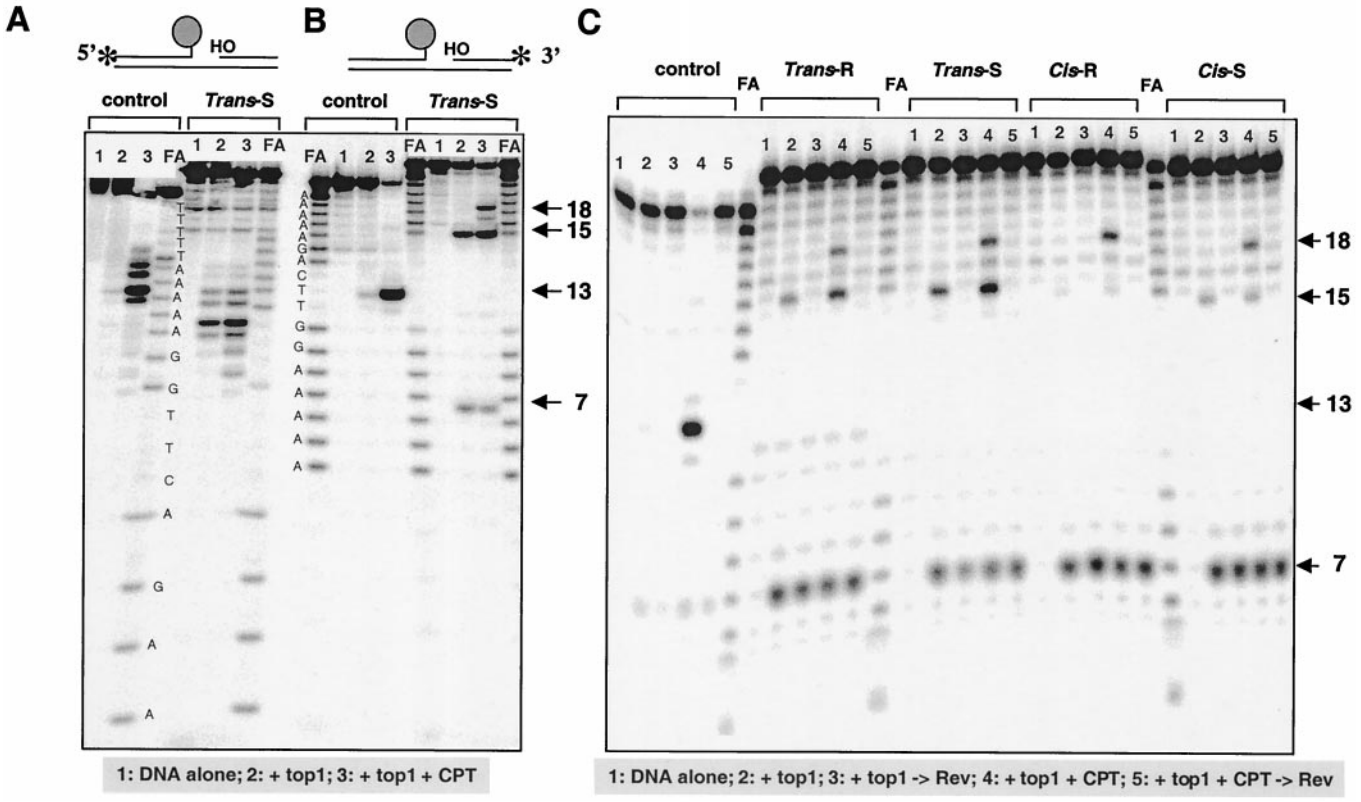

Fig. 2. Differential effects of the BaP DE-2 adducts on top1-mediated DNA cleavage. For oligonucleotide sequence and notation, see Fig. 1*B*. The upper strand of the oligonucleotide was end-labeled (asterisks) at either its 5′ (*A*) or 3′ (*B* and *C*) terminus. Note that the 3′-end label (cordycepin) introduces an additional A residue into the sequence shown in Fig. 1*B*. Lanes in *A* and *B*: 1, DNA alone; 2, + top1; 3, + top1 + 10 μM camptothecin (CPT). Lanes in *C*: 1, DNA alone; 2, + top1 for 15 min; 3, + top1 for 15 min, and then with 0.35 M NaCl for an additional 30 min; 4 and 5, same as lanes 2 and 3 + 10 μM camptothecin. FA, formic acid reaction to generate purine ladder. The size of the fragments generated by top1 with the 3′-end labeled oligonucleotide (including the cordycepin label) is indicated to the right.

gift from Yung-Chi Cheng, Yale University) according to standard procedures (33).

Results

A trans-*S* Adduct of BaP DE-2 Alters the Position of Top1-Mediated DNA Cleavage in the Near Vicinity of the Adduct. Consistent with previous data (10), we found that an unmodified, 22-mer oligodeoxynucleotide (Fig. 1*B*) was efficiently cleaved by human top1 at the expected site (Fig. 2). We then prepared oligonucleotides in which the dG residue immediately 3′ from the top1 cleavage site (position +1) was replaced by each of the four isomeric BaP DE-2 dG adducts, trans-*S*, trans-*R*, cis-*S*, and cis-*R* (Fig. 1*B*). The trans isomers are oriented in the DNA minor groove with the aromatic portion extending either in the direction of the 5′ terminus (trans-*S*) or the 3′ terminus (trans-*R*) of the adducted DNA strand. In contrast, the cis stereoisomers intercalate into the DNA such that the hydrocarbon displaces the adducted base and the base opposite to it (16) (Fig. 1*B*).

We examined the effect of a trans-*S* dG adduct on top1-mediated DNA cleavage in the upper strand of the duplex oligonucleotide (Fig. 2) by using oligonucleotides that were labeled either at their 5′-phosphate terminus with [γ-^{32}P]-ATP (Fig. 2*A*) or at their 3′ terminus with [α-^{32}P]-cordycepin (Fig. 2 *B* and *C*). Top1 cleavage of the 5′-end labeled oligonucleotide generates a top1-linked fragment, as top1 cleaves DNA by becoming covalently linked to the 3′-DNA end (Fig. 2*A*, upper scheme) (5, 6). Thus, reaction products need to be digested with proteinase K to be resolved in DNA sequencing gels. However, the proteinase K digestion is usually incomplete, and a single top1-mediated DNA cleavage site usually results in a cluster of closely spaced bands corresponding to subterminal digestion products (Fig. 2*A*, control lane 3). The same cleavage generates a single band from the 3′-end labeled oligonucleotide because the labeled fragment generated by top1 is not enzyme-linked (Fig. 2*B*, upper scheme). Single cleavage of the control oligonucleotide and effects of the labeling procedures can be seen by comparing lanes 3 for the control oligonucleotides in Fig. 2 *A* and *B*. Comparison of lanes 3 in Fig. 2 *A* and *B* demonstrates that the trans-*S* adduct altered top1 cleavage. First, it induced a new top1 cleavage site in the absence of camptothecin (Fig. 2 *A* and *B*, lanes 2). 3′-end labeling and comparison with sequencing reactions indicated that this new site corresponded to a 3′-end labeled 15-mer comprising 14-nucleotide residues of the initial sequence plus the cordycepin end-label (see summary in Fig. 5). Secondly, the trans-*S* adduct rendered the normal top1 cleavage site (13-mer site; see Fig. 5) undetectable. Finally, two new cleavage sites were induced by the trans-*S* adduct: (*i*) a camptothecin-independent site that produced a 7-mer fragment, and (*ii*) a camptothecin-dependent site corresponding to an 18-mer fragment labeled at the 3′-end (Fig. 2*B*). These results demonstrate that the presence of a single BaP DE adduct immediately 3′ from a preexisting top1 site completely blocks top1 cleavage at this site and induces new camptothecin-independent and -dependent cleavage sites within 6 nucleotides upstream and downstream from the adduct on the same modified DNA strand.

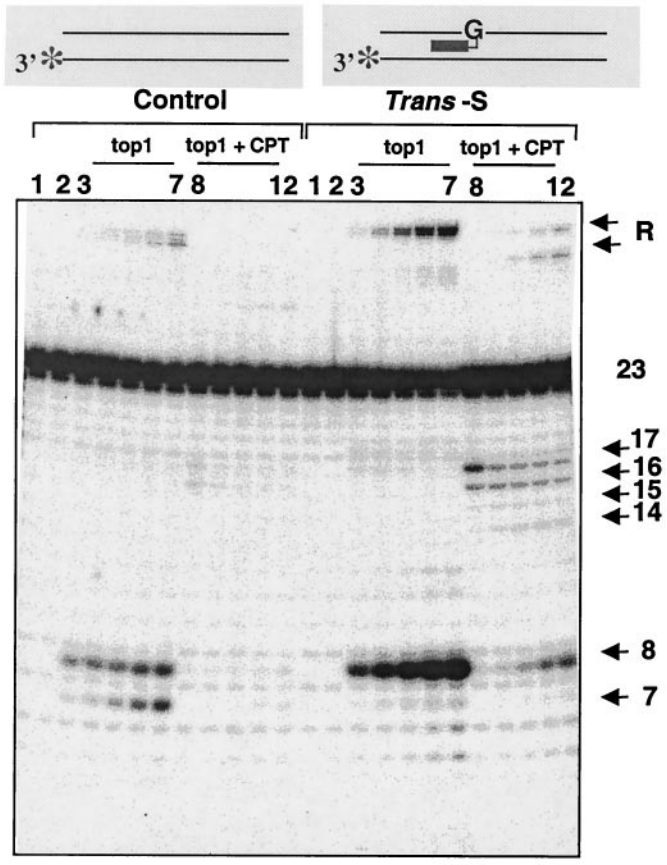

Fig. 3. Induction of top1-mediated DNA cleavage on the DNA strand opposite to a BaP DE-2 adduct and top1-mediated DNA recombination. Oligonucleotides (see Fig. 1*B*) without (control) or with the trans-*S* BaP DE-2 adduct (schematic representation at the top of the figure) were 3′-end labeled (asterisks) on the lower strand. Lanes: 1 and 2, DNA alone; 3–7, time course of top1-induced DNA cleavage in the absence of camptothecin; 8–12, same as lanes 3–7 with 10 μM camptothecin (CPT). Reaction times in lanes 3–7 and 8–12 were 15, 30, and 60 min and 2 and 4 h, respectively. The size of the fragments generated by top1 is indicated to the right (see Fig. 5). R, recombination products.

BaP DE-2 Adduct-Induced Top1-Mediated DNA Cleavage Depends on the Adduct Stereochemistry. Oligonucleotides with the four types of stereoisomeric BaP DE-2 adducts at the same dG residue (see Fig. 1) were tested for top1 cleavage (Fig. 2*C*). Cleavage at the control site (13-mer; see Fig. 5) was completely suppressed by the four adducts. By contrast, induction of the 15-mer site was most dependent on the type of adduct. Although the cis isomers induced detectable cleavage at this 15-mer site, it was too small to quantitate. In contrast, the trans isomers were markedly more efficient in inducing this site, and the trans-*S* isomer [derived from the carcinogenic (+)-BaP DE-2] was as much as 7-fold more efficient than the trans-*R* isomer derived from its noncarcinogenic enantiomer (Fig. 2*C*). These results demonstrate that suppression of top1 cleavage at the normal site does not depend on the stereochemistry of the BaP DE-2 adduct, but that adduct stereochemistry is critical for induction of top1 cleavage at the 15-mer site. In contrast to the 18-mer, this 15-mer accumulates in the absence of camptothecin, and thus cleavage at this position results in top1 poisoning.

Reversibility of the BaP DE-2 Adduct-Induced Top1 Cleavage Complexes. Religation of top1 cleavage complexes can be studied by increasing the salt concentration (8) or temperature (34),

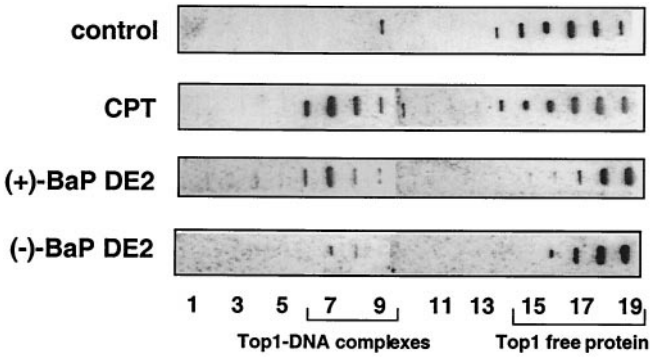

Fig. 4. Induction of top1 cleavage complexes by (+)- and (−)-BaP DE-2 in Chinese hamster V79 cells. After treatment (1 μM camptothecin or BaP DE-2 for 1 h), cell lysates were separated by cesium chloride gradients centrifugation. Fractions were collected from the bottom of the tubes (numbered 1–19 at the bottom of the figure). Presence of top1 protein was assayed in each of the gradient fractions by Western blotting with anti-top1 monoclonal antibody. Cellular DNA was contained in fractions 6–9, which correspond to the top1-DNA complexes.

which shift the top1-mediated DNA cleavage-religation toward religation. Fig. 2*C* shows that the BaP adduct-induced sites (generating the 15- and 18-mers) religated completely within 30 min in 0.35 M NaCl. Reversal was also observed with heat treatment (65°C for 30 min; data not shown). In addition, all four adducts induced cleavage at a site near the 3′-end of the upper strand (7-mer site; see Figs. 2 and 5). The cleavage complex at this site was neither enhanced by camptothecin (Fig. 2*C*, compare lanes 2 and 4) nor reversed by NaCl (compare lanes 3 and 5 to lanes 2 and 4). It was also resistant to heat (data not shown). This irreversibility (suicide reaction) (35, 36) probably results from the dissociation of the short DNA segment generated by top1 cleavage near the end of the oligonucleotide in an AT-rich sequence (see Fig. 5). Thus, these suicide complexes most likely result from the lack of an available partner for religation, rather than from inhibition of DNA religation by the adduct.

BaP DE-2 Adducts Induce Top1 Cleavage Complexes on the DNA Strands Opposite to the Adduct and Enhance Top1-Mediated DNA Recombination. Trans-BaP DE-2 adducts also effect the action of top1 on the strand opposite to the adducted strand (Fig. 3). In the control oligonucleotide, two relatively weak top1 cleavage sites were observed (7- and 8-mers derived from the lower strand 3′-end labeled with cordycepin). The 7- and 8-mer sites were salt- and heat-irreversible, presumably by the same mechanism as described above (suicide complexes). Cleavage at the site generating the 8-mer was markedly enhanced by the BaP DE-2 adducts and increased with incubation time (Fig. 3, lanes 3–7 of the trans-*S* section), indicating that BaP enhances the forward rate of top1 cleavage.

By contrast, camptothecin inhibited top1 cleavage at this suicide site (8-mer) in both the control and the BaP DE-2 adducted DNA. This inhibition is consistent with previous reports (8, 37) that camptothecin inhibits not only the religation but also the incision step of top1 reactions. In this respect, the enhancement of top1 cleavage by the BaP DE adducts differs markedly from the effects of camptothecin.

BaP DE-2 adducts also induced the formation of DNA products that migrated more slowly than the initial oligonucleotide (labeled R for "recombination" in Fig. 3). Such recombinations have previously been reported when top1 forms suicide products (7, 8). The appearance of these recombination products was correlated with the presence of the suicide sites (7- and

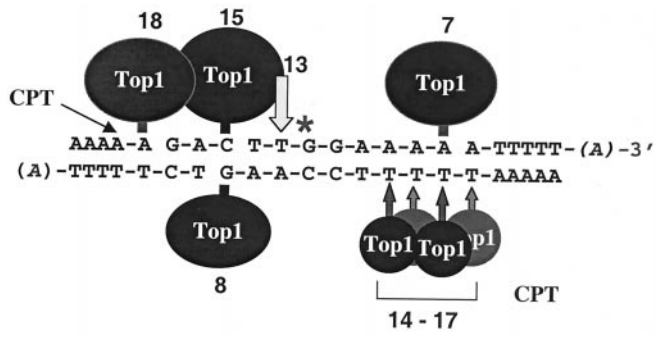

Fig. 5. Summary of the effects of BaP DE-2 dG adducts (position indicated by the asterisk) on top1 cleavage. Top1 cleavage complexes induced by the BaP DE-2 adducts are indicated by filled circles with linkage to the DNA 3′ terminus. The size of the circles is not meant to correlate with the extent of DNA cleavage. Numbers refer to the size of the fragments detected in DNA sequencing gels after DNA 3′-end labeling (see Figs. 2 and 3). Note that the 7-mer derived from the lower strand and shown in Fig. 3 (control) is not represented. Open arrow indicates the major top1 cleavage site in the control oligonucleotide, which is suppressed by the adducts. CPT, camptothecin-induced sites.

8-mer; Fig. 3). Both the suicide and the recombination products were enhanced in the presence of the BaP DE-2 adducts, and both were suppressed by camptothecin (Fig. 3). These results indicate that BaP DE-2 adducts can induce top1-mediated DNA cleavage and recombination.

BaP DE-2-Induced Top1 Cleavage Complexes Can Be Detected *in Vivo*. Chinese hamster V79 cells, which have been used previously to study mutagenesis by BaP DE adducts (30), were exposed to (+)-BaP DE-2 or to its noncarcinogenic isomer, (−)-BaP DE-2. Top1-DNA complexes were detected by using cesium chloride gradient centrifugation and Western blotting of the gradient fractions with anti-top1 monoclonal antibody (ICE bioassay) (31, 32). Fig. 4 shows the presence of top1 in the DNA-containing fractions from (+)-BaP DE-2-treated cells. Interestingly, top1-DNA complexes in the (−)-BaP DE-2-treated cells were much less. As expected, top1 complexes were detected in the same fractions from camptothecin-treated cells. These results show that (+)-BaP adducts can trap top1 in mammalian cells.

Discussion

The present study demonstrates that BaP DE adducts on the dG residue immediately 3′ from a top1 cleavage site (G + 1 in our oligonucleotide; see Fig. 1*B*) totally suppress normal top1 cleavage at this site while simultaneously inducing the formation of multiple new cleavage sites 3–6 base pairs away from the adduct. BaP DE dG adducts are mutagenic in a variety of bacterial and mammalian cell systems, and the frequency and types of mutations induced can depend both on the stereochemistry of the adduct and the DNA sequence context (22). Results reported here suggest a novel potential mechanism for genetic damage induced by BaP DE dG adducts in DNA: namely, induction of top1 cleavage complexes. Lesions derived from top1 cleavage complexes were far more evident in cells exposed to the carcinogenic (+)-BaP DE-2 than in cells exposed to its noncarcinogenic enantiomer. It has been proposed that top1 cleavage complexes can damage genomic DNA by generating replication-mediated DNA lesions (38). These lesions, in turn, can lead to cell death or illegitimate recombination after strand invasion and intermolecular religation by top1 (see Fig. 3) (7, 8). BaP DE-2 adducts represent very efficient DNA lesions for the induction of top1 cleavage complexes, with top1-mediated DNA cleavage taking place at several sites near a single dG adduct. This raises the possibility that top1 binding to such damaged DNA kills cells or promotes recombination. Recombination is indeed observed *in vitro*, with several of the top1 cleavage complexes formed from BaP DE-modified DNA (Fig. 3). Further investigations will be required to determine whether mutations by illegitimate recombination might be related to top1 trapping by BaP DE adducts.

The intercalated, cis-opened BaP DE adducts are very weak inducers of camptothecin-independent cleavage complexes (top1 poisons) in which the enzyme is bound to the third residue 5′ to the adduct site (15-mer). In contrast, the trans-opened dG adducts, which lie in the DNA minor groove, are efficient inducers of cleavage complexes at this 15-mer site, with the trans-*S* stereoisomer being significantly more active than the trans-*R* isomer. The trans-*S* dG adduct is the predominant adduct formed in DNA from the carcinogenic (+)-(*R*,*S*,*S*,*R*)-BaP DE-2 *in vitro*, as well as on metabolic activation of the parent hydrocarbon *in vivo*. This is an example on a molecular level of a clear enhancement of an activity associated with potential cell damage caused by this trans-*S* adduct, as opposed to the other three stereoisomeric (cis and trans, *R* and *S*) BaP DE-2 dG adducts. Orientation of the hydrocarbon in the trans-*S* dG adduct toward the 5′-end of the modified strand did not interfere with cleavage to form the 15-mer even though the adduct is only two nucleotides away from the phosphodiester bond that is cleaved. Interestingly, only this trans-*S* stereoisomer but not the trans-*R* stereoisomer has been reported to cause significant bending in oligonucleotide duplexes (39, 40).

Camptothecins also trap (poison) top1, resulting in their remarkable anticancer activity (6, 41, 42). However, the mechanism of top1 trapping by the BaP DE-DNA adducts appears to be quite different from that of camptothecins. The primary effect of camptothecins is to inhibit the religation of top1 cleavage complexes (43–46), although camptothecins can also inhibit top1 cleavage at higher concentrations (present study; Fig. 3) (37). In contrast, the primary effect of BaP DE-2 adducts is to enhance the formation of top1 cleavage complexes (forward reaction) while exhibiting minimal effects on religation (see Figs. 2 and 3).

The crystal structures of several fragments of top1 in a complex with an oligonucleotide comparable to the one used in the present study have recently been reported (11, 12). The enzyme encircles the DNA and shows tight contact with the DNA in the region immediately upstream (5′) from the nucleotide covalently linked to the enzyme (see Fig. 1*B*). The suppression of top1 cleavage by all four BaP DE-2 adducts at the cleavage site (Fig. 2) is consistent with the importance of DNA structure and contacts at this site for top1 action. The induction by the BaP DE adducts of top1 cleavage at new sites on both sides of the adducts and on both strands of the DNA demonstrates that DNA structure alterations can also stimulate cleavage of internucleotide bonds 3–6 nucleotides away from the nucleotide bearing the adduct.

Structural information on the interactions between DNA, ligands, and top1 should provide insights for the discovery of novel anticancer drugs. No structural data are presently available for the top1-DNA-camptothecin complex, and obtaining such data may be difficult because camptothecin itself does not bind covalently to the DNA and thus can dissociate from the complex. The most direct experimental evidence for camptothecin interaction with top1 cleavage complexes was obtained with an alkylating camptothecin derivative that forms an adduct with the guanine immediately downstream (G + 1 in Fig. 1) from the top1 cleavage site (47). Two models have recently been proposed for camptothecin binding in the top1

cleavage complex, one in which camptothecin is intercalated (15) in the DNA between the bases immediately flanking the cleavage site and another in which the G + 1 and the stacked camptothecin are flipped outside of the DNA (11). Both models implicate stacking of camptothecin with the G immediately 3′ from the cleavage site (G + 1). The present data provide a unique opportunity to model enzyme/DNA/drug complexes in which the ''drugs'' (BaP DEs) are covalently linked to the DNA to give adducts whose solution conformations in the absence of the enzyme are known. In contrast to cleavage complexes trapped by camptothecin, complexes containing covalent BaP DE adducts should persist. Because the trans-BaP DE-2 adducts are minor groove binders, their interactions with DNA and the enzyme in the ternary complex may well be quite different from those of the camptothecins but similar to those of the minor groove binders nogalamycin, aclarubicin, and ecteinascidin 743, which can act as top1 poisons (33, 48–50).

We dedicate this study to the memory of our friend and colleague Dr. Anthony Dipple.

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