Different Effects on Human Topoisomerase I by Minor Groove and Intercalated Deoxyguanosine Adducts Derived from Two Polycyclic Aromatic Hydrocarbon Diol Epoxides at or Near a Normal Cleavage Site*

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Topoisomerase I (top1) relieves supercoiling in DNA by forming transient covalent cleavage complexes. These cleavage complexes can accumulate in the presence of damaged DNA or anticancer drugs that either intercalate or lie in the minor groove. Recently we reported that covalent diol epoxide (DE) adducts of benzo[a]pyrene (BaP) at the exocyclic amino group of G(+1) block cleavage at a preferred cleavage site (−CTT—G(+1)G(+2)A−) and cause accumulation of cleavage products at remote sites. In the present study, we have found that the 10S G(+2) adduct of BaP DE, which lies toward the scissile bond in the minor groove, blocks normal cleavage, whereas the 10R isomer, which orients away from this bond, allows normal cleavage but blocks religation. In contrast to BaP, the pair of benzo[c]phenanthrene (BcPh) DE adducts at G(+2), which intercalate from the minor groove either between G(+1)/G(+2) or between G(+2)/A, allow normal cleavage but block religation. Both intercalated BcPh DE adducts at G(+1) suppress normal cleavage, as do both groove bound BaP DE adducts at this position. These studies demonstrate that these DE adducts provide a novel set of tools to study DNA topoisomerases and emphasize the importance of contacts between the minor groove and top1’s catalytic site.

Topoisomerase I (top1)1 is a nuclear enzyme that is essential for the regulation of DNA topology, transcription, replication, and probably DNA recombinations. The enzyme’s catalytic mechanism is well characterized. Nucleophilic attack on the DNA backbone by a catalytically essential tyrosine (Tyr723) of the enzyme breaks one of the DNA strands by forming a covalent phosphotyrosyl bond with the 3′-end of the DNA. This break provides a swivel point for DNA relaxation. Once supercoiling has been relieved, the 5′-hydroxyl of the cleaved DNA acts as a nucleophile toward the phosphotyrosyl bond and restores the DNA backbone, thus releasing the enzyme for further catalytic cycles (reviewed in Refs. 1 and 2).

Top1 can be trapped in a covalent complex with DNA by a variety of lesions including abasic sites, mismatches, oxidative lesions, base methylation (O6-methyl guanine, 5-methylcytosine), base alkylation (vinyl adducts), and DNA strand breaks (reviewed in Ref. 3). Top1 is an important target of several anticancer drugs. Camptothecin and its derivatives are believed to kill cancer cells by specifically trapping top1 and preventing the religation of the covalent top1 cleavage complexes. Several other DNA intercalating and minor groove-binding drugs have also been reported to trap these top1 cleavage complexes (reviewed in Ref. 4). However, little is known about how these noncovalently bound drugs block religation and so prevent release of functional top1. Such structural information should prove highly valuable in designing new top1 inhibitors.

We have recently utilized covalent DNA adducts (Fig. 1A) derived from trans ring opening of benzo[a]pyrene 7,8-diol-9,10-epoxides (BaP DE, two enantiomers of the diastereomer in which the benzylic 7-hydroxyl group and the epoxide oxygen are trans) by the exocyclic amino groups of the purine bases as probes of the catalytic activity of top1 (5, 6). These adducts were introduced into a 22-mer DNA sequence, which contains a single high affinity (7) top1 cleavage site (between T and G, Fig. 1B) and is derived from the oligonucleotide used for determination of the crystal structure of human top1 bound to this DNA substrate (8). These adducts are extremely powerful probes of enzyme-DNA contacts, since their solution conformations are known from two-dimensional NMR studies. For the BaP DE adducts at the exocyclic N9-amino group of dG (X = G, Fig. 1B), the trans 10S adduct (S-absolute configuration at the point of attachment of the amine to the hydrocarbon) lies in the minor groove with the aromatic portion pointing toward the 5′-end of the adducted strand, as shown in Fig. 2A (9). The 10R adduct lies toward the 3′-end (Fig. 2, A and B) (10). We previously found (5) that both of these distereomeric BaP DE trans adducts at G(+1) suppress normal top1 cleavage and induce new cleavages at positions 3–6 bases away from the adducted G. In marked contrast, when X = A the trans-opened BaP DE adducts at N9 are intercalated (10S toward the 3′-end) from the major groove (Fig. 2C) (11–13). Quite interestingly, these adducts initially appear invisible to the enzyme in that normal cleavage occurs but religation is blocked (6).

The present study was designed to investigate the effects of additional unique structural motifs on the activity of top1. Here we examine the consequences of placing the minor groove-bound trans 10R and 10S BaP dG adducts at the +2 position...
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**MATERIALS AND METHODS**

**Enzymes and Chemicals**—Human recombinant top1 was purified from Baculovirus-infected cells as described previously (15). Terminal deoxynucleotidyl transferase was purchased from Invitrogen. [α-32P]Cordycepin 5′-triphosphate was purchased from PerkinElmer Life Sciences; Polyacrylamide from Bio-Rad. Camptothecin was provided by Drs. M. C. Wani and M. E. Wall (Research Triangle Institute, Research Triangle Park, NC). Ten-mM aliquots of camptothecin in dimethyl sulfoxide (MeSO) were stored at −20°C and then thawed and diluted in reaction buffer just before use.

**Adducted Oligonucleotides**—The adducted oligonucleotides described in this and a previous study (5) are 22-mers, 5′-[32P]AAG ACT TG(+) and 1K(+) AAA AAT TTT Tj′-3′ with modified deoxyguanosine residues at G(+1) or G(+2) corresponding to the adducts formed upon trans opening of (+) or (−)-BaP DE at C10 and of (+) or (−)-BcPh DE at C1 (Fig. 1A). The oligonucleotides containing BaP adducts at G(+1) and their effects on top1 were described by us in previous work (5). The 22-mers modified at G(+2) with the trans-opened BaP and BcPh DE adducts, as well as shorter marker oligonucleotides corresponding to their potential cleavage products generated by top1, were synthesized and characterized in a separate study (16). The two diastereomeric 22-mers containing trans-opened BaP DE-2 adducts at G(+1) were prepared by the methods described therein. High performance liquid chromatography retention times, configurational assignments, and major CD bands for these two new oligonucleotides are given in Table I. The CD spectra of these 22-mers containing BaP DE-2 adducts at G(+1) are almost identical to the spectra for the corresponding oligonucleotides with these adducts at G(+2) (16). Each of the new BaP DE-2 adducted 22-mers (Table I) gave a mass of 7070 daltons (calculated for C228H326O10N6P2 7068 daltons). The adducted oligonucleotides were 3′-end-labeled with α-32P-labeled cordycepin, as described previously (15, 17). 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 (1 µM) for the indicated times at 25°C in 10 µl of reaction buffer (10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, 15 µg/ml bovine serum albumin, final concentrations). Reactions were stopped by adding sodium dodecyl sulfate (SDS) (final concentration 0.5%). For reversal experiments, the SDS stop was preceded by the addition of NaCl to a final concentration of 0.35 M followed by incubation for 30 min at 25°C. For the heat reversal experiments, the SDS stop was preceded by heating the samples to 65°C. Sequencing of oligonucleotides was performed by using the Maxam-Gilbert purine sequencing protocol (18). 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, and 1 mg/ml bromphenol 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 dried on Whatman No. 3MM paper sheets. Imaging and quantitations were performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**RESULTS**

**Position- and Isomer-specific Effects of BaP dG Adducts—**Fig. 3A shows our previously reported (5) results (repeated for comparison purposes) on top1-mediated DNA cleavage of the upper strand of the oligonucleotide containing BaP dG adducts at position G(+1), in comparison with a new experiment utilizing the same adducts at G(+2). In the absence of adduct, top1 did not produce detectable cleavage (lane 2, control), and camptothecin was required for observing the expected 13-mer cleavage product (lane 3, control). When BaP adducts were present at the G(+1) position, the formation of the 13-mer cleavage product was suppressed and top1 cleavage was shifted in such a way that a 15-mer product was observed independently of camptothecin, and an 18-mer product was observed in the presence of camptothecin. The trans-R adduct had a stronger effect than the trans-S adduct (see Fig. 3B for quantitation).

Different effects were observed when the BaP adduct was at the G(+2) position (right side of Fig. 3A). The trans-R adduct downstream from the scissile bond. We also examine the effects of DE dG adducts (Fig. 1A) derived from trans opening of benzölphenanthrene 3,4-diol 1,2-epoxide (BcPh DE) at positions +1 and +2. These BcPh adducts differ dramatically from the BaP adducts in that the aromatic portion of the hydrocarbon is intercalated from the minor groove into the DNA helix (14), as shown in Fig. 2 (trans-S adduct intercalated toward the 5′-end of the adducted strand, panel C) (14), and were consequently expected to have a very different "footprint" from the groove-bound BaP dG adducts in terms of their interactions with the enzyme at the cleavage site.

**FIG. 1.** A, structures of the trans-opened BaP and BcPh DE adducts at N° of dG utilized in this study. B, sequence of the oligodeoxynucleotide used in this study and position of the high affinity top1 cleavage site. Nomenclature of the residues at the +1 and +2 positions relative to the cleavage site is shown. X = G (present study) or A (6).
induced the accumulation of a large amount of cleavage product, which, in contrast to the control, did not require the presence of camptothecin. In the presence of camptothecin, cleavage at this site was almost irreversible in the presence of 0.35 M NaCl. The cleavage band migrated above the 13-mer cleavage product observed for the control oligonucleotide and below the 15-mer cleavage product observed for the oligonucleotides containing BaP G(1) adducts. Because the migration of the oligonucleotides containing BaP adducts was dependent on the presence and type of BaP adducts (see 23-mer bands), short BaP dG adduct-containing oligonucleotides of known length (14-, 15-, and 16-mers, including the labeled cordycepin residue) were used as markers to establish the length of this cleavage product. The cleavage product derived from the BaP trans-R G(1)-adducted oligonucleotide migrated in a position consistent with its being a 13-mer. Thus, the presence of a BaP trans-R dG adduct at the +2 position increased cleavage at the normal top1 high affinity site. In marked contrast to the trans-R G(1) adduct, a BaP trans-S G(1) adduct suppressed top1-mediated DNA cleavage at the 13-mer site and induced cleavage upstream, resulting in a 15-mer cleavage product (see sequence at the bottom of Fig. 3A) that was independent of camptothecin.

Further reversal experiments were carried out with the oligonucleotide containing the BaP trans-R dG(1) adduct. Heat reversal is commonly used to determine the stability of top1 cleavage complexes (19). Fig. 4 shows that a significant fraction of the top1 cleavage product (13-mer) from the BaP trans-R dG(1) adducted oligonucleotide migrated in a position consistent with its being a 13-mer. Thus, the presence of a BaP trans-R dG adduct at the +2 position increased cleavage at the normal top1 high affinity site. In marked contrast to the trans-R G(1) adduct, a BaP trans-S G(1) adduct suppressed top1-mediated DNA cleavage at the 13-mer site and induced cleavage upstream, resulting in a 15-mer cleavage product (see sequence at the bottom of Fig. 3A) that was independent of camptothecin.

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### Table I

| Parent diol epoxide | Absolute configuration at C1 | Retention time (min) | CD spectrum (nm intensity) |
|---------------------|----------------------------|----------------------|---------------------------|
| (+)-1R,2S,3R,4S-DE-2 | R                          | 17.9                 | 254 (12.8); 279 (12.3)    |
| (-)-1R,2S,3R,4R-DE-2 | S                          | 18.9                 | 246 (10.3); 262 (15.9)    |

* Assignments are based on CD spectra of the individual N²-dG adducts (36, 37) after enzymatic digestion (38), as well as on comparison of the CD spectra for the present oligonucleotides with those of the analogous oligonucleotides with the trans opened R and S adducts of BcPh DE-2 at G(1) (16).

* On a Higgins DNA column (4.6 × 100 mm, 5 μm) at 40 °C eluted at 1.5 ml/min with a gradient of acetonitrile in 0.1 M (NH₄)₂CO₃, pH 7.5, that increased the acetonitrile composition from 5 to 11% over 20 min.

* In MeOH, normalized to 1.0 absorbance unit at 260 nm.

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**Fig. 3.** A, effects of BaP dG adducts on top1 cleavage. Control is the unmodified oligonucleotide, G(+1) and G(+2) correspond to the oligonucleotides with adducts at the +1 and +2 position, respectively, and (S) and (R) indicate the configuration at the site of attachment of the base to the hydrocarbon (see Fig. 1). Convention for each lane: A, DNA alone; B, DNA + top1 for 15 min at 21 °C; C, DNA + top1 + camptothecin for 15 min at 21 °C; Cₛ, same after an additional 15 min following addition of 0.35 M NaCl (final concentration); Bₛ, same reversal experiment in the absence of camptothecin. The high affinity top1 cleavage site (13-mer) and the 15-mer cleavage site are indicated on the oligonucleotide sequence. Labeling was at the 3'-end of the upper strand with α-32P-labeled cordycepin (annotated as A in the sequence). B, quantitation of lanes A–C of the gel shown in A. The number under each set corresponds to the cleavage site (13- or 15-mer).
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Induction of Top1 Cleavage Sites on the DNA Strand Complementary to the Adduct-containing Strand—We previously reported (6) that a BaP dG adduct at the +1 position of the oligonucleotide induces the formation of top1 cleavage sites on the lower strand, complementary to the adducted strand. We compared these results with the effects of the BaP dG(+2) adducts. In the unmodified oligonucleotide, cleavage of the lower strand by top1 is minimal (Fig. 5, lane 3, Control). The presence of BaP dG adducts (both S and R) at positions +1 or +2 induced top1 cleavage of the lower strand upstream from the normal cleavage site, resulting in 8-, 16-, and 17-mer cleavage products (Fig. 5). The 16-mer was observed only in the presence of camptothecin, whereas the 8-mer was observed in the absence of camptothecin. The 8-mer presumably represent “suicide” products resulting from diffusion away of the short, T-rich 8-mer fragment so that the cleavage complex cannot religate. Thus, the presence of BaP dG adducts either at the +1 or +2 position results in the formation of top1 cleavage products at positions flanking the adducts on both strands of the modified oligonucleotide. The adducts at G(+2) appeared to be somewhat more effective in promoting 16-mer formation than were the adducts at G(+1).

Effects of BcPh DE Adducts at Positions +1 and +2—We then tested the effects of the intercalated BcPh dG adducts at position +1 of the same oligonucleotide (Fig. 6). As in the case of the BaP G(+1) adducts, top1-mediated DNA cleavage was: 1) completely suppressed at the high affinity 13-mer site, 2) induced upstream in the absence of camptothecin (15-mer cleavage product), and 3) induced at the 18-mer site in the presence of camptothecin. Cleavage at both of these sites was reversible (Fig. 6). Next we looked at the effects of BcPh dG adducts at G(+2) (Fig. 7). Both the trans-S and the trans-R-adducted oligonucleotides trapped top1 at the high affinity (13-mer) site independently of camptothecin. However, the oligonucleotide containing the trans-S adduct showed little cleavage at any site, even in the presence of camptothecin. Cleavage of the trans-R adducted oligonucleotide was markedly more efficient. Reversal experiments in the presence of 0.35 M NaCl showed that the cleavage observed with the BcPh trans-R dG(+2) adduct reversed more slowly than cleavage of the correspond-

G(+2) adduct resisted heat treatment. This result is consistent with the stabilization of this cleavage product to salt reversal (see Fig. 3A and results above) (5). Together, these observations suggest that the enhancement of top1 cleavage by the BaP trans-R G(+2) adduct is due at least in part to an inhibition of top1-mediated DNA religation.

Fig. 4. Trapping of top1 cleavage complex by trans-R BaP dG(+2) adduct. Lane 1, DNA alone; lanes 2 and 3, + top1; lanes 4 and 5, + top1 + camptothecin (CPT). In lanes 1, 2, and 4, reactions were for 15 min at 21 °C. In lanes 3 and 5, after 15 min, reactions were heated to 65 °C for 30 min before being stopped with SDS (0.5% final concentration). In this and the following figures, the migration position(s) of the cleavage product(s) are indicated by the arrows (see Fig. 3A for the sequence).

Fig. 5. Induction of top1-mediated DNA cleavage on the lower strand of the oligonucleotide duplex containing BaP dG adducts on the upper strand. Reactions were for 15 min at 21 °C. Lanes A, DNA alone; lanes B, + top1; lanes C, + top1 + camptothecin; lanes D, purines as revealed by formic acid reactions.

Fig. 6. Modifications of top1 cleavage in the presence of BcPh adducts at the G(+1) position. The oligodeoxynucleotide was labeled on the upper strand with α-[32P]-labeled cordycepin (shown as [32P]-A). Control, unmodified oligodeoxynucleotide; BcP(S), trans-S-BcPh adduct at G(+1); BcP(R), trans-R-BcPh adduct at G(+1). DNA was reacted with top1 in the absence of camptothecin in lanes 2, 8–13, and 15–20 and with top1 and camptothecin in lanes 14 and 21 (lanes labeled T+C). Top1 reactions were for 15 min at 21 °C in lanes 2, 3, 9, 14, 16, and 21. Reversibility of the top1 cleavage complexes was studied after addition of 0.35 M NaCl (lanes 4–7, 10–13, and 17–20) for 1, 3, 10, and 30 min, as indicated above the lanes.)

ing unmodified oligonucleotide observed in the presence of camptothecin (Fig. 8). Similarly, the cleavage product from the BcPh trans-R-adducted oligonucleotide failed to undergo religation upon treatment at 65 °C. These results demonstrate that, as in the case of the BaP adduct which lies in the minor groove, a BcPh adduct intercalated in the DNA from the minor
the top1 cleavage complexes was studied after addition of 0.35 M NaCl. Reactions were for 15 min at 21 °C.

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FIG. 7. Top1 cleavage complexes observed in the presence of BcPh adducts at the G(+2) position. The oligodeoxynucleotide was labeled on the upper strand with α-32P-labeled cordycepin (A). control, unmodified DNA; BcP(S), trans-S-BcPh adduct at G(+2); BcP(R), trans-R-BcPh adduct at G(+2). Lanes A, DNA alone; lanes B, + top1 lanes C, + top1 + camptothecin. Reactions were for 15 min at 21 °C. 13, 14, and 15 correspond to 13-, 14-, and 15-mer oligodeoxynucleotides labeled at their 3′-end with α-32P-labeled cordycepin and containing a trans-S- or trans-R-BcPh adduct at their G(+2) position.

FIG. 8. Trapping of top1 cleavage complexes observed in the presence of the trans-R-BcPh G(+2) adduct. control, unmodified DNA. Lane 1, DNA, no treatment; lanes 2 and 9–13, DNA + top1 in the absence of camptothecin; lanes 3–7, DNA + top1 + camptothecin. Reactions were for 15 min at 21 °C in lanes 2, 3, and 9. Reversibility of the top1 cleavage complexes was studied after addition of 0.35 M NaCl (lanes 4–6 and 10–13) for 3, 10, and 30 min as indicated above the lanes or after heating the samples at 55 °C for 30 min.

groove one base downstream from the high affinity top1 cleavage site traps the cleavage complex by inhibiting its religation and does so more effectively than does camptothecin.

DISCUSSION

When incorporated near the scissile bond of a DNA substrate, covalent adducts derived from trans ring opening of BaP and BcPh DE-2 by the exocyclic amino groups of purines have remarkable effects on the nicking-closing activity of human top1 (summarized in Fig. 2). Depending on their position in the DNA sequence relative to the top1 cleavage site and their orientation in the DNA, these adducts either: 1) prevent top1 from cleaving its DNA substrate at the normal position while enhancing cleavage at other, remote sites (Fig. 2A) or 2) permit cleavage at the normal site but inhibit religation, resulting in accumulation (trapping) of the top1-DNA cleavage complex, even in the absence of camptothecin (Fig. 2, B and C). Structures for all these adducts are known from solution NMR studies (9–14, 20, 21). As shown in Fig. 2, the trans-opened adducts from BaP and BcPh DE-2 may either lie in the minor groove (BaP DE dG adducts), intercalate from the minor groove (BcPh DE dG adducts), or intercalate from the major groove (dA adducts from both hydrocarbons). Their orientation relative to the DNA strand depends on the configuration at the site of attachment of the hydrocarbon to the base as shown in Fig. 2. In the case of trans-opened dG adducts, both minor groove-bound BaP S-adducts and intercalated BcPh S-adducts (9, 14) orient toward the 5′-end of the adducted strand, whereas the corresponding R-adducts (10, 14) orient toward the 3′-end. For trans-opened dA adducts from both hydrocarbons, the orientations relative to the adducted strand are opposite from those of the corresponding dG adducts, such that S-dA adducts intercalate toward the 3′-end (11, 20, 22) and R-adducts toward the 5′-end (12, 13, 21).

Our aim was to use these BaP and BcPh DE adducts placed at or near a top1 cleavage site as specific probes of top1 function. We previously reported suppression of top1-mediated DNA cleavage at the normal site (Fig. 2A) and enhancement of cleavage at new sites by minor groove-bound trans-S and trans-R BaP adducts at G(+1), immediately downstream from the scissile bond (5). The present study examines the effects of these same adducts one base farther downstream, at G(+2). We have also examined BcPh DE dG adducts, which have a different binding, namely intercalation from the minor groove, at both G(+1) and G(+2).

Our present results indicate that the BaP trans-S adduct at the G(+2) position suppresses top1 cleavage at the normal site (13-mer, Fig. 3A) and leads to top1-mediated DNA cleavage at a position upstream from it (15-mer) in the absence of camptothecin. NMR studies have shown that the hydrocarbon portion of a trans-opened BaP trans-S dG adduct lies in the minor groove toward the 5′-end of the adducted strand and covers the flanking base pair immediately upstream from the adduct (9), which corresponds to G(+1) and its complement in the present top1 substrate (see Fig. 2A). In contrast, the BaP trans-R adduct at G(+2) orients in the opposite direction (10), such that the hydrocarbon in the minor groove overlies the base pair corresponding to the +3 position on the cleaved strand, well removed from the normal cleavage site. This R adduct permits top1 cleavage to occur at the normal site (Fig. 3A). Thus, the results observed with both the BaP R and S adducts at G(+1) (5) and the BaP S adduct at G(+2) demonstrate that minor groove occupancy in close proximity to the base pair (corresponding to G(+1)) immediately downstream from the cleavage site, but not further downstream, prevents top1 from cleaving its substrate at the normal site (13-mer) and leads instead to cleavage at a different site (15-mer). This suggests that nucleophilic attack by the catalytic tyrosine (Tyr723 for human top1) cannot take place when the minor groove is occupied at the scissile bond (positions −1 and +1). Our observations are consistent with the top1-DNA crystal structure from Redimbo et al. (23) showing the importance of minor groove interactions at the scissile bond in the top1-DNA covalent complex. Both lysine 532 and arginine 364 were found to occupy the DNA minor groove and to form hydrogen bonds with the −1 base pair (O2 position of 5-iodouridine and the N3 position of adenine, respectively).

In contrast to the BaP adducts, trans-opened BcPh DE-2 adducts of dG, although covalently bonded from the minor groove, have their aromatic rings intercalated into the DNA rather than lying outside in the groove (14). Both trans-S and -R BcPh adducts at G(+1) behave similarly to their BaP analogs by suppressing cleavage at the normal cleavage site and enhancing cleavage at a different (15-mer) site. In marked contrast to these BcPh dG(+1) adducts (intercalated from the minor groove), which suppress normal cleavage, both the S and R BaP dA adducts at the same +1 position (X = A in Fig. 1B), which intercalate from the major groove, are initially “invisible” to the enzyme and do not suppress cleavage, but poison the enzyme by irreversibly blocking religation once normal cleavage has occurred (Fig. 2C). This difference is consistent with top1-DNA contacts that make adducts intercalated from the
min grove more visible to the enzyme (such that normal cleavage cannot occur) relative to adducts that are intercalated from the major groove. In the crystal structure (24), the major groove of the DNA has minimal contacts around the +1 base pair.

The BcPh trans-R adduct at G(+2), which intercalates between G(+2) and its 3' nearest neighbor, G(+3), well away from the scissile bond, does not suppress normal cleavage at this site. In this respect it behaves similarly to the corresponding BaP trans-R adduct at the same (G(+2)) position. Interestingly, however, there is a marked difference between the trans-S G(+2) adducts from the two hydrocarbons. With the BaP trans-S adduct, top1 cleavage at the high affinity site is completely suppressed, even in the presence of camptothecin (Fig. 3), whereas in the case of the BcPh trans-S adduct, normal top1-mediated DNA cleavage occurs, albeit at a low level relative to the BcPh trans-R adduct at the same position (Fig. 7). The BaP trans-S adduct lies in the minor groove in the 5' direction (9) adjacent to the +1 base in the oligonucleotide, whereas the corresponding BcPh dG adduct intercalates between the G(+2) and G(+1) base pairs (14) (see Fig. 2) and hence does not impinge upon the scissile bond. Thus, for the S adducts, the BaP dG(+2) adduct, but not the BcPh dG(+2) adduct, is "seen" by top1 as close enough to the scissile bond to alter the enzyme-DNA contacts and possibly the noncovalent binding of the enzyme to the DNA. This is consistent with the amino acid contacts (see above) in the DNA minor groove at the cleavage site (23, 24).

Although the BaP and BcPh trans-R G(+2) adducts do not prevent top1 from cleaving its DNA substrate at the normal scissile bond, both of these adducts inhibit top1-mediated DNA religation activity, resulting in the observed accumulation of cleavage products independently of camptothecin. Our interpretation is that these adducts might distort the DNA structure after top1 has cleaved the DNA backbone and produce a misalignment of the +1 base including the 5'-hydroxyl at the end of the cleaved DNA. The net result would be an inhibition of top1-mediated DNA religation. A similar but much weaker trapping effect was observed with the BcPh trans-S adduct.

The determination of the binding site of inhibitors in the top1-DNA complex is important for rational drug design as a result of the discovery that top1 is the target of camptothecin and its derivatives (reviewed in Refs. 4 and 25). The present observation that camptothecin has no effect on the accumulation of the cleavage complex in the presence of the BcPh trans-S adduct at G(+2) (see Fig. 7) may be due either to inefficient cleavage of the scissile bond or to failure of camptothecin to trap the cleavage complex. The latter interpretation is consistent with the importance of drug interactions with the +1 base for camptothecin activity. In the current models proposed for the binding of camptothecin derivatives in the top1-DNA complex (24, 26–28) camptothecin stacks between the +1 and −1 base of the DNA scissile strand and interacts both with the enzyme and the DNA. Our data are also consistent with the importance of minor groove interactions, which was suggested from cross-linking studies showing that a camptothecin derivative bearing an alkylating arm at the 7-position of the drug formed a covalent complex with the +1 guanine N3 position (29). We also found that the BaP trans-R G(+2) adduct stabilized camptothecin-trapped cleavage complexes (Fig. 3A, lane 18). Since this adduct lies in the minor groove downstream from the top1 cleavage site, this stabilization might be due to the propagation of DNA distortions upon the −1 base, which would further stabilize the interaction of camptothecin with the enzyme-DNA complex.

Noncovalent, minor groove binders have been found to enhance the accumulation of top1 cleavage complexes and are a potential source for novel top1 poisons (30–32). However, the position of the drug binding site relative to the top1-DNA cleavage complex has remained unknown due to the reversible binding of these inhibitors. The present study and our recent report (5) demonstrate the usefulness of oligonucleotides containing covalently bound polycyclic aromatic hydrocarbon adducts at defined positions to determine the molecular interactions between minor groove ligands and top1-DNA complexes.

In contrast, ligands that lie in or intercalate from the minor groove at position +1 suppress top1-mediated DNA cleavage at the scissile +1/−1 bond and induce cleavages distal to this site.

The present study is the first demonstration that BcPh dG adducts result in marked accumulation of top1 cleavage complexes, both at the normal high affinity site (see above) and also at new sites (Figs. 3, 5, and 6). Polycyclic aromatic hydrocarbons are environmental pollutants giving rise to carcinogenic DEs during mammalian metabolism (33). Their carcinogenic potential has been linked to their persistence in DNA (34). For instance, fjord region polycyclic aromatic hydrocarbons are refractory to repair, whereas the generally less carcinogenic bay region adducts are more readily repaired by the nucleotide excision repair system (35). In the absence of DNA repair, these adducts would be likely to trap top1. Because top1 is a ubiquitous enzyme that acts throughout the human genome during transcription and replication (1), trapping of top1 as cleavage complexes by polycyclic aromatic hydrocarbon DE adducts could kill cells, as do anticancer drugs such as camptothecin and its derivatives (4, 25) that target top1. Top1 trapping can also lead to recombibinations (Ref. 5 and reviewed in Ref. 3), and this mechanism might also contribute to the carcinogenic activity of polycyclic aromatic hydrocarbons.

In summary, the present report extends our previous studies with BaP DE adducts (5, 6) to the intercalated BcPh DE dG adducts and suggests that top1 might be targeted by such adducts in vivo, especially by some of the adducts that are poorly repaired by the nucleotide excision repair system.

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Different Effects on Human Topoisomerase I by Minor Groove and Intercalated Deoxyguanosine Adducts Derived from Two Polycyclic Aromatic Hydrocarbon Diol Epoxides at or Near a Normal Cleavage Site

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J. Biol. Chem. 2002, 277:13666-13672. doi: 10.1074/jbc.M200209200 originally published online February 6, 2002

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