Effect of DNA Base Sequence on the Configuration of Deoxyadenosine Adducts Formed by the Fjord Region Diol Epoxide, (+)-(1*R*,2*S*,3*R*,4*S*)-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene†

Albert M. Cheh,*,‡,§ Haruhiko Yagi,§ and Donald M. Jerina§

Department of Chemistry, American University, Washington, D.C. 20016, and Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received May 10, 1994; Revised Manuscript Received August 18, 1994®

ABSTRACT: The carcinogen (+)-(1*R*,2*S*,3*R*,4*S*)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (in which the 4-OH group and epoxide oxygen are cis) was reacted with duplexes formed from self-complementary oligodeoxyribonucleotides, producing 1*S* or 1*R* configured adducts through trans or cis epoxide ring opening, respectively, by the exocyclic amino group of a central target A. Sequences containing 5′-AT-3′ generated much higher *S vs R* ratios than the average of 3.38 observed with calf thymus DNA samples, while sequences containing 5′-TA-3′ generated much lower ratios. Sequences with G in the position immediately 5′ to the central AT or TA, and C in the position immediately 3′, generated moderately higher ratios than did sequences with adjacent 5′ C and 3′ G. When thymidine was replaced by deoxyuridine in several sequences, the ratios of *S vs R* configured dA adducts and dA adducts *vs* dG adducts were substantially and uniformly reduced, but otherwise varied with the choice of nearest neighbors in patterns similar to those observed with the T containing sequences. Two hypothetical mechanisms are proposed to explain the effect of nearest neighbors on the *S vs R* dA adduct ratio; in both, diol epoxide intercalation precedes covalent bonding. In one mechanism, intercalation to the 5′ side of the target A yields an *S* adduct while intercalation to the 3′ side yields an *R* adduct, and the extent of adduct formation follows the nearest neighbor series G > C > T. In the other, which is more consistent with the limited evidence available, intercalation to the 5′ side of the target A yields an *R* adduct while intercalation to the 3′ side yields an *S* adduct, and the extent of adduct formation follows the nearest neighbor series T > C > G.

Polycyclic aromatic hydrocarbons (PAHs)[1] are ubiquitous environmental carcinogens (Dipple *et al.*, 1984). Bay-region diol epoxides have been shown to be ultimate carcinogenic metabolites of PAHs (Jerina *et al.*, 1976, 1977, 1984; Thakker *et al.*, 1988). Covalent modification of DNA by electrophilic metabolites such as diol epoxides is presumed to be the first step in tumorigenesis (Miller & Miller, 1967; Jerina *et al.*, 1991). Four optically active bay-region diol epoxide isomers (enantiomers of a diastereomeric pair) may be formed from a given hydrocarbon (Figure 1). For several hydrocarbons, the diol epoxide isomer with the *R,S,S,R*-configuration reading from the benzylic hydroxyl position to the benzylic epoxide position (Figure 1) exhibits by far the greatest carcinogenicity, although other configurational isomers of benzo[*c*]phenanthrene diol epoxide also exhibit significant carcinogenicity (Jerina *et al.*, 1986).

The exocyclic amino groups of purine bases in DNA are the principal targets of PAH diol epoxides (Jerina *et al.*, 1991). Cis or trans addition of the amino group to the benzylic epoxide position of the diol epoxide produces adducts that have either retained or inverted configuration, respectively, at the point of attachment to the hydrocarbon moiety. Figure 2 shows a diol epoxide with *R* configuration at the benzylic epoxide (C-1) position, (+)-(*S,R,S,R*)-BcPhDE-1, forming a 1*R* adduct by cis addition or a 1*S* adduct by trans addition.

In a previous study we proposed that dA adducts with *R* or *S* configuration at the point of attachment to the hydrocarbon might have opposite orientations within DNA, as indicated by their abilities to inhibit the action of 5′- or 3′-exonucleases, respectively (Cheh *et al.*, 1990). The adducts in question were formed in calf thymus DNA by the sets of four isomeric bay-region BcPhDEs and benz[*a*]anthracene diol epoxides. It was not known if the specificity of nuclease inhibition resulted from opposite adduct orientations in duplex DNA or in the short single-stranded products of initial endonuclease digestion, or both. Recent evidence indicates that at least some *R* and *S* adducts have opposite orientations in both single- and double-stranded DNA. Mao *et al.* (1993) reported that a *trans*-dG adduct formed by (+)-(*R,S,S,R*)-

† This research was supported in part by NIH Grant CA52043 to A.M.C. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

‡ American University.

§ National Institutes of Health.

® Abstract published in *Advance ACS Abstracts,* October 1, 1994.

[1] Abbreviations: PAH, polycyclic aromatic hydrocarbon; BcPhDE, 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene of unspecified absolute configuration; (+)-(*S,R,S,R*)-BcPhDE-1, (+)-(1*R*,2*S*,3*R*,4*S*)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene; (−)-(*R,S,R,S*)-BcPhDE-1, (−)-(1*S*,2*R*,3*S*,4*R*)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene; (+)-(*S,R,R,S*)-BcPhDE-2, (+)-(1*S*,2*R*,3*R*,4*S*)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo-[*c*]phenanthrene; (+)-(*R,S,S,R*)-BPDE-2, (+)-(7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; (−)-(*S,R,R,S*)-BPDE-2, (−)-(7*S*,8*R*,9*R*,10*S*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; (±)-BPDE-2, racemic mixture of (+)-(*R,S,S,R*)-BPDE-2 and (−)-(*S,R,R,S*)-BPDE-2. In the abbreviations, the absolute configurations of the benzo-ring carbons of the diol epoxides are read from the benzylic hydroxyl-bearing carbon to the benzylic epoxide-bearing carbon; in the BPDE series, this is the same as the order of reading in the IUPAC name, but in the BcPhDE series, it is the reverse. DNase I, deoxyribonuclease I; SVPD, snake venom phosphodiesterase; APase, alkaline phosphatase. DNA base sequences and target bases for diol epoxides in DNA are indicated by G, A, T, and C. Where U appears in a sequence, it is understood to be attached to deoxyribose. Adducts are recovered as nucleosides and are referred to as dA, dG, or dC adducts because the sugar is attached. Base positions within a sequence are indicated with a # symbol, while the sequences are referred to by a number only.

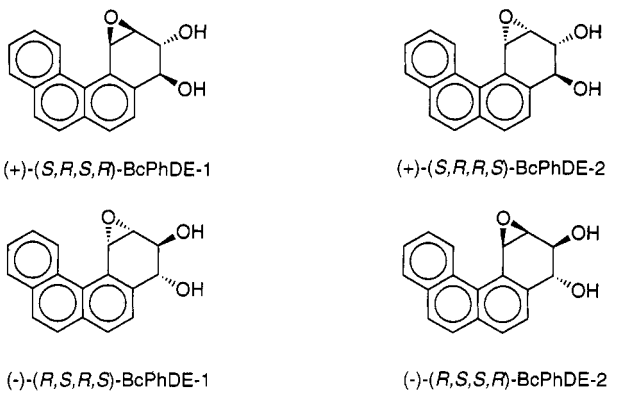

FIGURE 1: Stereochemistry of the optically isomeric bay-region diol epoxides of benzo[*c*]phenanthrene. Absolute configurations are designated from the benzylic hydroxyl-bearing carbon to the benzylic epoxide-bearing carbon. DE-1 and DE-2 diastereomers have the benzylic hydroxyl and epoxide groups cis and trans to each other, respectively.

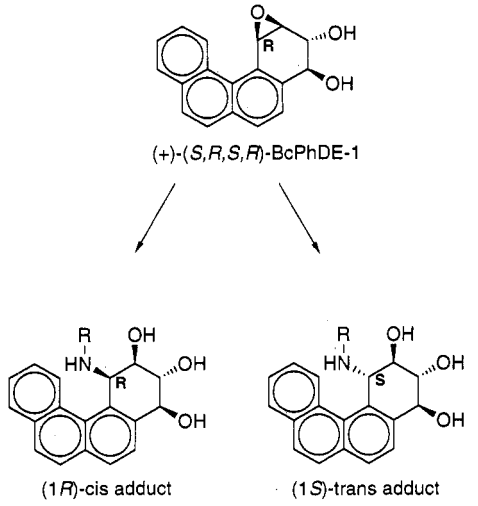

FIGURE 2: Formation of either a cis ring opened adduct with *R*-configuration at the site of attachment to the hydrocarbon (C-1) or a trans ring opened adduct with *S*-configuration at the site of attachment to the hydrocarbon (C-1), by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1.

BPDE-2 in a single-stranded sequence (*S* configuration at C-10, the point of attachment to the hydrocarbon moiety) selectively inhibited the activity of a 3′-exonuclease, while a *trans*-dG adduct formed by (−)-(*S*,*R*,*R*,*S*)-BPDE-2 in the same sequence (*R* configuration at C-10) selectively inhibited the activity of a 5′-exonuclease operating in the opposite direction. They also showed that, with these adducts of opposite absolute configurations, phosphodiester linkages on opposite sides of the adducted G were resistant to digestion and concluded that the opposite absolute configurations of the adducts resulted in their orienting in opposite directions in the single strand. NMR data and energy minimization calculations indicate that the *trans*-N^2-dG adducts of (+)-(*R*,*S*,*S*,*R*)-BPDE-2 (Cosman *et al.*, 1992) and (−)-(*S*,*R*,*R*,*S*)-BPDE-2 (de los Santos *et al.*, 1992), which have opposite *S* and *R* configurations, respectively, at the point of attachment to the hydrocarbon, lie in opposite directions in the minor groove of duplex DNA (in the 10*S* adduct the pyrenyl moiety faces the 5′ end of the adducted strand, while in the 10*R* adduct it faces the 3′ end). The ability of a diol epoxide to form adducts with specific orientations or configurations may be related to its carcinogenicity (Cosman *et al.*, 1992; de los Santos *et al.*, 1992; Cheh *et al.*, 1993).

The sequence of the DNA target plays a role in determining the extent of diol epoxide adduct formation in DNA (Boles & Hogan, 1985; Reardon *et al.*, 1989; Kootstra *et al.*, 1989; Rill & Marsch, 1990; Dittrich & Krugh, 1991a,b; Ross *et al.*, 1993; Margulis *et al.*, 1993; Schwartz *et al.*, 1994). The next question is whether sequence affects the types of adducts that are formed, which themselves may have different biological effects. Margulis *et al.* (1993) have noted that the ratio of *S vs R* configured adducts formed by (+)-(*R*,*S*,*S*,*R*)-BPDE-2 in native DNA is much higher than the ratio for adducts formed in poly(dG-dC)·poly(dG-dC) or poly(dG)·poly(dC) and have suggested that sequence effects might underlie this observation. In this study we demonstrate a linkage between the sequence flanking a target A in an oligodeoxyribonucleotide duplex and the configuration of adducts formed from (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 at the target.

An examination of a space-filling model of B DNA suggested that thymine methyl groups might be sterically prominent features that are encountered by diol epoxides forming adducts at the N^6 of A in the major groove of DNA. We hypothesized that if major groove binding rather than intercalation preceded covalent bonding, then placing a T to the 5′ side of a target A in a DNA duplex might inhibit the formation of adducts of one configuration, while placing a T to the 3′ side might inhibit the formation of adducts of the opposite configuration. Oligodeoxyribonucleotide duplexes formed by self-complementary sequences containing a central 5′-TA-3′ or 5′-AT-3′ and flanked by various combinations of G and C, plus additional sequences where U replaced T, were used to test this hypothesis. The adducts formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 have been fully characterized (Agarwal *et al.*, 1987). This diol epoxide isomer forms the greatest proportion of dA adducts compared to dG adducts of any of the bay-region diol epoxide isomers of four different hydrocarbons (Cheh *et al.*, 1993). Since it also forms appreciable amounts of both *S* and *R* configured dA adducts and is highly carcinogenic on mouse skin (Levin *et al.*, 1986), it was chosen to react with the self-complementary duplexes.

MATERIALS AND METHODS

Oligodeoxyribonucleotides. Oligodeoxyribonucleotides were synthesized with an Applied Biosystems Model 392 synthesizer. dU phosphoramidite was obtained from Cruachem, Inc. (Sterling VA). Purification of oligomers followed methods developed by Applied Biosystems (1987). Tritylated oligomers obtained from the synthesizer were evaporated to remove excess ammonia and were purified by HPLC at 65 °C, using a Hamilton PRP-1 column (1 × 30 cm) and a gradient of 19–28% acetonitrile in aqueous 0.05 M triethylammonium hydrogen carbonate, pH 8.5, developed over 27 min with a flow rate of 3 mL/min. Tritylated oligomers were treated with 80% acetic acid, evaporated to dryness, dissolved in 0.01 M Tris-HCl, pH 7.4, extracted with ethyl acetate, and evaporated again. The resulting detritylated oligomers were chromatographed at 65 °C on the same column with a gradient starting at 7% (hexamers and octamers) or 9% (decamers and dodecamers) acetonitrile in 0.05 M triethylammonium acetate, pH 7.4, and increasing by 0.2%/min with a flow rate of 3 mL/min. Oligomers were dried in vacuo, dissolved in 0.01 M Tris-HCl, pH 7.4, and stored frozen at −20 °C until use. Oligomer concentrations were determined by absorbance at 260 nm using an estimated factor of 1 absorbance unit = 40 μg/mL for short oligomeric duplexes. Annealing of self-complementary oligomers to form duplexes was checked by electrophoresis at 4 °C in 20% polyacrylamide gels containing

0.5× Tris–borate–EDTA (Sambrook *et al.*, 1989) and 50 mM NaCl.

Reactions with (+)-(S,R,S,R)-BcPhDE-1. (+)-(*S,R,S,R*)-BcPhDE-1 was synthesized and purified as described previously (Yagi *et al.*, 1983). Reaction mixtures (0.5 mL final volume) contained 0.5 mg/mL DNA oligomer, 0.15 M NaCl, 0.01 M Tris-HCl, pH 7.4, 30 μg/mL diol epoxide, and 10% acetonitrile. Oligomer, NaCl, and Tris-HCl were preincubated at room temperature for 15 min to enable duplex formation before the diol epoxide in acetonitrile was added. In some experiments, oligomer solutions were preincubated by heating to about the T_m predicted by the *OLIGO* program (Rychlik, 1991) and slowly cooling to room temperature, but this made no difference in the adduct distribution. Gel electrophoresis also indicated that slow annealing was largely unnecessary. Reactions proceeded at room temperature for 36–48 h. Comparison reactions substituted 0.5 mg/mL calf thymus DNA for oligomer.

Determination of Adducts. Reaction mixtures were diluted to 2 mL with water. Extractions to remove noncovalently bound hydrolysis products of the diol epoxide, enzymatic digestions to nucleosides and nucleoside adducts using DNase I, SVPD, and APase, and recovery of nucleoside adducts with Sep-paks were performed as described previously (Cheh *et al.*, 1990), except SVPD was increased to 0.25 unit/ 0.25 mg of calf thymus DNA or oligomer to ensure adequate release of adducts. Adducts were dissolved in 0.25 mL of methanol followed by 0.25 mL of water and separated by HPLC at 1 mL/min on a 0.46 × 25 cm Thomson ODS-1 column (Thomson Instrument Co., Springfield, VA) or a Beckman Ultrasphere C-18 column, using a linear gradient running from 55% water, 3% tetrahydrofuran, and 42% methanol to 30% water, 3% tetrahydrofuran, and 67% methanol over 25 min. Relative amounts of *S* and *R* configured adducts were determined by peak areas measured at 255 nm.

It was found that an unidentified adduct produced in very small amounts in the reaction between (+)-(*S,R,S,R*)-BcPhDE-1 and DNA and appearing as a very small chromatographic peak between the cis ring opened (*R* configured) dG and trans ring opened (*S* configured) dA adducts (Dipple *et al.*, 1987) was produced in much larger quantities in the oligomer duplexes and was not resolved from the cis (*R* configured) dG adduct by the Thomson column. We previously observed that columns from different manufacturers and even different columns of the same type from the same manufacturer will show different selectivities with diol epoxide adducts (Cheh *et al.*, 1993). The unknown adduct has a spectrum similar to the other dG adducts, but different from the dA adducts. It could be an N-7 adduct (Osborne *et al.*, 1981; RamaKrishna *et al.*, 1992; Cheh *et al.*, 1993) or a dC adduct. For this study, to calculate the % adduct formation corresponding to dA adducts, the area counts for the unknown adduct were added to the total area counts for dG adducts. Cochromatography of the unknown adduct with the *R* configured dG adduct in the majority of experiments precluded analysis of the *S vs R* ratio for dG adducts, however.

RESULTS

Ratio of S vs R dA Adducts Formed by (+)-(S,R,S,R)-BcPhDE-1 in Dodecameric Oligodeoxyribonucleotide Duplexes. Table 1 shows the 24 self-complementary dodecamers, containing either A or T in base position #6 (counting from the 5′ end) and varying G and C sequences in base positions #1 through #5 (with self-complementarity the first six bases specify the remaining ones), that were employed as duplexes. Controls consisting of (+)-(*S,R,S,R*)-BcPhDE-1 reactions with calf thymus DNA were included in all experiments with oligomers. The ratio of *S vs R* configured dA adducts formed in 18 different control runs with calf thymus DNA ranged from 2.96 to 3.85; the average was 3.38 with a standard deviation of 0.27 and a coefficient of variation of about 8%. Oligomer sequences containing 5′-AT-3′ (odd-numbered oligomers in Table 1) all produced a higher *S vs R* dA adduct ratio than did the parallel calf thymus DNA reaction, while

Table 1: Configuration and Proportion of dA Adducts Formed by (+)-(*S,R,S,R*)-BcPhDE-1 in Duplex Dodecamers[a]

Oligomer Number	Sequence[b]	Ratio of *S vs R* Adducts	%dA Adducts[c]
	Calf Thymus DNA	3.38	84.5
1	CCCG CATG CGGG	4.45	61.7
2	CCCG CTAG CGGG	1.58	58.1
3	CCCG GATC CGGG	5.49	33.5
4	CCCG GTAC CGGG	2.08	53.8
5	CCGC CATG GCGG	3.88	54.5
6	CCGC CTAG GCGG	1.54	50.7
7	CCGC GATC GCGG	7.18	44.4
8	CCGC GTAC GCGG	2.75	52.1
9	CGCC CATG GGCG	4.13	51.3
10	CGCC CTAG GGCG	1.80	46.0
11	CGCC GATC GGCG	5.42	39.2
12	CGCC GTAC GGCG	2.36	53.6
13	GCCC CATG GGGC	3.90	59.8
14	GCCC CTAG GGGC	1.71	53.4
15	GCCC GATC GGGC	6.45	44.4
16	GCCC GTAC GGGC	2.45	62.1
17	GGCG CATG CGCC	4.61	59.1
18	GGCG CTAG CGCC	1.61	54.3
19	GGCG GATC CGCC	4.81	33.0
20	GGCG GTAC CGCC	2.17	49.1
21	GCGG CATG CCGC	3.75	53.1
22	GCGG CTAG CCGC	1.75	51.0
23	GCGG GATC CCGC	5.33	27.8
24	GCGG GTAC CCGC	2.95	42.6

[a] Averages of two or more determinations. [b] Results are obtained with self-complementary duplexes; for simplicity, a complementary strand is not shown. [c] Calculated by dividing the HPLC peak area associated with dA adducts by the total adduct area for dA + dG.

sequences containing 5′-TA-3′ and identical flanking G and C sequences (each next even-numbered oligomer in Table 1) all produced *S vs R* dA adduct ratios lower than the parallel calf thymus DNA reaction. The effect of reversing the positions of A and T is consistently large compared to the experimental error indicated by the variation in the calf thymus DNA ratio and can be seen in sequences that are C rich (oligomers 1–16) or G rich (oligomers 17–24) in the 5′ end.

The *S vs R* dA adduct ratio is further altered by whether base positions #5 and #8 in the dodecamer, adjacent to the target A and its neighboring T, contain G or C. Comparisons between the first *vs* the third or the second *vs* the fourth members of each set of four oligomers shown in Table 1 reveal that those sequences which have a C in position #5, and therefore G in position #8, consistently exhibit lower ratios of *S vs R* dA adducts than those containing a G in position #5 and a C in position #8, with the rest of the sequence held constant.

Ratio of S vs R dA Adducts Formed by (+)-(S,R,S,R)-BcPhDE-1 in Decameric, Octameric, and Hexameric Oligodeoxyribonucleotide Duplexes. Table 2 shows the shorter DNA oligomer sequences that were reacted as duplexes with (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1, the *S vs R* ratio of the resulting dA adducts, and the relative amount of dA *vs* dG adducts that were formed. In all cases, regardless of oligomer length or flanking sequence, a central 5′-AT-3′ is seen in Table 2 to yield a larger ratio of *S* configured *vs R* configured dA adducts than 5′-TA-3′, the same observation made with the dodecamers shown in Table 1. Octameric oligomers 33–36 and 37–40 demonstrate the effects of changing G for C in base position #3 and C for G in #6, adjacent to the central 5′-AT-3′ or 5′-TA-3′, while the flanking positions (#1, #2, #7, and #8) are held constant. As with the dodecamers, C in position #3 with G in position #6 lowers the ratio of *S vs R* dA adduct compared to the reverse sequence.

A number of comparisons may be made to show the effects of sh ·tening the oligomer sequence on the *S vs R* dA adduct ratio, e.g., oligomers 1 (Table 1), 25, 33, and 43 (Table 2) or 2 (Table 1), 26, 34, and 44 (Table 2). Generally, a small upward trend seems to result from shortening; however, the magnitude of change is no larger than experimental error. The total amount of adducts (dA + dG) formed in sequences 43 and 44 was much less than in the longer oligomers, so additional hexamers were not tested.

Ratio of S vs R dA Adducts Formed by (+)-(S,R,S,R)-BcPhDE-1 in Oligodeoxynucleotide Duplexes Where T Was Replaced by U. To test further the hypothesis that the methyl groups on nearest neighboring thymines steer dA adduct formation toward *S* or *R* configuration, several oligodeoxyribonucleotides were synthesized with deoxyuridine in place of thymidine. These sequences are listed in Table 3, where they carry the same oligomer number as the corresponding T containing one (Table 1), plus a U indicating replacement of T by U in the sequence. The ratio of *S vs R* dA adducts formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 in the U containing duplexes (Table 3) is always much lower than in the corresponding T containing ones (Table 1); in some cases the formation of *R* adducts is favored. In spite of this universal decrease in the ratio of *S vs R* dA adducts, the ratio still depends on the choice of nearest neighbors, in a pattern similar to that observed with the oligomeric duplexes containing T. The *S vs R* ratio is higher with 5′-AU-3′ compared to 5′-UA-3′ and with G in base position #5 compared to C in the same position. The resulting pattern for the *S vs R* ratio of 5′-GAUC-3′ > 5′-CAUG-3′ > 5′-GUAC-3′ > 5′-CUAG-3′ follows exactly the pattern observed with the analogous sequences containing T.

Table 2: Configuration and Proportion of dA Adducts Formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 in Decameric, Octameric, and Hexameric Oligodeoxyribonucleotide Duplexes[a]

Oligomer Number	Sequence[b]	Ratio of *S vs R* Adducts	%dA Adducts[c]
25	CCG CATG CGG	4.52	67.7
26	CCG CTAG CGG	1.62	63.1
27	GGC GATC GCC	6.88	56.3
28	GGC GTAC GCC	2.94	69.7
29	CGC CATG GCG	3.80	58.7
30	CGC CTAG GCG	1.63	55.6
31	GCG GATC CGC	4.63	40.2
32	GCG GTAC CGC	2.05	56.8
33	CG CATG CG	4.63	69.5
34	CG CTAG CG	1.62	63.5
35	CG GATC CG	5.59	52.4
36	CG GTAC CG	2.68	71.7
37	GC CATG GC	4.11	77.8
38	GC CTAG GC	1.88	74.9
39	GC GATC GC	6.81	68.9
40	GC GTAC GC	2.78	79.1
41	CC GATC GG	6.26	56.9
42	CC GTAC GG	3.62	75.2
43	G CATG C	4.90	70.5
44	G CTAG C	2.00	54.5

[a] Averages of two or more determinations. [b] Results are obtained with self-complementary duplexes; for simplicity, a complementary strand is not shown. [c] Calculated by dividing the HPLC peak area associated with dA adducts by the total adduct area for dA + dG.

Relative Amounts of dA vs dG Adducts Formed by (+)-(S,R,S,R)-BcPhDE-1 in Oligodeoxyribonucleotide Duplexes. Table 1 also shows for each dodecamer the percent of adducts that are dA adducts. The average shown for 18 calf thymus DNA samples is 84.5% dA adducts, with a standard deviation of 1.3%. Within each of the six sets of four oligomers containing identical flanking sequences in base positions #1 to #4 and #7 to #12 (Table 1), the central sequence 5′-GATC-3′ in base positions #5 through #8 always shows the least amount of dA adduct compared to dG adduct. The rank order for the three other central sequences in the sets of four varies. 5′-CATG-3′ generates the greatest or second greatest proportion of dA adduct compared to dG adduct; it ranks first particularly if there is a G in base position #4. 5′-CTAG-3′

Table 3: Configuration and Proportion of dA Adducts Formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 in Self-Complementary Dodecamers Containing U in Place of T[a]

Oligomer Number	Sequence[b]	Ratio of *S vs R* Adducts	%dA Adducts[c]
9U	CGCC CAUG GGCG	2.04	41.4
10U	CGCC CUAG GGCG	0.821	34.4
11U	CGCC GAUC GGCG	3.09	32.4
12U	CGCC GUAC GGCG	1.16	39.4
21U	GCGG CAUG CCGC	1.82	40.9
22U	GCGG CUAG CCGC	0.914	38.3
23U	GCGG GAUC CCGC	2.19	21.7
24U	GCGG GUAC CCGC	1.10	29.7

[a] Averages of two or more determinations. [b] Results are obtained with self-complementary duplexes; for simplicity, a complementary strand is not shown. [c] Calculated by dividing the HPLC peak area associated with dA adducts by the total adduct area for dA + dG.

ranks second if G is in base position #4, and third if C is in base position #4. 5′-GTAC-3′ ranks first, second, or third, lowest if G is in base position #4.

As with the longer duplexes, the pairwise comparisons of decamer and octamer sequences shown in Table 2 indicate that 5′-CATG-3′ produces a greater proportion of dA adduct than does 5′-CTAG-3′, while 5′-GTAC-3′ produces a greater proportion of dA adduct than does 5′-GATC-3′, regardless of the length or sequence of the flanking region. Four-way comparisons of octamers (33–36 and 37–40) show that, as with the dodecamers (Table 1), the central sequence 5′-GATC-3′ produces the lowest % amount of dA adduct, but unlike the dodecamers, 5′-CATG-3′ with an adjacent 5′ G ranks second, not first, in the % dA adduct formed.

The percentages of all adducts that are formed on dA are uniformly lower (about 10% or so) in the sequences containing U (Table 3) compared to the corresponding ones containing T (Table 1). As is the case with the corresponding sequences containing T, 5′-GAUC-3′ generates the lowest ratio of dA *vs* dG adduct, while the rank order of the other central four-base sequences containing U is similar but not identical to the rank order of the corresponding sequences containing T.

DISCUSSION

Nearest Neighbor Effect on the Ratio of S vs R dA Adducts Formed by (+)-(S,R,S,R)-BcPhDE-1. The first effect of sequence on the *S vs R* ratio of dA adducts to be examined involves reversal of the central A and T. With self-complementary sequences containing a single A flanked by one T, and every other base a G or C, the number of possible sequences of length $n = 2^{(n/2)}$. Although it should be noted that only 24 of 64 possible dodecameric sequences, 8 of 32 possible decameric sequences, 10 of 16 possible octameric sequences, and 2 of 8 possible hexameric sequences were tested, all of them yielded a higher ratio of *S vs R* dA adducts from the reactions with (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 if the T was on the 3′ side of the target A than if it was on the 5′ side; this effect is considerably larger than experimental error. Changing the location of a methyl group does not appear to be the reason for the effect of reversing the positions of A and T,

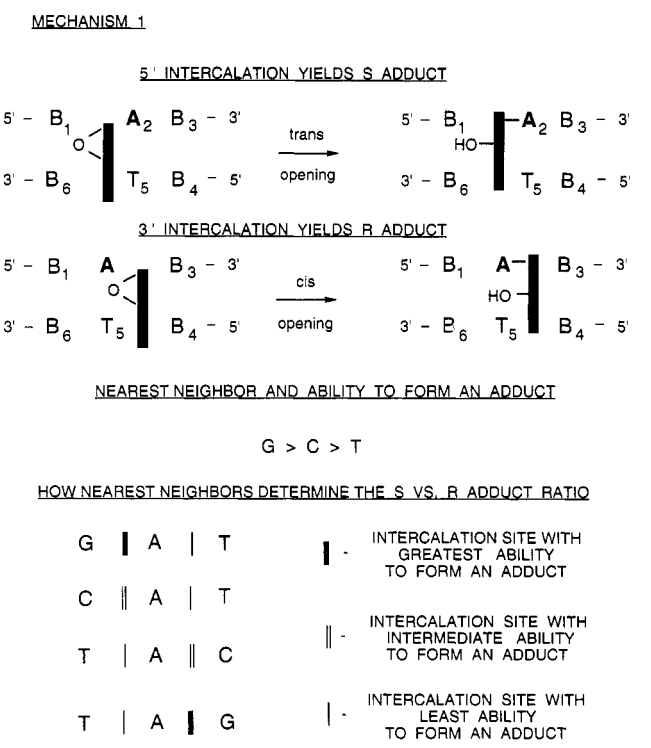

FIGURE 3: First of two mechanistic explanations of how nearest neighbors affect the *S vs R* ratio of dA adducts formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 in self-complementary duplexes. The top of the figure shows the sequence of the target strand reading $B_1A_2B_3$, 5′ to 3′, left to right, with the complementary strand reading $B_6T_5B_4$, 3′ to 5′, left to right; the target A is in bold. The diol epoxide is represented schematically by the heavy vertical line with an attached epoxide; it is shown intercalating between the target A·T pair and a neighboring base pair, with the epoxide oriented toward the 5′ end of the target strand. At the bottom, the nearest neighbor sequences are arranged in vertical order by the ratios of *S vs R* configured dA adducts produced in them; 5′-GAT-3′ generates the largest ratio, while 5′-TAG-3′ generates the smallest ratio.

because in the sequences where U replaced T, reversing the positions of A and U shows an analogous effect. Instead, loss of thymine methyl groups affects the *S vs R* dA adduct ratios by uniformly lowering them, an effect that presumably is caused by removal of the methyl group from the T that base-pairs with the target A, and not the T that is adjacent to the target A because it is independent of the position of the latter. The hypothesis that adjacent thymine methyl groups steer the incoming (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 by steric hindrance is not tenable, and an alternative explanation for the observed sequence effects must be sought.

The greatest effect of sequence on the ratio of *S vs R* dA adduct formation is that exerted by nearest neighbors to the target A. The observed rank order of *S vs R* dA adduct formation as a function of nearest neighbors is 5′-GAT-3′ > 5′-CAT-3′ > 5′-TAC-3′ > 5′-TAG-3′; this rank order holds regardless of whether the 5′ flanking region is G rich or C rich, or short or long. Geacintov (Geacintov, 1986; Margulis *et al.*, 1993) has discussed mechanisms whereby, in 0.15 M NaCl, intercalation of diol epoxides could be an important precursor to covalent bonding. Two illustrated mechanisms (Figures 3 and 4) are proposed to explain the rank order of nearest neighbor sequences for *S vs R* dA adduct formation by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1. In both mechanisms it is assumed that intercalation precedes covalent bonding. It is also assumed that intercalation leading to covalent bond formation occurs with the epoxide facing a *specific* end of the strand containing the target A, while the reverse orientation,

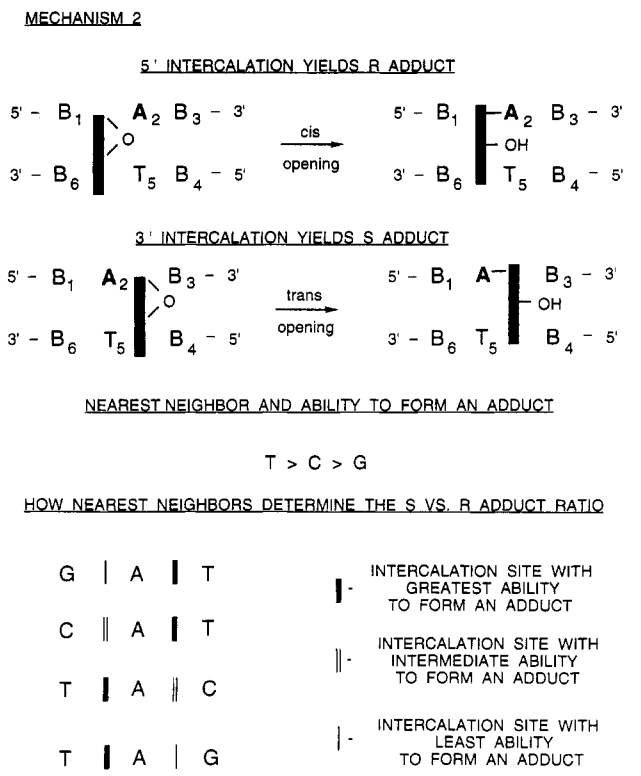

FIGURE 4: Second of two mechanistic explanations of how nearest neighbors affect the *S vs R* ratio of dA adducts formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 in self-complementary duplexes. The top of the figure shows the sequence of the target strand reading $B_1A_2B_3$, 5′ to 3′, left to right, with the complementary strand reading $B_6T_5B_4$, 3′ to 5′ left to right; the target A is in bold. The diol epoxide is represented schematically by the heavy vertical line with an attached epoxide; it is shown intercalating between the target A·T pair and a neighboring base pair, with the epoxide oriented toward the 3′ end of the target strand. At the bottom, the nearest neighbor sequences are arranged in vertical order by the ratios of *S vs R* configured dA adducts produced in them; 5′-GAT-3′ generates the largest ratio, while 5′-TAG-3′ generates the smallest ratio.

where the hydrocarbon has flipped over to face the opposite end of the target strand, is less favorable for adduct formation. In such a model, intercalation on opposite sides of the target A preferentially presents opposite faces of the hydrocarbon to the A, resulting in opposite (trans *vs* cis) opening of the epoxide and formation of adducts of opposite (*S vs R*) absolute configurations. Both mechanisms show how (a) intercalating on a specific side of the target A leads to an adduct of a specific absolute configuration, coupled with (b) the degree of adduct formation depends systematically on the identity of the nearest neighbor, giving rise to the observed dependence of the *S vs R* adduct ratio on the nearest neighboring sequence around the target A. Finally, in the mechanisms the proper alignment of diol epoxide and the N^6 of the target A is the key to adduct formation, rather than just the strength of noncovalent, intercalative binding with the nearest neighboring base pair.

In mechanism 1 (Figure 3), intercalation has the epoxide of (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 face the 5′ end of the target strand. Then, intercalation on the 5′ side of the target A produces an *S* adduct by trans epoxide opening while intercalation to the 3′ side produces an *R* adduct by cis epoxide opening. The ability to form covalent adducts on the target A after intercalation is greatest if the nearest neighbor to the A is G, less if it is C, and least if it is T (it should be noted that intercalation involves base *pairs*, so a nearest neighboring T actually means a T·A *pair* neighboring the target A·T). The mechanism explains the observation that reversing A and T while keeping the rest of the sequence the same has a greater effect than reversing the G and C in the positions adjacent to the target A and T. Reversing G and C changes 5′-CATG-3′ (the nearest neighbors to the target A are underlined) to 5′-GATC-3′ or vice versa, or 5′-CTAG-3′ to 5′-GTAC-3′ or vice versa. This reversal changes *one* of the two nearest neighbors, which alters the *S vs R* ratio of dA adducts. On the other hand, reversing A and T changes 5′-CATG-3′ to 5′-CTAG-3′ or vice versa, or 5′-GATC-3′ to 5′-GTAC-3′ or vice versa. This changes *both* nearest neighbors, with *additive* effects on the *S vs R* dA adduct ratio.

Mechanism 2 (Figure 4) is the inverse of mechanism 1. Intercalation has the epoxide face the opposite (3′) end of the target strand, while the ability of the diol epoxide to bond covalently to the target A follows the opposite nearest neighbor rank order of T > C > G. The result of reversing both the rank order and the configuration of the adduct formed after intercalation on a given side of the target A is to produce the same relationship between sequence and the ratio of *S vs R* adducts as mechanism 1.

Next to be considered is what evidence might support one mechanism over the other. Cosman *et al.* (1993b) determined the structure of a *trans*-(+)-(*S*,*R*,*R*,*S*)-BcPhDE-2 dA adduct with *R* configuration as it lies in DNA [the majority of dA adducts formed in calf thymus DNA by this BcPhDE isomer have *R* rather than *S* configuration (Dipple *et al.*, 1987)]. They report that the adduct lies intercalated to the 5′ side of the adducted A in DNA. Although the BcPhDE isomer and the DNA sequence used by Cosman *et al.* are different from the ones employed in this study, if it is correct to assume that the intercalation site for the adduct reflects the intercalation site of the reacting diol epoxide, then mechanism 2 predicts that diol epoxide intercalation to the 5′ side of the adducted A will produce the observed *R* configured adduct. The evidence currently available favors (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 adduct formation at A following mechanism 2 over mechanism 1.

Mechanisms 1 and 2 as written imply that the *S vs R* dA adduct ratio would be greater than 1 if the sequence were 5′-GAT-3′ or 5′-CAT-3′, and less than 1 if it were 5′-TAC-3′ or 5′-TAG-3′. Clearly superimposed upon the sequence effect is an overall bias that (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 has toward the formation of *S* configured dA adducts, such that the average for all the sequences tested is around 3.6; this bias is what is uniformly reduced for all sequences when U replaces T. It is not known what causes this uniform reduction or whether it is at all related to the intercalation schemes of mechanisms 1 and 2.

The sites of strongest intercalation are not necessarily the best ones for adduct formation. On the basis of the extent of red shift with poly(dA-dT)·poly(dA-dT) versus poly(dG-dC)·poly(dG-dC), racemic (±)-BPDE-2 intercalates best with alternating dA·dT and dT·dA pairs (Geacintov *et al.*, 1988), but forms adducts largely at G. The benzylic epoxide carbon of the diol epoxide and the exocyclic purine amino group target must be properly aligned for covalent bonding to occur (Harvey & Geacintov, 1988). Therefore, in both mechanisms 1 and 2, nearest neighbors are discussed in terms of the ability of a diol epoxide intercalated with a particular nearest neighbor to form adducts rather than the strength of the intercalative association with that nearest neighbor. The structure of the *trans*-(+)-(*S*,*R*,*R*,*S*)-BcPhDE-2 dA adduct reported by Cosman *et al.* (1993b) shows the intercalated hydrocarbon moiety overlapping mainly with the neighboring G on the *opposite*

strand. This emphasizes the importance of remembering that the discussion of nearest neighboring bases actually means nearest neighboring *base pairs*.

Whether a diol epoxide alignment is appropriate for adduct formation presumably depends on its intercalative stacking with the target A·T and neighboring base pairs, subject to possible steric or hydrogen bonding constraints applied by the highly ordered DNA structure and the specifically configured diol. In this regard, it should be noted that both (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 and (–)-(*R*,*S*,*R*,*S*)-BcPhDE-1 are unusual among DE-1 species in adopting a pseudodiequatorial conformation for their hydroxyl groups; diol epoxide carcinogenicity is associated with the adoption of a pseudodiequatorial as opposed to pseudodiaxial conformation (Sayer *et al.*, 1981; Chang *et al.*, 1987). Also, in order for adduct formation to occur preferentially when the epoxide faces a specific end of the target strand, as is required by the mechanisms, we propose that flipping the hydrocarbon in the intercalation site (making the epoxide face the other end of the target strand) causes an interaction with either the phosphodiester backbone or the overhang of base pairs (the latter caused by the helical twist of the DNA) such that the epoxide cannot align as well for covalent bonding. Then, the same preferred orientation of the epoxide is likely to hold for both the intercalation site 5′ to the target A and the one that is 3′. This latter requirement distinguishes the proposed mechanisms 1 and 2 from an alternative model that would tie the direction the epoxide faces to the identity of the nearest neighbor.

Some evidence may be cited to support this interpretation. In DNA (Dipple *et al.*, 1987) and in duplex oligodeoxyribonucleotides (Cheh, unpublished experiments), (–)-(*R*,*S*,*R*,*S*)-BcPhDE-1 reactions at A are skewed toward cis ring opening (*S* adduct formation), compared to the trans ring opening preferred by its enantiomer, (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1, as described in the present work. Thus, the hydrocarbon moiety of (–)-(*R*,*S*,*R*,*S*)-BcPhDE-1 must be intercalating the same way as the hydrocarbon moiety of (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1, except with the epoxide on the opposite side, cis ring opening is now favored instead of the trans ring opening. This suggests that, for optimum adduct formation, some manner of steric constraint does favor one particular hydrocarbon intercalation geometry over the one where the hydrocarbon has flipped over.

It should be noted that the observation of a particular *adduct* geometry (intercalated or groove-bound) neither proves nor disproves the intermediacy of noncovalent intercalated species. Some diol epoxide adducts are found to be intercalated in DNA [a *cis*-dG adduct of (+)-(*R*,*S*,*S*,*R*)-BPDE (Cosman *et al.*, 1993a), a *trans*-dA adduct of (–)-(*S*,*R*,*R*,*S*)-BPDE (Schurter *et al.*, 1994), and a *trans*-dA adduct of (+)-(*S*,*R*,*R*,*S*)-BcPhDE-2 (Cosman *et al.*, 1993b)]; this does not prove that, with these diol epoxide isomers [or with (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1], intercalation precedes covalent bonding. *trans*-dG adducts from (+)-(*R*,*S*,*S*,*R*)-BPDE-2 (Cosman *et al.*, 1992) and (–)-(*S*,*R*,*R*,*S*)-BPDE-2 (de los Santos *et al.*, 1992) are found in the DNA minor groove rather than intercalated between base pairs; this does not disprove a mechanism in which intercalation of the diol epoxide precedes covalent bonding.

Effects of the Sequence beyond Nearest Neighbors. Margulis *et al.* (1993) have noted that long range sequence effects may play a role in diol epoxide bonding to DNA. With the self-complementary oligomers employed in this study, changing the sequence at positions beyond the nearest neighbors to the target A appears at first glance to have a systematic effect.

Table 4: Theoretical Proportion of Adducts Formed by (+)-(*S*,*R*,*S*,*R*)-BcPhDE-1 on dA in Oligomeric Duplexes of Different Lengths[a]

oligomer length	% of adducts on dA	% of dA that forms adducts	% of doubly adducted duplexes
12-mer	53	27	7.4
10-mer	59	25	6.3
8-mer	65	22	5.0
6-mer	74	19	3.6

[a] Based on reactions containing 0.25 mg of oligomer (nucleotide MW = 331) and 0.015 mg of diol epoxide (MW = 278), in which it is assumed that 60% of the diol epoxide forms adducts, 85% of adducts are formed on dA (and the rest on dG) in a sequence having a GC content of 50%, and the extent of reaction on a given purine is independent of its position in the sequence.

Comparisons of the *S vs R* dA adduct ratios in Table 1 for the oligomers numbered *n*, *n* + 4, *n* + 8, and *n* + 12, where *n* = 1, 2, 3, or 4, suggest that when positions #5 to #8 are held constant and a single G to the 5′ side of the central four-base sequence is shifted from base position #4 to #3 to #2 to #1 (with a corresponding shift of a single C in the 3′ flanking region), there is a zigzag pattern of small changes in *S vs R* dA adduct ratio, with the shape of the zigzag depending on whether G or C occupies base position #5. However, unlike the impact resulting from switching nearest neighbors, the observed impact of any long distance changes in sequence is really of the same magnitude as experimental error; therefore, no conclusions about long distance effects may be drawn from these experiments. As was noted above, shortening the sequence by eliminating nucleotides at both ends may cause a small increase in the ratio of *S vs R* dA adducts, but the apparent effect is also no greater than experimental error.

The Effect of Sequence on the Relative Proportions of dG and dA Adducts Produced. DNA sequence also plays a role in setting the balance between dA *vs* dG adduct formation (e.g., the four-base central sequence 5′-GATC-3′ consistently produces the lowest % dA adduct regardless of the flanking sequence). However, the mechanisms underlying these observations are unknown, particularly because there is no consistent rank order for the other three four-base central sequences.

In the duplexes formed from self-complementary oligomers, two target dA sites exist, one on each strand, so it is possible that double adducts could be formed in a duplex, with the first adduct either assisting or sterically hindering the formation of a second, thereby affecting the % dA adduct formed or the *S vs R* configuration of the second adduct. The % dA adduct formed would also be altered if target Gs at the ends of the sequence exhibited heightened or reduced reactivity. To minimize the effect that an adduct on one of the two As might have on adduct formation at the other A, the ratio of diol epoxide to DNA or oligodeoxynucleotide was reduced in this study from the previous 0.1 mg of diol epoxide/mg of DNA to 60% of that.

An analysis may be done to see if this was successful. Table 4 shows, as a function of oligomer length, calculations of the statistically expected amounts of dA *vs* dG adducts, and from this, the extents of adduct formation on the available dA targets and the extents of double adduct formation in the duplexes. The amount of double adduct formation theoretically would not have exceeded about 4–8% unless the first adduct aided the formation of a second adduct, or the Gs at the ends of the duplexes had unusually low reactivities.

The majority of dodecamers tested produced less than the theoretical proportion of dA adducts listed in Table 4 (the

average observed % dA was 50%). However, only 24 of 64 possible self-complementary dodecamer sequences were tested; it is possible that those which were not tested might have produced much greater proportions of dA adducts. (±)-BPDE-2 tends to form G adducts in GC rich regions of duplex DNA (Boles & Hogan, 1986; Osborne, 1990; Dittrich & Krugh, 1991a,b; Schwartz *et al.* 1994). If this were true for (+)-(*S,R,S,R*)-BcPhDE-1, it could explain a production of less than the theoretical % of dA adducts. In the dodecamers under study, the flanking regions are solely composed of G and C, with runs of G common. A study that examined the sites in DNA where (+)-(*S,R,S,R*)-BcPhDE-1 formed dG adducts (Ross *et al.*, 1993), however, is inconclusive in this regard. The decamers and octamers, whose average % dA were 58% and 68%, respectively, generated % dA adduct proportions that were higher compared to the theoretical amount (Table 4), bearing in mind that only 8 of 32 possible decamers and 10 of 16 possible octamers were tested. These sequences do not have as long or as many runs of G as do the dodecamers. In any event, a similarity between predicted and observed % dA adducts formed in the sequences under study suggests there are no special effects stemming from having two adduct sites in the duplex or unusual reactivities of the Gs at the ends of the duplex.

Significance of Sequence Dependent Changes in the Ratio of S vs R dA Adducts. A linkage between DNA adduct formation and carcinogenesis is assumed, but because of the multiplicity of adducts that may be formed, enormous complications may be encountered when PAH diol epoxides are used to investigate this linkage. Diol epoxide adducts may arise by cis or trans opening of the epoxide ring, by reaction at different sites on a DNA base, such as at the exocyclic amino group or N-7 of a purine, and by reaction with either G, C, or A. On top of this, the types of adducts that are formed may be influenced by the DNA sequence surrounding each target base.

Sequence specificity in adduct formation is an important determinant of carcinogenicity, since given sites within a sequence could be more or less effective in activating oncogenes or inactivating tumor suppressor genes. The ability to form certain types of adducts, perhaps with specific orientations in the DNA helix, and consequent differential biological processing of the damage could also be associated with diol epoxide carcinogenicity.

It is possible to determine the relative amounts of different types of adducts formed in bulk in a DNA, by performing a digestion and quantitation of adduct peak areas in an HPLC chromatographic profile. In doing so, the sequence context of adduct formation is lost. Conversely, various methods such as polymerase or nuclease arrest (Ross *et al.*, 1993) or uvrABC incision (Tang *et al.*, 1992) may be used to position diol epoxide adducts and show how the amount of adduct that is formed varies with sequence, but without necessarily knowing what kinds of adducts are formed at each site. The results of this study indicate that DNA sequence and adduct type can be closely linked and need to be studied simultaneously.

ACKNOWLEDGMENT

We thank Dr. Ryszard Jankowiak for providing his conditions for determining single-*vs* double-stranded oligomers by electrophoresis and Dr. Jane Sayer for helpful discussions.

REFERENCES

Agarwal, S. K., Sayer, J. M., Yeh, H. J. C., Pannell, L. K., Hilton, B. D., Pigott, M. A., Dipple, A., Yagi, H., & Jerina, D. M. (1987) *J. Am. Chem. Soc. 109*, 2497–2504.

Applied Biosystems (1987) User Bulletin for DNA Synthesizer Models 380/381, Issue 13—Revised.

Boles, T. C., & Hogan, M. E. (1986) *Biochemistry 25*, 3039–3043.

Chang, R. L., Wood, A. W., Conney, A. H., Yagi, H., Sayer, J. M., Thakker, D. R., Jerina, D. M., & Levin, W. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8633–8636.

Cheh, A. M., Yagi, H., & Jerina, D. M. (1990) *Chem. Res. Toxicol. 3*, 545–550.

Cheh, A. M., Chadha, A., Sayer, J. M., Yeh, H. J. C., Yagi, H., Pannell, L. K., & Jerina, D. M. (1993) *J. Org. Chem. 58*, 4013–4022.

Cosman, M., de los Santos, C., Fiala, R., Hingerty, B. E., Singh, S. B., Ibanez, V., Margulis, L. A., Live, D., Geacintov, N. E., Broyde, S., & Patel, D. J. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1914–1918.

Cosman, M., de los Santos, C., Fiala, R., Hingerty, B. E., Ibanez, V., Luna, E., Harvey, R., Geacintov, N. E., Broyde, S., & Patel, D. J. (1993a) *Biochemistry 32*, 4145–4155.

Cosman, M., Fiala, R., Hingerty, B. E., Laryea A., Lee, H., Harvey, R. G., Amin, S., Geacintov, N. E., Broyde, S., & Patel, D. (1993b) *Biochemistry 32*, 12488–12497.

de los Santos, C., Cosman, M., Hingerty, B. E., Ibanez, V., Margulis, L. A., Geacintov, N. E., Broyde, S., & Patel, D. J. (1992) *Biochemistry 31*, 5245–5252.

Dipple, A., Moschel, R., & Bigger, C. A. H. (1984) in *Chemical Carcinogens, Second Edition, Volume 2* (Searle, C. E., Ed.) ACS Monograph 182, pp 41–163, American Chemical Society, Washington, DC.

Dipple, A., Pigott, M. A., Agarwal, S. K., Yagi, H., Sayer, J. M., & Jerina, D. M. (1987) *Nature 327*, 535–536.

Dittrich, K. A., & Krugh, T. R. (1991a) *Chem. Res. Toxicol. 4*, 270–276.

Dittrich, K. A., & Krugh, T. R. (1991b) *Chem. Res. Toxicol. 4*, 277–281.

Geacintov, N. E. (1986) *Carcinogenesis 7*, 759–766.

Geacintov, N. E., Shahbaz, M., Ibanez, V., Moussaoui, K., & Harvey, R. G. (1988) *Biochemistry 27*, 8380–8387.

Harvey, R. G., & Geacintov, N. E. (1988) *Acc. Chem. Res. 21*, 66–73.

Jerina, D. M., & Lehr, R. E. (1977) in *Microsomes and Drug Oxidations: 3rd International Symposium* (Ullrich, V., Roots, I., Hildebrandt, A. G., Estabrook, R. W., & Conney, A. H., Eds.) pp 709–723, Pergamon Press, Oxford, England.

Jerina, D. M., Lehr, R. E., Yagi, H., Hernandez, O., Dansette, P. M., Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., & Conney, A. H. (1976) in *In Vitro Metabolic Activation In Mutagenesis Testing* (de Serres, F. J., Fouts, J. R., Bend, J. R., & Philpot, R. M., Eds.) pp 159–177, Elsevier/North-Holland Biomedical Press, Amsterdam.

Jerina, D. M., Yagi, H., Thakker, D. R., Sayer, J. M., van Bladeren, P. J., Lehr, R. E., Whalen, D. L., Levin, W., Chang, R. L., Wood, A. W., & Conney, A. H. (1984) in *Foreign Compound Metabolism* (Caldwell, J., & Paulson, G. D., Eds.) pp 257–266, Taylor and Francis Ltd., London.

Jerina, D. M., Sayer, J. M., Agarwal, S. K., Yagi, H., Levin, W., Wood, A. W., Conney, A. H., Preuss-Schwartz, D., Baird, W. M., Pigott, M. A., & Dipple, A. (1986) in *Biological Reactive Intermediates III* (Kocsis, J. J., Jollow, D. J., Witmer, C. M., Nelson, J. O., & Snyder, R., Eds.) pp 11–30, Plenum Press, New York.

Jerina, D. M., Chadha, A., Cheh, A. M., Schurdak, M. E., Wood, A. W., & Sayer, J. M. (1991) in *Biological Reactive Intermediates IV* (Witmer, C. M., Snyder, R., Jollow, D. J., Kalf, G. F., Kocsis, J. J., & Sipes, I. G., Eds.) pp 533–553, Plenum Press, New York.

Kootstra, A., Lew, L. K., Nairn, R. S., & MacLeod, M. C. (1989) *Mol. Carcinog. 1*, 239–244.

Levin, W., Chang, R. L., Wood, A. W. Thakker, D. R., Yagi, H., Jerina, D. M., & Conney, A. H. (1986) *Cancer Res. 46*, 2257–2261.

Mao, B., Li, B., Amin, S., Cosman, M., & Geacintov, N. E. (1993) *Biochemistry 32*, 11785–11793.
Margulis, L. A., Ibanez, V., & Geacintov, N. E. (1993) *Chem. Res. Toxicol. 6*, 59–63.
Miller, E. C., & Miller J. A. (1967) *Proc. Soc. Exp. Biol. and Med. 124*, 915.
Osborne, M. (1990) *Chem.-Biol. Interact. 75*, 131–140.
Osborne, M. R., Jacobs, S., Harvey, R. G., & Brookes, P. (1981) *Carcinogenesis 2*, 553–558.
RamaKrishna, N. V. S., Gao, F., Padmavathi, N. S., Cavalieri, E. L., Rogan, E. G., Cerny, R. L., & Gross, M. L. (1992) *Chem. Res. Toxicol. 5*, 293–302.
Reardon, D. B., Bigger, C. A. H., Strandberg, J., Yagi, H., Jerina, D. M., & Dipple, A. M. (1989) *Chem. Res. Toxicol. 2*, 12–14.
Rill, R. L., & Marsch, G. A. (1990) *Biochemistry 29*, 6050–6058.
Ross, H., Bigger, C. A. H., Yagi, H., Jerina, D. M., & Dipple, A. (1993) *Cancer Res. 53*, 1273–1277.
Rychlik, W. (copyright) (1991) *OLIGO*, distributed by National Biosciences, Plymouth MN.
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., p 6.7, Cold Spring Harbor Laboratory Press, Plainview, NY.
Sayer, J. M., Yagi, H., Croisy-Delcy, M., & Jerina, D. M. (1981) *J. Am. Chem. Soc. 103*, 4970–4972.
Schurter, E. J., Yeh, H. J. C., Sayer, J. M., Lakshman, M. K., Yagi, H., Jerina, D. M., & Gorenstein, D. G. (1994) *Biochemistry* (in press).
Schwartz, J. J, Lau, H. H. S., & Baird, W. M. (1994) *Chem. Res. Toxicol. 7*, 29–40.
Tang, M. S., Pierce, J. R., Doisy, R. P., Nazimiec, M. E., & MacLeod, M. C. (1992) *Biochemistry 31*, 8429–8436.
Thakker, D. R., Levin, W., Wood, A. W., Conney, A. H., Yagi, H., & Jerina, D. M. (1988) in *Drug Stereochemistry—Analytical Methods and Pharmacology* (Wainer, I. W., & Drayer, D. E., Eds.) pp 271–296, Marcel Dekker, New York.
Yagi, H., Thakker, D., Ittah, Y., Croisey-Delcy, M., & Jerina, D. M. (1983) *Tetrahedron Lett. 24*, 1349–1352.