Fidelity of Translesional Synthesis past Benzo[*a*]pyrene Diol Epoxide−2′-Deoxyguanosine DNA Adducts: Marked Effects of Host Cell, Sequence Context, and Chirality[†]

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ABSTRACT: We have used a site-specific approach to investigate the mutagenic potential of (+)- and (−)-*trans-anti*-benzo[*a*]pyrene diol epoxide (BPDE) DNA adducts. Oligodeoxyribonucleotides (5′TCCTCCTG$_1$G$_2$-CCTCTC), modified at the exocyclic amino groups of G_1 or G_2, were incorporated into a single-stranded shuttle vector and introduced into *Escherichia coli* or simian kidney (COS) cells. This experimental system permits translesional synthesis to proceed in the absence of DNA repair. The presence of (+)- or (−)-BPDE-N^2-dG adducts strongly inhibited translesional synthesis in *E. coli;* induction of cellular SOS functions reduced this blocking effect. Vectors containing (+)-BPDE adducts at G_1 or G_2 generated mutation frequencies of 19% and 3%, respectively; these values were not altered significantly by induction of SOS functions. In COS cells, (+)-BPDE-modified vectors generated mutation frequencies of 13% at G_1 and 45% at G_2. In *E. coli*, the (−)-BPDE adduct generated mutation frequencies of ≤2% at G_1 and G_2 and, in COS cells, 13% at G_1 and 21% at G_2. The predominant mutations in *E. coli* and COS cells were G→T transversions targeted to the site of the lesion; however, when G_2 was modified, a significant number of targeted G→A and G→C mutations were observed in COS cells. We conclude from this study that (+)- and (−)-BPDE-N^2-dG adducts pair preferentially to dCMP and dAMP during translesional synthesis in a process that is strongly influenced by the stereochemistry of the adduct, by the bases flanking the lesion, and by host cell factors.

Benzo[*a*]pyrene belongs to an ubiquitous class of environmental mutagens known as polycyclic aromatic hydrocarbons. Although characterized intrinsically by low chemical reactivity, benzo[*a*]pyrene is metabolized by cellular monooxygenases to highly reactive epoxides and diol epoxides (for reviews, see: Conney, 1982; Harvey, 1991). The biologically active metabolite, 7*r*,8*t*-dihydroxy-9,10*t*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [(±)-*anti*-BPDE],[1] reacts predominantly with guanine residues in native DNA to form covalent adducts (Cheng *et al*., 1989; Meehan & Straub, 1979). Unless these lesions are repaired prior to replication, translesional DNA synthesis may result in mutations.

The effects of stereochemistry on the tumorigenic (Buening *et al*., 1978; Slaga *et al*., 1979) and mutagenic (Wood *et al*., 1977; Stevens *et al*., 1985; Brooks & Osborne, 1982; Rodriguez & Loechler, 1993; Wei *et al.*, 1994) activities of the *anti*-BPDE stereoisomers are striking (Conney, 1982) and ultimately may provide clues to the molecular mechanism(s) of BPDE mutagenesis. Site-specifically placed lesions derived from the covalent binding of (+)- and (−)-*anti*-BPDE enantiomers to guanine residues in the sequence context of codons 60 and 61 of the human c-Ha-*ras1* protooncogene have been described by Liu *et al*. (1996). The availability of positionally and stereochemically defined lesions incorporated into oligonucleotides prompted us to compare their mutagenic potentials in bacterial and mammalian cell model systems, using a site-specific mutagenesis approach (Singer & Essigmann, 1991). This strategy, using a single-stranded vector, minimizes the contribution of DNA repair. The mutations observed can be traced directly to adducts of known stereochemistry located at defined positions in DNA. Our findings in this study suggest that the consequences of a stereochemically defined lesion depend not only on the sequence context in which the adduct is embedded, but also on the nature of the cell in which mutagenicity is expressed.

The mutagenic specificity of racemic *anti*-BPDE has been examined in bacterial (Bernelot-Moens *et al*., 1990; Eisenstadt *et al*., 1982), rodent, and human cells (Brookes & Osborne, 1982; Carothers & Grunberger, 1990; Keohavong & Thilly, 1992; Yang *et al*., 1987). The predominant mutations observed are G→T transversions. A few studies have been conducted with the optically active enantiomer (+)-*anti*-BPDE, with 7*R*,8*S*,9*S*,10*R* stereochemistry (Rodriguez & Loechler, 1993; Wei *et al*., 1991), and with (−)-*anti*-BPDE, with the 7*S*,8*R*,9*R*,10*S* configuration (Wei *et al*., 1994).

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[1] Abbreviations: BPDE, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; **G**$^+$, (+)-*trans-anti*-BPDE-N^2-dG; **G**$^-$, (−)-*trans-anti*-BPDE-N^2-dG; ss, single stranded; ϵdC, 3,N^4-etheno-2′-deoxycytidine.

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The binding of (+)-*anti*-BPDE to cellular DNA is approximately 6 times greater than that of (−)-*anti*-BPDE (Brookes & Osborne, 1982). Both enantiomers react predominantly with the exocyclic amino group of purines in DNA, forming *trans-anti*- and *cis-anti*-BPDE-N^2-dG and -N^6-dA adducts (Cheng *et al.*, 1989; Meehan & Straub, 1979). Due to the multiplicity of adducts formed, it has been difficult to correlate individual lesions with specific mutational events. However, in *Escherichia coli*, site-specific mutagenesis studies by Mackay *et al*. (1992) and Jelinsky *et al*. (1995) on (+)-*trans-anti*-BPDE-N^2-dG adducts, and by Chary *et al*. (1995) on six different stereoisomeric BPDE-N^6-dA adducts, have shown the feasibility of this approach. Precise definition of relationships between mutagenic specificity and DNA adduct structure is important, especially for molecular epidemiological investigations in which the pattern of mutations observed in human tumor suppressor genes is correlated with mutagenic specificity of environmental mutagens (Harris, 1991).

One of us has developed a shuttle vector system in which a single DNA adduct can be incorporated at a defined position in single-stranded (ss) DNA (Moriya, 1993). The ss nature of the construct minimizes DNA repair and permits quantitative analysis of translesional events for various DNA adducts. The modified vector is introduced into *E. coli* or simian kidney (COS) cells, and has been used to quantify mutation frequencies of a variety of chemically-defined lesions (Moriya, 1993; Moriya *et al.*, 1994; Pandya & Moriya, 1996). Following insertion of a mutagenic or nonmutagenic base opposite the lesion, the complementary strand may be extended. This template is utilized in subsequent rounds of DNA synthesis. Since all molecules contain the same DNA adduct positioned at the same site, the frequency of bases incorporated opposite the adduct during translesional synthesis can be calculated. Chain extension on a misaligned template may produce deletions in the nascent strand (Kunkel, 1990; Shibutani & Grollman, 1993). If the adduct blocks DNA synthesis, failure to extend the nascent chain is manifested as a killing event.

In this study, a site-specific approach was used to establish the specificity and frequency of mutations induced by (+)-*trans-anti*- and (−)-*trans-anti*-BPDE-N^2-dG adducts, positioned in the sequence context of codons 60 and 61 of the human c-Ha-*ras1* protooncogene. We find that the stereochemistry of the *trans-anti*-BPDE-N^2-dG adducts, the nucleotide sequence context in which these lesions are positioned, and the type of host cell strongly influence translesional synthesis past BPDE-N^2-dG, thereby altering the relative frequency of bases incorporated opposite the adduct.

MATERIALS AND METHODS

Modified Oligodeoxyribonucleotides. Racemic *anti*-BPDE was synthesized as previously described (Yagi *et al*., 1977). (+)- and (−)-*anti*-BPDE enantiomers were prepared from racemic *anti*-BPDE, using chiral stationary phase HPLC (Pirkle column) techniques (Weems & Young, 1989). The oligodeoxyribonucleotide d(TCCTCCTG$_1$G$_2$CCTCTC) was modified post-synthetically, introducing (+)- or (−)-*anti*-BPDE at G_1 or G_2 (Cosman *et al*., 1990; Shibutani *et al*., 1993). Products containing *trans*-adducts were isolated by three successive HPLC cycles using different elution protocols, as described elsewhere (Liu *et al*., 1996). Minor amounts of modified oligodeoxyribonucleotides containing other adducts, including *cis* adducts, were also observed, but were not isolated or studied further. Further HPLC separation cycles of the major adducts produced single-peak elution profiles. Each of the adducts was further purified by electrophoresis on denaturing 20% polyacrylamide gels, yielding single bands.

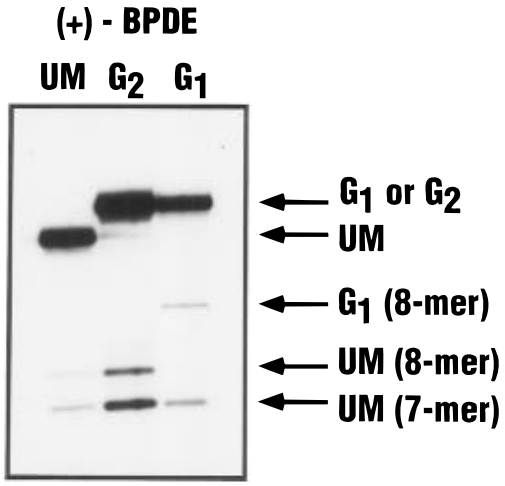

FIGURE 1: Analysis of unmodified (UM) and two (+)-*anti*-BPDE-modified 15-mer oligonucleotides (G_1 and G_2) by the G-cleavage reaction of Maxam−Gilbert sequencing method. The bands are identified as follows (from top to bottom): G_1 or G_2, full 15-mers with BPDE residues either at G_1 or G_2; G_1 (8-mer), cleavage fragment with 8 bases and with a BPDE residue at G_1; UM (8-mer); and UM (7-mer). The latter fragments are 8 or 7 bases long with no BPDE residues. In lane G_2, the cleavage pattern is identical to that in lane UM; thus, the (+)-*anti*-BPDE-N^2-dG lesion is at G_2. In lane G_1, the mobility of one of the fragments is retarded; thus, the lesion is at G_1 (see Mao *et al.*, 1992 for details). The two (−)-*anti*-BPDE-modified oligonucleotides gave analogous cleavage patterns (data not shown).

Adduct sites were identified by modified Maxam−Gilbert sequencing techniques, focusing on the guanine (G)-cleavage reaction (Liu *et al.*, 1996) (Figure 1). In lane UM, products of G-cleavage give rise to three different bands on a polyacrylamide gel; the upper band represents the uncleaved 15-mer, while the two lower bands are shorter oligonucleotide fragments cleaved at one of the guanine residues. In lane G_2, the oligonucleotide bears the BPDE residue on the guanine residue closer to the 3′-end (adduct G_2^+) since the mobility pattern is identical to that in lane UM of the Maxam−Gilbert G-cleavage reaction (G-cleavage destroys modified and unmodified guanine residues). In lane G_1 (from adduct G_1^+), the second band from the top represents the fragment d(TCCTCCTG$_1$[BPDE]) containing the BPDE residue on G_1; the mobility of this 8-mer containing a single BPDE residue is considerably slower than that of an unmodified 8-mer fragment (second band from the top in the lane UM). The large difference arises because the effect of a single BPDE residue on mobility is greater on shorter than on longer oligonucleotide fragments. If the BPDE residue is positioned at guanine G_2, only unmodified cleavage fragments are observed since guanines (modified or unmodified) are destroyed by the Maxam−Gilbert G-cleavage reaction. Thus, the site of binding at G_1 can be identified (Mao *et al.*, 1992).

The stereochemistry of BPDE adducts was determined by digesting modified oligodeoxyribonucleotides with snake venom phosphodiesterase and bacterial alkaline phosphatase,

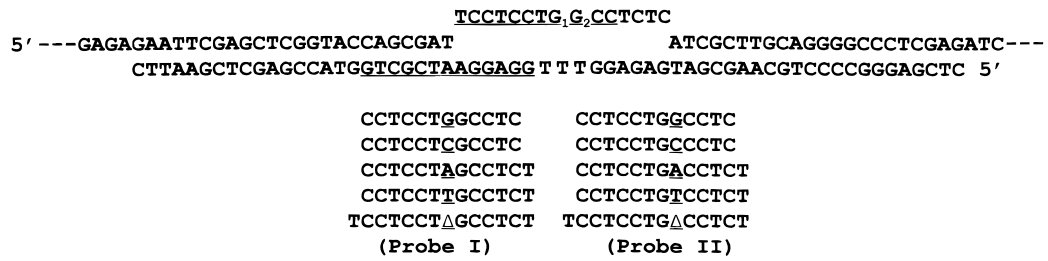

FIGURE 2: Ligation of a modified 15-mer to *Eco*RV-digested ss pMS2 and probes used for analysis. The upper strand is part of a ss pMS2 sequence. Underlined nucleotides in the inserted oligomer correspond to a sequence containing codon 60−63 of the noncoding strand of the human c-Ha-*ras1* gene. G_1 and G_2 in the insert are modified with BPDE. G_1 corresponds to the first base of codon 61, and G_2 to the third base of codon 60. The underlined 13-mer sequence of the 61-mer (bottom strand) was used to determine the concentration of ss DNA construct, as described by Moriya (1993). This 61-mer was removed by digestion with T4 DNA polymerase and exonuclease III after ligation. Three base mismatches at and adjacent to modified Gs (TG_1G_2/TTT) serve as a genetic marker to identify products of DNA replication. Probes I and II were used to determine by differential oligonucleotide hybridization (Moriya & Grollman, 1993) bases at the G_1 and G_2 modification sites, respectively, in progeny plasmids.

and then separating the resulting mixture of nucleosides by reverse phase HPLC (as described by Liu *et al*., 1996). The relative proportions of dC:dT:dG:BPDE-N^2-dG were approximately 8:5:1:1, as predicted by the base composition of the modified oligonucleotide. The BPDE-N^2-dG adducts co-eluted with authentic (+)-*trans*- or (−)-*trans*-BPDE-N^2-dG mononucleoside adduct standards (Mao *et al.*, 1992), prepared by the method of Cheng *et al*. (1989).

Construction of Vectors and Mutagenesis Studies. BPDE-modified oligodeoxyribonucleotides were incorporated into the ss shuttle vector pMS2, as described previously (Moriya, 1993; Moriya *et al.*, 1994), with ligation reactions conducted at 4 °C, rather than 9 °C. The nomenclature used in this paper is as follows: pMS2($\mathbf{G_1}^+$), pMS2($\mathbf{G_1}^-$), pMS2($\mathbf{G_2}^+$), and pMS2($\mathbf{G_2}^-$), where $\mathbf{G}^+$ and $\mathbf{G}^-$ represent (+)- and (−)-*trans-anti*-BPDE-N^2-dG adducts, respectively, and $\mathbf{G_1}$ and $\mathbf{G_2}$ indicate the relative position of the modified base (Figure 2). Methods used for transforming *E. coli*, inducing SOS functions, and transfecting simian kidney (COS7) cells have been described (Moriya, 1993; Moriya *et al.*, 1994). Following transformation of *E. coli*, the mixture was plated immediately to assure the independence of each transformant. Progeny plasmids were analyzed by a differential oligonucleotide hybridization technique (Moriya & Grollman, 1993), using probes listed in Figure 2. In experiments with COS7 cells, progeny plasmids were recovered 48 h after transfection (Hirt, 1967), treated with nuclease S1 to digest input ss DNA, and then used to transform *E. coli* DH10B for mutational analysis. Detailed procedures are described elsewhere (Pandya & Moriya, 1996).

RESULTS

In *E. coli* AB1157, the number of transformants recovered in experiments with pMS2($\mathbf{G_1}^+$), pMS2($\mathbf{G_1}^-$), pMS2($\mathbf{G_2}^+$), and pMS2($\mathbf{G_2}^-$) were 0.5%, 4%, 1%, and 11%, respectively, of values obtained with the unmodified vector pMS2-(TG_1G_2C) (Table 1). The analysis of progeny plasmids showed that the fraction of scaffold-derived plasmids was small (≤2.2%) in SOS-induced AB1157 and COS cells. In SOS-uninduced AB1157, however, this fraction was large; for example, accounting for 70% of transformants for G_1^+. The fraction of scaffold-derived plasmids was larger for G^+ adducts than G^- adducts, in parallel with the stronger blocking effects (lower yield of transformants) of G^+ (Table 1). Data presented in Table 1 have been corrected for progeny plasmids derived from the scaffolding oligonucleotide. The number of transformants recovered relative to the control increased, in some cases 90-fold, following induction of SOS functions. In the experiments with COS cells, no marked differences in the recovery of progeny plasmid were observed between the control and the modified constructs; for example, 1.5×10^5 and 1.3×10^5 transformants per recovered DNA were obtained for two transfections with the control construct, and 1.3×10^5 and 0.8×10^5 transformants for two transfections with the G_1^+ construct, which blocked translesional synthesis most strongly in *E. coli*. Unlike the *E. coli* experiments, these values do not necessarily represent the efficiency of translesional synthesis, since plasmids are amplified in COS cells and collectively recovered from transfected cells.

Table 1: Transformation Efficiency of *E. coli* AB1157 with ssDNA Constructs Containing (+)- and (−)-*trans-anti*-BPDE-N^2-dG (G^+, G^-) Adducts

ssDNA[a]	SOS induction[b]	no. of transformants[c] N = G^+ expt 1	N = G^+ expt 2	N = G^-
pMS2(TG_1G_2C)	−	3615 (100)	4879 (100)	4856 (100)
	+	965 (100)	1032 (100)	3796 (100)
pMS2(TNG_2C)	−	20 (0.5)		198 (4)
	+	435 (45)		1703 (45)
pMS2(TG_1NC)	−		54 (1)	542 (11)
	+		708 (69)	1067 (28)

[a] 50 ng of circularized ssDNA per transformation; N is G^+ or G^-. [b] AB1157 in log-phase growth was irradiated with UV at 20 J/m^2 prior to transformation (Moriya *et al*., 1994). [c] Expressed per transformation; values reflect plasmids having correctly inserted sequence. The number in parentheses represents percent of the corresponding control value. Each number was corrected for plasmid derived from the scaffolding oligonucleotide.

Mutational analysis of AB1157 transformants from pMS2-($\mathbf{G_1}^+$) revealed that approximately 20% of translesional events in *E. coli* were associated with targeted mutations in the presence and absence of induced SOS functions (Table 2). The strong blocking effect of G^+ adducts in the absence of induced SOS functions resulted in a limited number of transformants. Mutational events consisted primarily of G→T transversions and a smaller number of G→A and G→C mutations. Much lower mutation frequencies, 2% and 3% under SOS-inducing and -noninducing conditions, respectively, were found for pMS2($\mathbf{G_2}^+$). The mutation spectrum for $\mathbf{G_2}^+$ also contained G→T, G→A, and G→C mutations. Mutation frequencies for pMS2($\mathbf{G_1}^-$) and pMS2($\mathbf{G_2}^-$) were

Table 2: Mutagenicity of Site-Specifically Placed (+)- and (−)-*trans-anti*-BPDE-N^2-dG Adducts in *E. coli* and COS7 Cells[a]

ssDNA	host[c]	no. of expts	G	T	A	C	Δ[d]	frequency of targeted mutations,[b] %
pMS2(G_1^+)	AB1157	3	25 (81)	5 (16)	0 (0)	1 (3)	0	19
	AB1157 + UV	3	318 (80)	75 (19)	4 (1)	2 (0.5)	0	20
	COS7→DH10B	3	197 (87)	27 (12)	2 (0.9)	0 (0)	0	13
pMS2(G_2^+)	AB1157	2	70 (97)	1 (1)	1 (1)	0 (0)	0	3
	AB1157 + UV	2	249 (98)	2 (0.8)	1 (0.4)	2 (0.8)	0	2
	COS7→DH10B	4	183 (55)	119 (36)	24 (7)	5 (2)	0	45
pMS2(G_1^-)	AB1157	3	272 (99)	4 (1)	0	0	0	1
	AB1157 + UV	3	343 (98)	4 (1)	0	1 (0.3)	2 (0.6)	2
	COS7→DH10B	3	217 (87)	29 (12)	1 (0.4)	2 (0.8)	0	13
pMS2(G_2^-)	AB1157	3	330 (100)	0	0	0	0	<0.3
	AB1157 + UV	3	349 (99)	2 (0.6)	1 (0.3)	0	0	1
	COS7→DH10B	4	253 (79)	40 (12)	10 (3)	18 (6)	0	21

[a] Number in parentheses represents percentage of each targeted event. [b] [(No. of T+A+C+Δ) ÷ total] × 100. [c] AB1157 was irradiated with UV, as described in Table 1. In COS7 experiments, progeny plasmid was transferred to *E. coli* DH10B for mutational analysis. [d] Deletion of G, this can be a deletion of the modified or neighboring G.

low (≤2%). The predominant mutations in all *E. coli* experiments were G→T transversions, targeted to the site of the lesion.

Mutational analysis of plasmids replicated in COS cells, representing data combined from at least three independent experiments, is given in Table 2. Translesional synthesis on ss pMS2($\mathbf{G_1}^+$) and pMS2($\mathbf{G_2}^+$) was associated with mutation frequencies of 13% and 45%, respectively. The mutation frequency for pMS2($\mathbf{G_1}^+$) in COS cells (13%) is comparable to mutation frequencies of 19−20% observed in *E. coli*, while the mutation frequency for pMS2($\mathbf{G_2}^+$) in COS cells (45%) is much higher than values of 2−3% observed for this vector in *E. coli* (Table 2). Similarly, the high mutation frequencies observed for pMS2($\mathbf{G_1}^-$) (13%) and pMS2($\mathbf{G_2}^-$) (21%) in COS cells contrast with values of ≤2% obtained for these vectors in *E. coli*. The predominant mutations in COS cell experiments were also G→T transversions. Significant numbers of G→A and G→C mutations were observed for $\mathbf{G}^+$ and $\mathbf{G}^-$ when $\mathbf{G}_2$ was modified; the combined frequencies of these mutations in COS cells were 0.9% and 1.2% for pMS2($\mathbf{G_1}^+$) and pMS2($\mathbf{G_1}^-$), respectively; whereas, frequencies of approximately 9% were observed for pMS2($\mathbf{G_2}^+$) and pMS2($\mathbf{G_2}^-$). G→A transitions were more common than G→C transversions for pMS2($\mathbf{G_2}^+$); G→C transversions predominated for pMS2($\mathbf{G_2}^-$).

Analysis of 360 transformants obtained with and without SOS induction in experiments where plasmids were constructed with unmodified oligomers, and of 270 transformants recovered from COS7 cells transfected with the unmodified construct, revealed no mutations in the regions probed by oligonucleotide hybridization. Thus, the background mutation frequency for this study is <0.4%.

DISCUSSION

Nonmutagenic events and G→T transversions targeted to the site of the lesion predominate during translesional synthesis in this mutational study of positionally-defined (+)- and (−)-*trans-anti*-BPDE-dG adducts. Our observations are consistent with previous reports of BPDE-induced mutagenesis in bacterial and mammalian cells and may be attributed to preferential pairing of (+)- and (−)-BPDE-dG adducts with dCMP or dAMP during DNA replication. In B-DNA, (+)- and (−)-BPDE-dG adducts are readily accommodated by Watson−Crick pairing to dC(*anti*) when the modified base is also in the *anti* conformation (Cosman *et al*., 1992; de los Santos, 1992; Fountain & Krugh, 1995). dG(*anti)*·dA(*anti),* dG(*anti)*·dA(*syn),* and dG(*syn)*·dA(*anti)* base pairs have been reported in X-ray crystallographic studies of DNA duplexes containing G:A mismatches (Brown *et al*., 1986, 1989; Privé *et al*., 1987). Analogous pairing arrangements could stabilize the BPDE−dG:dAMP pair in the replication fork. Extrahelical displacement of BPDE−dG on the template strand (Shibutani & Grollman, 1993) is likely to account for the (rare) frameshift mutations observed in the present investigation.

In our experiments, mutation frequencies were strikingly influenced by the host cell. With the exception of $\mathbf{G_1}^+$, which is equally mutagenic in both hosts, $\mathbf{G}^+$ and $\mathbf{G}^-$ are significantly more mutagenic (13−70-fold) in COS cells than in *E. coli* when similar sequences are compared. These results confirm our earlier observation (Shibutani *et al*., 1991; Moriya *et al*., 1994) that the mutation frequency of a given DNA adduct in bacteria and mammalian cells may vary considerably, presumably as a result of intrinsic differences between DNA polymerases that copy past the lesion.

In *E. coli*, (+)-BPDE-dG adducts appear to be more mutagenic than adducts derived from the (−)-enantiomer; $\mathbf{G_1}^+$ was more than 10-fold more mutagenic than $\mathbf{G_1}^-$, although a large difference between $\mathbf{G_2}^+$ and $\mathbf{G_2}^-$ was not observed. In COS cells, there is relatively little difference between the two isomers. Thus, preferential binding of (+)-BPDE to DNA (Cheng *et al*., 1989; Meehan & Straub, 1979) may account for the higher carcinogenicity reported for (+)-*anti*-BPDE in animal models (reviewed in Conney, 1982). The full spectrum of mutations produced by BPDE may be interpreted more fully when the mutagenic potential of (+)-*cis*- and (−)-*cis*-BPDE has been established and further information on the effects of flanking bases on mutagenesis by BPDE−dG adducts is available.

Activated *ras* genes in chemically-induced animal tumors often contain mutations in codons 12, 13, and/or 61 (Barbacid, 1987). Human c-Ha-*ras1* sequences, modified with racemic *anti*-BPDE and analyzed in NIH 3T3 cells, are mutated at several positions in codons 12 or 61 (Vousden *et al*., 1986). In our study, $\mathbf{G_1}^+$ and $\mathbf{G_1}^-$, corresponding to the first position of codon 61, generated C:G→A:T transversions. Thus, translesional synthesis past $\mathbf{G_1}^+$ and $\mathbf{G_1}^-$ in COS cells would activate the c-Ha-*ras* protooncogene at frequencies of 12% if incorporated in this gene (Table 2); other base changes at this position do not result in activation. The frequency of mutations induced by (+)- and (−)-BPDE adducts in COS cells was generally higher at $\mathbf{G_2}$ than at $\mathbf{G_1}$;

however, $\mathbf{G}_2$ corresponds to the third base of codon 60, and mutations at this position are silent.

Induction of SOS functions reduces the blocking effects of $\mathbf{G}^+$ and $\mathbf{G}^-$ on translesional synthesis in *E. coli* (Table 1); the increased number of transformants was not accompanied by a parallel increase in mutation frequency for either sequence or adduct ($\mathbf{G_1}^+$, $\mathbf{G_2}^+$, $\mathbf{G_1}^-$, $\mathbf{G_2}^-$) tested (Table 2). Protocols employed in our experiments have been used in studies of exocyclic DNA adducts; in this case, mutation frequencies were affected significantly by SOS induction (Moriya *et al*., 1994). In the two-step model proposed by Bridges and Woodgate (1984, 1985) for error-prone synthesis, activated RecA and UmuC/D′ facilitate extension of a primer stalled opposite a DNA adduct, rather than incorporation of a base opposite the adduct. Our previous studies of 3,N^4-etheno-2′-deoxycytidine (ϵdC) revealed 2% and 32% mutation frequencies in SOS-uninduced and -induced AB1157, respectively. Interestingly, this exocyclic adduct shows 33% mutation frequency in SOS-uninduced NR9232, a 3′→5′ proofreading-defective strain (Moriya *et al.*, 1994). These results indicate that mispaired bases leading to targeted mutations are inserted opposite ϵdC at a substantial frequency, while mispaired bases are preferentially removed in proofreading-competent cells. In the proofreading-defective strain, incorporated bases are not edited, resulting in a higher mutation frequency. In SOS-induced proofreading-competent cells, induced gene products allow extension of mispairings, resulting in a higher mutation frequency. Unlike ϵdC, the four BPDE adducts did not show increased mutagenicity in NR9232 (data not shown), indicating that mispaired bases are not preferentially removed by the proofreading activity. Therefore, induction of SOS functions can increase survival without influencing mutation frequency. One study (Jacobsen & Humayun, 1990) showed that effects of SOS induction on the mutation frequency of an exocyclic DNA adduct depend on local sequence context, suggesting that effects of induced SOS functions are not homogeneous. This might explain the difference between our data and those of Mackay *et al.* (1992). Furthermore, the dominant G→T transversions induced by (+)-*trans-anti*-BPDE-N^2-dG lesions in Mackay's experiments were observed in a different sequence context and in a different *E. coli* replication system. Since these G adducts, especially G^+, are strong blocks to DNA synthesis, few transformants were collected in the absence of SOS induction (Table 1). Further experiments may be needed to clarify this point.

Mutagenicity of $\mathbf{G}^+$ is markedly affected by the bases flanking the lesion. In *E. coli*, T$\mathbf{G}^+$G is 10-fold (SOS-induced) and 6-fold (SOS-noninduced) more mutagenic than G$\mathbf{G}^+$C, and in COS cells, G$\mathbf{G}^+$C is 3.5-fold more mutagenic than T$\mathbf{G}^+$G. $\mathbf{G}^-$ is significantly mutagenic only in COS cells, and the effect of flanking bases on adduct-induced mutagenesis in this host cell is less marked. G→T transversions dominate the mutational spectrum in our studies; this mutation is most apparent when the adduct is in a T$\mathbf{G}^+$G sequence context. In COS cells, when $\mathbf{G}_2$ was modified, a significant number of G→A and G→C mutations were also observed in G$\mathbf{G}^+$C and G$\mathbf{G}^-$C sequences (Table 2). In *E. coli*, the mutation frequency was too low to reliably establish mutational heterogeneity. However, in two other studies of the mutagenic potential of (+)-*trans-anti*-BPDE in *E. coli*, G→T transversions were found almost exclusively in a T$\mathbf{G}^+$ sequence context (Mackay *et al.*, 1992), while in a C$\mathbf{G}^+$G sequence context, G→T, G→A, and G→C mutations were observed (Jelinsky *et al.*, 1995). The model used by the authors to rationalize these results is that the BPDE-N^2-dG adduct can assume several different conformations at the replication fork, each producing a different kind of mutation.

The elucidation of the structural basis underlying mutagenic phenomena must take into account the structure and orientation of the single strand−double strand junction at the replication fork and the influence of DNA polymerases on this structure. Progress is being made in understanding the structural characteristics of adducts in various sequence contexts in the absence of enzymes. Cosman *et al*. (1995) recently described the solution structure of the (+)-*trans-anti*-BPDE-N^2-dG adduct at a single strand−double strand junction. Adduct conformation was found to be quite different from the structure of the same adduct in full duplexes (Cosman *et al.*, 1992). Other studies of the spectroscopic and gel electrophoretic mobilities of oligonucleotides containing BPDE-N^2-dG demonstrate that neighboring bases can exert striking effects on the properties of the adducted DNA. Suh *et al.* (1994) reported that carcinogen−base stacked and external adduct conformations coexist in DNA duplexes where $\mathbf{G}^+$ is flanked by G on the 5′-side. However, an external, presumably minor groove conformation (Cosman *et al*., 1992) predominates when $\mathbf{G}^+$ is flanked by T (Fountain & Krugh, 1995). Likewise, gel electrophoresis studies of duplexes revealed that a large bend is induced at the site of the $\mathbf{G}^+$ when it is flanked on the 5′-side by another G. On the other hand, the $\mathbf{G}^-$ adduct appears somewhat more bent than an unmodified sequence, but the extent of bending is unaffected by a 5′-flanking G (Liu *et al*., 1996). These studies strongly suggest that both adduct stereochemistry and base sequence context influence conformation of the adducts in duplex DNA. A better understanding of the consequences of adduct conformation on DNA replication must await the results of analogous studies at single strand−double strand junctions.

In summary, we conclude from our observations that translesional synthesis past BPDE−DNA adducts is strongly influenced by the stereochemistry of the adduct, the nucleotide sequence context of the modified base, and the DNA replication complex of the host cell. Conformational flexibility of the adduct at the replication fork plays an important role in determining the base incorporated opposite the lesion. In our study, adducts with the same flanking bases generate different mutation frequencies and specificities in *E. coli* and COS cells. Thus, translesional events are determined by a complex interplay between the DNA adduct, flanking bases, and the replication complex; the precise mechanism(s) by which structural features of the lesion and DNA polymerase functions are manifested remains to be elucidated.

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REFERENCES

Barbacid, M. (1987) *Annu. Rev. Biochem. 56*, 779−827.

Bernelot-Moens, C., Glickman, B. W., & Gordon, A. J. E. (1990) *Carcinogenesis 11*, 781−785.

Bridges, B. A., & Woodgate, R. (1984) *Mol. Gen. Genet. 196*, 364−366.

Bridges, B. A., & Woodgate, R. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4193−4197.
Brookes, P., & Osborne, M. R. (1982) *Carcinogenesis 3*, 1223−1226.
Brown, T., Hunter, W. N., Kneale, G. G., & Kennard, O. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2402−2406.
Brown, T., Leonard, G. A., Booth, E. D., & Chambers, J. (1989) *J. Mol. Biol. 207*, 455−457.
Buening, M. K., Wislocki, P. G., Levin, W., Yagi, H., Thakker, D. R., Akagi, H., Koreeda, M., Jerina, D. M., & Conney, A. H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5358−5361.
Carothers, A. M., & Grunberger, D. (1990) *Carcinogenesis 11*, 189−192.
Chary, P., Latham, G. J., Robberson, D. L., Kim, S. J., Han, S., Harris, C. M., Harris, T. M., & Lloyd, R. S. (1995) *J. Biol. Chem. 270*, 4990−5000.
Cheng, S. C., Hilton, B. D., Roman, J. M., & Dipple, A. (1989) *Chem. Res. Toxicol. 2*, 334−340.
Conney, A. H. (1982) *Cancer Res. 42*, 4875−4917.
Cosman, M., Ibanez, V., Geacintov, N. E., & Harvey R. G. (1990) *Carcinogenesis 11*, 1667−1672.
Cosman, M., de los Santos, C., Fiala, R., Hingerty, B. E., 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., Hingerty, B., Geacintov, N. E., Broyde, S., & Patel, D. J. (1995) *Biochemistry 34*, 15334−15550.
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.
Eisenstadt, E., Warren, A. J., Porter, J., Atkins, D., & Miller, J. H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1945−1949.
Fountain, M. A., & Krugh, T. R. (1995) *Biochemistry 34*, 3152−3161.
Harris, C. C. (1991) *Cancer Res.* (*Suppl.*) *51*, 5023s−5044s.
Harvey, R. G. (1991) *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*, Cambridge University Press, Cambridge.
Hirt, B. (1967) *J. Mol. Biol. 26*, 365−369.
Jacobsen, J. S., & Humayun, M. Z. (1990) *Biochemistry 29*, 496−504.
Jelinsky, S. A., Liu, T., Geacintov, N. E., & Loechler, E. L. (1995) *Biochemistry 34*, 13545−13553.
Keohavong, P., & Thilly, W. G. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 4623−4627.
Kunkel, T. A. (1990) *Biochemistry 29*, 8003−8011.
Liu, T., Xu, J., Tsao, H., Li, B., Yang, C., Amin, S., Moriya, M., & Geacintov, N. E. (1996) *Chem. Res. Toxicol. 9*, 255−261.
Mackay, W., Benasutti, M., Drouin, E., & Loechler, E. L. (1992) *Carcinogenesis 13*, 1415−1425.
Mao, B., Margulis, L. A., Li, B., Ibanez, V., Lee, H., Harvey, R. G., & Geacintov, N. E. (1992) *Chem. Res. Toxicol. 5*, 773−778.
Meehan, T., & Straub, K. (1979) *Nature 277*, 410−412.
Moriya, M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1122.
Moriya, M., & Grollman, A. P. (1993) *Mol. Gen. Genet. 239*, 72−76.
Moriya, M., Zhang, W., Johnson, F., & Grollman, A. P. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 11899−11903.
Pandya, G. A., & Moriya, M. (1996) *Biochemistry 35*, 11487−11492.
Privé, G. G., Heinemann, U., Chandrasegaran, S., Kan, L. S., Kopka, M. L., & Dickerson, R. E. (1987) *Science 238*, 498−504.
Rodriguez, H., & Loechler, E. L. (1993) *Biochemistry 32*, 1759−1769.
Shibutani, S., & Grollman, A. P. (1993) *J. Biol. Chem. 268*, 11703−11710.
Shibutani, S., Takeshita, M., & Grollman, A. P. (1991) *Nature 349*, 431−434.
Shibutani, S., Margulis, L. A., Geacintov, N. E., & Grollman, A. P. (1993) *Biochemistry 32*, 7531−7541.
Singer, B., & Essigmann, J. M. (1991) *Carcinogenesis 12*, 949−955.
Slaga, T. J., Bracken, W. J., Gleason, G., Levin, W., Yagi, H., Jerina, D. M., & Conney, A. H. (1979) *Cancer Res. 39*, 67−71.
Stevens, C. W., Bouck, N., Burgess, J. A., & Fahl, W. E. (1985) *Mutat. Res. 152*, 5−14.
Suh, M., Jankowiak, R., Ariese, F., Mao, B., Geacintov, N. E., & Small, G. J. (1994) *Carcinogenesis 15*, 2891−2898.
Vousden, K. H., Bos, J. L., Marshall, C. J., & Phillips, D. H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1222−1226.
Weems, H. B., & Yang, S. K. (1989) *Chirality 1*, 276−283.
Wei, S.-J. C., Chang, R. L., Wong, Bhachech, N., Cui, X. X., Hennig, E., Yagi, H., Sayer, J. M., Jerina, D. M., Preston, B. D., & Conney, A. H. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 11227−11230.
Wei, S.-J. C., Chang, R. L., Hennig, E., Cui, X. X., Merkler, K. A., Wong, C.-Q., Yagi, H., Jerina, D. M., & Conney, A. H. (1994) *Carcinogenesis 15*, 1729−1735.
Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Thakker, D. R., Jerina, D. M., & Conney, A. H. (1977) *Biochem. Biophys. Res. Commun. 77*, 1389−1396.
Yagi, H., Thakker, D. R., Hernandez, O., Koreeda, M., & Jerina, D. M. (1977) *J. Am. Chem. Soc. 99*, 1604−1611.
Yang, J.-L, Maher, V. M., & McCormick, J. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3787−3791.

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