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Chloride ions catalyze the formation of cis adducts in the binding of *anti*-benzo[*a*]pyrene diol epoxide to nucleic acids

(carcinogen/polycyclic aromatic hydrocarbon/DNA adducts/chlorohydrin)

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ABSTRACT **The alkylation of DNA by racemic 7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-BPDE) exhibits a strong preference for formation of trans adducts between the N^2 deoxyguanosine alkylation site and the (+)-enantiomer of *anti*-BPDE. In the presence of 10 mM buffer with no added salt, 98% of the adducts formed with native calf thymus DNA result from trans opening of the epoxide ring. The strong selectivity for trans adduct formation obtained with duplex DNA at low salt concentration is found to a lesser degree with poly(G) but is nearly absent with dAMP. When DNA adducts are formed in 10 mM $MgCl_2$ or 1 M NaCl, the proportion of cis adducts increases to ≈7 and ≈26%, respectively. At low salt, 10 mM $MgCl_2$, and 1 M NaCl, deoxyguanosine adducts are approximately 1%, 6%, and 24% cis, whereas deoxyadenosine adducts are approximately 11%, 14%, and 37% cis, respectively. NaCl also increases the proportion of cis adducts formed with poly(G) and dAMP. It is proposed that the increase in cis-adduct formation due to salt results from S_N1 attack of chloride ion on the BPDE carbocation, forming a trans chlorohydrin, followed by S_N2 attack of DNA.**

Benzo[*a*]pyrene is a potent carcinogen and ubiquitous environmental pollutant which is metabolically activated to form several diastereomeric diol epoxide intermediates capable of alkylating DNA, RNA, and protein (1–7). The most mutagenic and carcinogenic of these diastereomers is 7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [(+)-*anti*-BPDE] (7–9). The primary alkylation site of the diol epoxides in DNA is the N^2 position of deoxyguanosine (N^2-dGuo) (5–7, 10–12), and the principal adduct formed at this site *in vivo* is due to (+)-*anti*-BPDE (4). The purine is attached to the C-10 position of the hydrocarbon and is trans to the C-9 hydroxy substituent of the benzo[*a*]pyrene moiety (a trans adduct). Although trans adducts predominate in DNA, those with the 9 and 10 substituents of the hydrocarbon moiety in the cis configuration (cis adducts) also occur (12–15). The interaction of *anti*-BPDE with DNA involves physical association and a DNA-catalyzed hydrolysis reaction that competes with the alkylation reaction. The mechanisms of these reactions have been reviewed (16, 17), as have the characteristics of the covalent adducts (18).

The conformations of the trans N^2-dGuo adducts of (+)-*anti*-BPDE and 7*S*,8*R*-dihydroxy-9*R*,10*S*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [(−)-*anti*-BPDE] in DNA have recently been determined by NMR, and both adducts have been found to be situated in the minor groove, causing minimal distortion of the duplex (19, 20). The hydrocarbon moieties of the two adducts point in opposite directions [toward the 5′ end of the modified strand in the (+)-BPDE adduct, and toward the 3′ end in the (−)-BPDE adduct]. The cis (+)-*anti*-BPDE N^2-dGuo adduct has an intercalated conformation, leading to disruption of a base pair (21). The conformation of the cis (−)-*anti*-BPDE N^2-dGuo adduct is believed, on the basis of less direct spectroscopic evidence, to be similar (22). A variety of correlative evidence suggests that the less disruptive trans adducts are more carcinogenic than cis adducts (22).

The mechanism of DNA alkylation by chemical carcinogens has been worked out for a large number of compounds (23). Many of the most important carcinogens require metabolic activation prior to exhibiting biological activity. The ultimate carcinogenic form of benzo[*a*]pyrene is *anti*-BPDE, and the identification of this intermediate has led to the development of the bay-region theory of polycyclic aromatic hydrocarbon activation (7). Other important carcinogens have also been shown to alkylate DNA through the formation of proximate epoxides (24). These concepts have dominated our picture of carcinogen initiation mechanisms for over two decades.

In an effort to understand the mechanism of adduct formation we have studied the effect of salts on this reaction with DNA, homopolymer forms of RNA, and dAMP. These effects have been used to elucidate a role for chloride in the alkylation of DNA by polycyclic aromatic hydrocarbons. We have found that chloride salts induce the formation of substantial amounts of cis adducts. Chloride, bromide, and iodide salts also catalyze cis opening of the epoxide during *anti*-BPDE hydrolysis, and this effect does not depend on the nature of the cation. It is widely accepted that BPDE-DNA adducts are formed by nucleophilic attack of exocyclic amino groups on the C-10 carbocation of the epoxide (or the epoxide itself). Our evidence now suggests that, at least in the case of cis adducts, this reaction can also occur via the formation of a (trans) chlorohydrin intermediate which then undergoes S_N2 nucleophilic attack by DNA.

MATERIALS AND METHODS

Chemicals and Supplies. Racemic (±)-*anti*-BPDE and racemic *anti*-[G-^{3}H]BPDE were prepared as described (25, 26). Resolved (+)- and (−)-*anti*-[1-^{3}H]BPDE were obtained from the Chemical Carcinogen Repository of the National Cancer Institute and were in some cases diluted with unlabeled (+)- and (−)-*anti*-BPDE, which were prepared as described (27). C_{18} reverse-phase Sep-Pak cartridges were obtained from Waters. Extinction coefficients ($M^{-1}\cdot cm^{-1}$, with nucleic acid concentrations in terms of phosphate) used were as follows: poly(G), ε_{254} = 9800 (28); calf thymus DNA, ε_{260} = 6550 (29);

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Abbreviations: (+)-*anti*-BPDE, 7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; (−)-*anti*-BPDE, 7*S*,8*R*-dihydroxy-9*R*,10*S*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; *cis*- and *trans*-tetrol, racemic 7r,8t,9t,10t- and racemic 7r,8t,9t,10c-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, respectively.

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dAMP at pH 7.0, ε_{260} = 15,300 (30), and *anti*-BPDE in tetrahydrofuran, ε_{345} = 46,600 (our measurement).

Preparation of BPDE-Modified Calf Thymus DNA. Adducts between BPDE and calf thymus DNA were generated in samples containing 5 ml of 0.01 M potassium phosphate (pH 7.5), 3 mM (1 mg/ml) nucleic acid, 10 μM (±)-*anti*-[^{3}H]BPDE, 10% (vol/vol) acetone and NaCl or $MgCl_2$ as indicated. The reaction was started by the addition of (±)-*anti*-BPDE, and the samples were incubated at 5°C for 20 hr. At the end of the incubation period the polynucleotide sample was extracted five times with 3 volumes of water-saturated ethyl acetate to remove tetrol {*cis*-tetrol and *trans*-tetrol (racemic 7r,8t,9t,10t- and 7r,8t,9t,10c-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene) are the hydrolysis products of *anti*-BPDE}. DNA was precipitated at 0°C by the addition of NaCl (final concentration, 0.1 M) and 1 volume of 2-propanol. After precipitation, the sample was boiled for 5 min and then centrifuged. The pellet was suspended in a 0.1 M NaCl/2-propanol (1:1, vol/vol) and again boiled and centrifuged. The pellet was dried with a stream of nitrogen.

Enzymatic Hydrolysis of BPDE-Modified Calf Thymus DNA. Modified DNA was dissolved (1 mg/ml) in 0.01 M sodium acetate, pH 5.5/0.1 mM EDTA. It was incubated at 37°C with hydrolytic enzymes added according to the following schedule (activities given are for 1 mg of DNA): 0 and 8 hr, 50 units of DNase II; 16 and 28 hr, 0.5 unit of spleen phosphodiesterase II; 40 and 52 hr, 50 units of alkaline phosphatase. Prior to the first addition of alkaline phosphatase, the pH of the sample was adjusted to ≈10 by the addition of 0.1 M glycine (pH 10.4), and $MgCl_2$ and $ZnCl_2$ were added to final concentrations of 1 mM. Incubation was halted after 64 hr.

Preparation of BPDE-Modified Poly(G) and dAMP. Adducts between BPDE and poly(G) or dAMP were generated in samples containing 3 ml of 10 mM sodium cacodylate (pH 7.2), 5 mM (in terms of bases) polymer or monomer, 0.1 mM EDTA, 10% (vol/vol) acetone, and between 0 and 1 M NaCl. Resolved (+)- or (−)-*anti*-[^{3}H]BPDE (≈10 μM) was added to each sample. The specific activities of the (+)- and (−)-*anti*-BPDE stocks used were 890 and 740 Ci/mol, respectively (1 Ci = 37 GBq). The samples were allowed to react at room temperature overnight in the dark. The monomer samples were then brought to pH 9.5 by the addition of 120 μl of 1 M Tris (pH 8.1) and 14 μl of 5 M NaOH. Each sample was extracted four or five times with 2 ml of water-saturated 1-butanol and then twice with 2 ml of diethyl ether, to remove tetrol. The monomer samples were then brought to pH 10 by the addition of 100 μl of 0.2 M Tris base and 60 μl of 0.2 M KOH. To remove any remaining tetrol from the polymer samples, they were transferred to dialysis tubing (Spectrapor, 2000-Da molecular mass cutoff) and dialyzed for 36 hr at 4°C in 250 ml of 10 mM sodium cacodylate, pH 7.2/100 mM NaCl/1 mM EDTA (to inhibit RNases by removing traces of Mg^{2+}). The dialysate was changed five times in this interval (EDTA was omitted from the last change). $MgCl_2$ was then added (10 mM) to the polymer samples, followed by 2.5 volumes of ethanol. The samples were then chilled to −20°C, and the precipitated RNA was recovered by centrifugation for 10 min at 1000 × *g*. The pellets were dried and dissolved in 1.2 ml of water plus 0.3 ml of 1.5 M KOH.

Hydrolysis of BPDE-Modified Poly(G) and dAMP. RNA hydrolysis was carried out by boiling modified homopolymer samples in 0.3 M KOH for 15 min; the pH of these samples was then lowered to 10 by addition of Tris·HCl. $MgCl_2$ and $ZnCl_2$ were then added (1 mM each) to all samples. Alkaline phosphatase was added in four equally spaced doses (4 × 40 units for each sample) over a period of 24 hr in the dark at room temperature.

Isolation of BPDE-Nucleoside Adducts. Modified nucleosides were separated from unmodified nucleosides with C_{18} Sep-Pak cartridges. Aqueous samples were brought to neutral pH and loaded onto the Sep-Pak; the cartridge was then washed with water to remove unmodified nucleosides and the nucleoside adducts were eluted with methanol. The samples were concentrated with a stream of nitrogen or with a rotary evaporator. The mixture of modified nucleosides was resolved into individual adducts by reverse-phase HPLC using a 0.46 × 25 cm C_{18} column and an isocratic methanol/water mobile phase. DNA adduct samples were eluted with 50% (vol/vol) methanol at 0.7 ml/min from a DuPont Zorbax column. Adducts formed between poly(G) and resolved BPDE enantiomers were eluted from a 5-μm Rainin (Woburn, MA) Microsorb column by the same protocol. Adducts derived from reactions of dAMP and resolved BPDE enantiomers were eluted isocratically with 52% methanol at 0.8 ml/min from the latter column. Detection was by fluorescence and radioactivity. For fluorescence detection, the column effluent was excited at 245 nm, with emission being measured at wavelengths > 320 nm by using a cutoff filter. For detection of tritium, 1- or 0.5-min fractions were collected for liquid scintillation counting. Adduct quantitation was based on a summation of the disintegrations per minute (dpm) in a peak. Adducts were identified from their chromatographic and spectroscopic properties on the basis of previous work in this (11, 14, 26) and other laboratories (12, 15). Adduct samples stored for more than a few weeks were kept at −80°C.

RESULTS

Effect of Salt on Adduct Distribution. The distributions of adducts formed between ^{3}H-labeled *anti*-BPDE and calf thymus DNA (42% G+C content) in the presence of buffer only, or with the addition of 10 mM $MgCl_2$ or 1 M NaCl, are shown in Table 1. These results are representative of 20 experiments carried out under a variety of conditions, with several DNA substrates, which consistently showed a higher proportion of cis adducts at higher salt concentrations. Adduct elution was monitored by both radioactivity and fluorescence. Quantum yields of different BPDE nucleoside adducts vary significantly, so the values for the relative amounts of different adducts in Tables 1 and 2 are based on radioactivity. In the absence of added salt, ≈98% of the total adducts formed with dGuo and dAdo exhibit the trans configuration.

The formation of adducts can be significantly affected by changes in salt concentration. The amount of cis dGuo adducts as a fraction of total dGuo adducts is estimated to be

Table 1. Effects of salts on the proportions of cis and trans adducts from reaction of (±)-*anti*-BPDE with calf thymus DNA

	No added salt	$MgCl_2$ (10 mM)	NaCl (1 M)
	% of total adducts recovered from HPLC		
Adduct*			
trans dGuo	93	83	66
cis dGuo	≈1	5	21
trans dAdo	5.7	10	7.8
cis dAdo	0.7	1.6	4.5
	Fraction of cis adducts		
Adduct†			
(+) dGuo	≈0	≈0	0.14
(−) dGuo	≈0.07	0.36	0.65
(+) dAdo	0.15	0.17	0.49
(−) dAdo	0.09	0.12	0.26

*Epoxide ring opening configuration and alkylation site.
†*anti*-BPDE enantiomer and alkylation site.

Table 2. Effect of NaCl concentration (0, 0.25, or 1 M) on the cis fraction of *anti*-BPDE adducts formed with poly(G) and dAMP

Alkylation target	BPDE enantiomer	Fraction of cis adducts		
		0 M	0.25 M	1 M
dAMP	+	0.35		0.87
	−	0.56		0.84
poly(G)	+	0.145	0.173	
	−	0.174	0.296	

1% with no added salt, 6% with 10 mM $MgCl_2$, and 24% with 1 M NaCl (Table 1). Compared to samples with no added salt, the amount of cis dGuo plus cis dAdo adducts as a fraction of total adducts goes up 4-fold with 10 mM $MgCl_2$ and 15-fold with 1 M NaCl. The fraction of dAdo adducts that form with the cis configuration more than triples with 1 M NaCl (from 0.11 to 0.37) but increases only slightly with 10 mM $MgCl_2$ (Table 1). The amount of cis dAdo adducts as a fraction of total adducts (dAdo plus dGuo) goes up 2-fold with 10 mM $MgCl_2$ and 6-fold with 1 M NaCl. The total cis adduct yield goes from <2% with no added salt to 26% at 1 M NaCl.

The HPLC profiles of adducts formed between (±)-*anti*-BPDE and dAMP in the presence and absence of 1 M NaCl are shown in Fig. 1. Unlike poly(G) and DNA, dAMP exhibits little selectivity for trans adduct formation at low salt concentration (the trans/cis adduct ratios are between 0.75 and 2 for both BPDE enantiomers). However, as in the case of DNA, larger proportions of cis adducts are formed at higher salt concentration with both poly(G) and dAMP (Table 2). The salt concentration used with poly(G) was limited by the tendency of the nucleic acid to precipitate.

Adduct Recovery. A potential problem in making measurements of the ratios of different DNA adducts is the possibility that the hydrolytic enzymes used to release the adducts from the polynucleotide might be selective, which could lead to spurious results if digestion were incomplete. The maintenance of low levels of DNA modification and the use of large excesses of hydrolytic enzymes can obviate this concern by releasing the adducts quantitatively (26). We employed this approach in the present work and, in addition, determined that there was no significant loss of adducts during HPLC. The total recovery of injected radioactivity (dpm) in the adduct fractions collected from the HPLC eluate ranged from 80% to 100%.

Mechanism of Chloride-Catalyzed Cis-Adduct Formation. Fig. 2 depicts the principal mechanism of chloride-catalyzed cis-adduct formation. The chlorohydrin forms via S_N1 attack of chloride ion on the BPDE carbocation. Halohydrin formation can also occur via S_N2 attack of halide ion on BPDE (data not shown). The kinetics of the hydrolysis reaction indicate that the relative importance of the S_N1 and S_N2 reaction pathways varies with the nature of the halide ion and its concentration (40). Because it is less nucleophilic, chloride ion participates less in the S_N2 pathway than iodide or bromide.

DISCUSSION

The most widely accepted mechanism for the formation of adducts from benzo[*a*]pyrene, since the demonstration that

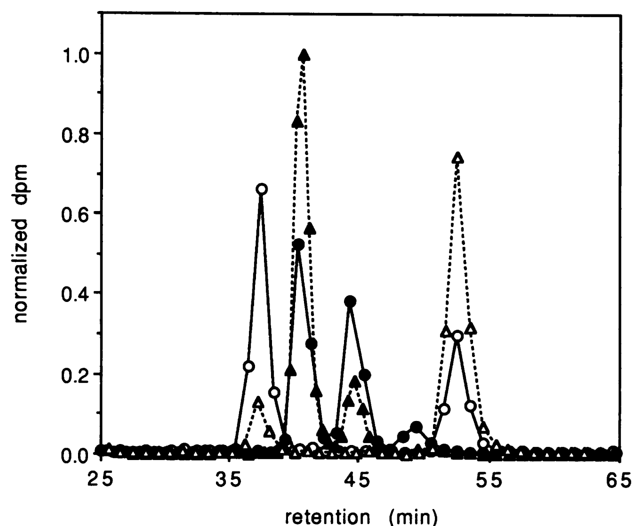

FIG. 1. Reverse-phase HPLC elution profiles of covalent adducts formed between dAMP and (−)-*anti*-[³H]BPDE in 0 M (—●—) or 1 M (- -▲- -) NaCl and between dAMP and (+)-*anti*-[³H]BPDE in 0 M (—○—) or 1 M (- -△- -) NaCl. All chromatograms were normalized to the same total amount of radioactivity, and the ordinate scale was adjusted to give a maximum peak height of 1. The retention times of the (−)-enantiomer, 0 M, and (+)-enantiomer, 1 M, profiles were multiplied by 1.02 and 0.965, respectively. The adducts are eluted in the following order: trans (+), cis (−), trans (−), cis (+).

BPDE is a metabolically activated intermediate in this process, involves direct attack of the C-10 carbocation of BPDE (or BPDE itself) on nucleophilic sites in DNA, RNA, or protein (7). Indeed, this mechanism has been proposed for many xenobiotic chemicals, which have been shown to undergo metabolic conversion to form epoxides (7, 23). Our results indicate, however, that at least in the case of cis BPDE adducts, the alkylation of DNA can occur via chlorohydrin intermediates. Both NaCl and $MgCl_2$ markedly increase the proportion of cis adducts obtained by alkylation of duplex DNA with *anti*-BPDE. In the absence of added chloride ion, cis dGuo adducts in DNA are nearly undetectable, whereas about 10% of the dAdo adducts exhibit a cis configuration. There is a direct relationship between the concentration of chloride ion and the level of cis adducts derived from both dGuo and dAdo.

We have found that iodide, bromide, and chloride ions catalyze the formation of *cis*-tetrol during hydrolysis of *anti*-BPDE. From studies of the chloride ion concentration dependence of the kinetics and product ratio of the hydrolysis reaction, we conclude that chloride catalysis of *cis*-tetrol formation results primarily from S_N1 attack by chloride ion on the C-10 carbocation of BPDE, resulting in the formation of a trans chlorohydrin, followed by an S_N2 attack by water, returning the C-10 position of the product to the original configuration. The mechanism proposed for the "chloride effect" observed in the hydrolysis of model epoxides (31–34) is similar but involves formation of the chlorohydrin via an S_N2 chloride attack on the epoxide as the rate-determining step. Attack of halide ions on *anti*-BPDE itself also occurs,

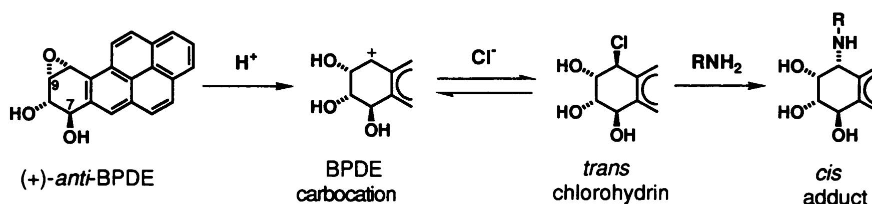

FIG. 2. Proposed mechanism of chloride-catalyzed cis adduct formation in the alkylation of DNA by the ultimate carcinogen *anti*-BPDE.

and the relative importance of this pathway increases going from chloride to bromide to iodide. Since chloride ion catalyzes the formation of cis products during both BPDE hydrolysis and BPDE–nucleic acid alkylation, we propose that the mechanism of the latter reaction is analogous, with the exocyclic amino group of a base making an S_N2 attack on a trans chlorohydrin. Heterolytic C—X bond energies are linearly related to the logarithm of the concentration of halide which provides half-maximal formation of *cis*-tetrol (r^2 = 0.985), which is consistent with the properties of halides as leaving groups in S_N2 reactions.

The S_N1 attack of halide ions on the BPDE carbocation should be capable of producing both trans and cis halohydrin intermediates, leading to cis and trans products, in a ratio that is difficult to predict. One difference between the halide catalysis of the hydrolysis and alkylation reactions is that in the former, the fraction of cis products never exceeds 40%, while in the latter, it can reach nearly 90% (Table 2).

Alkylation of DNA by chlorohydrin derivatives of BPDE undoubtedly occurs *in vivo*, and this reaction would tend to shift adduct formation toward the less tumorigenic, cis configuration. However, the significance of this pathway is limited by the low intracellular concentration of chloride ion (5–15 mM) (35). The extracellular chloride concentration is ≈110 mM, so the formation of chlorohydrin intermediates would be more prevalent in this region. These intermediates would participate in passive detoxification of BPDE by increasing formation of *cis*-tetrol. They could also react with nucleophiles, such as thiols, or with nucleophilic sites in proteins, to form adducts. The chloride effect would be primarily on product ratios, rather than on kinetics. The extent to which these reactions take place is not known. The sites of the multiple activation steps in the formation of BPDE from benzo[*a*]pyrene have not been determined, nor has the extent to which BPDE exists outside the cell. BPDE is more stable in a hydrophobic environment and would have a relatively long half-life when dissolved in membranes or bound to hydrophobic regions of proteins. Thus, transport of partially or fully activated benzo[*a*]pyrene to sites distant from its formation is possible. Similarly, chlorohydrins formed in other compartments might be able to alkylate nuclear DNA.

At low salt concentrations, alkylation of poly(G) yields more cis adducts than DNA, but the homopolymer still exhibits considerable selectivity for trans-adduct formation. The (−)- and (+)-enantiomers of *anti*-BPDE form 17% and 14% cis adducts, respectively. Poly(G) is thought to be a parallel-stranded tetraplex (36, 37). It is not enantioselective in its binding of *anti*-BPDE and thus differs in this respect from duplex DNA. We found very little trans adduct selectivity with dAMP, and a similar finding for dGMP has been reported (12). Poly(A), which is single-stranded at neutral pH (38), also is not enantioselective, and its trans-adduct selectivity is less than that of poly(G) (40).

A steric explanation has been proposed for the highly selective formation of trans *anti*-BPDE adducts by duplex DNA (12). *anti*-BPDE, as well as its carbocation, can adopt conformations in which its hydroxy groups are pseudodiaxial (conformer I) or pseudodiequatorial (conformer II) (12, 39). The conformations of the 7 and 8 hydroxy groups in cis and trans adducts have been shown to be pseudodiaxial and pseudodiequatorial, respectively, and it appears that the adduct benzo ring conformation is retained from that of the alkylating BPDE conformer (12). This led to the suggestion (12) that the less bulky conformer II intercalates into DNA, giving rise to trans adducts, whereas conformer I is sterically constrained to react from the outside of the helix, forming cis adducts. In support of this idea, it was noted that while mostly trans adducts were formed with double-stranded DNA (as we have also found for reactions at low chloride levels), roughly equal amounts of cis and trans adducts were formed with dAMP and dGMP. The reported data were obtained in reaction mixtures containing 95 mM chloride (12). However, our data on adduct formation with dAMP in the absence of chloride ion are consistent with this proposal.

The same proposal may also account for the fact that at both dGuo and dAdo alkylation sites in duplex DNA, the BPDE enantiomer that forms more adducts [the (+)-enantiomer in the case of dGuo and the (−)-enantiomer in the case of dAdo] forms a higher proportion of trans adducts. The enantiomer that alkylates a given site to a greater degree may be the one more able to intercalate there, or better able to attack the target amino group when intercalated. The enantiomer less able to alkylate via an intercalation complex would be more likely to alkylate via an alternative, nonintercalative pathway.

In conclusion, it is likely that the proportion of BPDE-nucleic acid adducts that form with the cis configuration is influenced by a number of factors, including the target secondary structure, the conformational distribution of the participating diol epoxide molecules, and the concentration of nucleophilic halide ions. Since cis and trans adducts apparently have different biological activities, it is important to understand the role of each of these factors in adduct formation.

Note Added in Proof. The accuracy for estimating minor adducts is improved by the use of normalized fluorescence content, and this technique results in a larger salt effect on cis adduct formation than reported here. Thus, the 3-fold difference in the occurrence of cis dAdo adducts formed in the presence of low and high salt increases to 4-fold when calculated on the basis of fluorescence content.

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1. Sims, P., Grover, P. L., Swaisland, A., Pal, K. & Hewer, A. (1974) *Nature (London)* **252**, 326–328.
2. Huberman, E., Sacks, L., Yang, S. K. & Gelboin, H. V. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 607–611.
3. Meehan, T., Straub, K. & Calvin, M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1437–1441.
4. Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H. & Nakanishi, K. (1976) *Science* **193**, 592–595.
5. Koreeda, M., Moore, P. D., Yagi, H., Yeh, H. J. & Jerina, D. M. (1976) *J. Am. Chem. Soc.* **98**, 6720–6722.
6. King, H. W. S., Osborne, M. R., Beland, F. A., Harvey, R. G. & Brookes, P. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2679–2681.
7. Thakker, D. R., Yagi, H., Levin, W., Wood, A. W., Conney, A. H. & Jerina, D. M. (1985) in *Bioactivation of Foreign Compounds*, ed. Anders, M. W. (Academic, New York), pp. 177–242.
8. 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.
9. 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. USA* **75**, 5358–5361.
10. Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Weinstein, I. B., Beland, F. A., Harvey, R. G., Kasai, H., Miura, I. & Nakanishi, K. (1976) *J. Am. Chem. Soc.* **98**, 5714–5715.
11. Straub, K. M., Meehan, T., Burlingame, A. L. & Calvin, M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5285–5289.
12. Cheng, S. C., Hilton, B. D., Roman, J. M. & Dipple, A. (1989) *Chem. Res. Toxicol.* **2**, 334–340.
13. Jeffrey, A. M., Grzeskowiak, K., Weinstein, I. B., Nakanishi, K., Roller, P. & Harvey, R. G. (1979) *Science* **206**, 1309–1311.
14. Yamamoto, J., Subramaniam, R., Wolfe, A. R. & Meehan, T. (1990) *Biochemistry* **29**, 3966–3972.
15. Sayer, J. M., Chadha, A., Agarwal, S. K., Yeh, H. J., Yagi, H. & Jerina, D. M. (1991) *J. Org. Chem.* **56**, 20–29.
16. Geacintov, N. E. (1988) in *Polycyclic Aromatic Hydrocarbon*

Carcinogenesis: Structure-Activity Relationships, eds. Yang, S. K. & Silverman, B. D. (CRC, Boca Raton, FL), Vol. 2, pp. 181–206.
17. Harvey, R. G. & Geacintov, N. E. (1988) *Acc. Chem. Res.* **21,** 66–73.
18. Gräslund, A. & Jernström, B. (1989) *Q. Rev. Biophys.* **22,** 1–37.
19. 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. USA* **89,** 1914–1918.
20. 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.
21. 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. (1993) *Biochemistry* **32,** 4145–4155.
22. Geacintov, N. E., Cosman, M., Mao, B., Alfano, A., Ibanez, V. & Harvey, R. G. (1991) *Carcinogenesis (London)* **12,** 2099–2108.
23. Miller, E. C. & Miller, J. A. (1985) in *Bioactivation of Foreign Compounds*, ed. Anders, M. W. (Academic, New York), pp. 3–19.
24. Essigmann, J. M., Green, C. L., Croy, R. G., Fowler, K. W., Büchi, G. H. & Wogan, G. N. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47,** 327–337.
25. Harvey, R. G. & Fu, P. P. (1978) in *Polycyclic Hydrocarbons and Cancer*, eds. Gelboin, H. V. & T'so, P. O. P. (Academic, New York), Vol. 1, pp. 133–165.
26. Meehan, T. & Straub, K. (1979) *Nature (London)* **277,** 410–412.
27. Yagi, H., Akagi, H., Thakker, D. R., Mah, H. D., Koreeda, M. & Jerina, D. M. (1977) *J. Am. Chem. Soc.* **99,** 2358–2359.
28. Englander, J. J., Kallenbach, N. R. & Englander, S. W. (1972) *J. Mol. Biol.* **63,** 153–169.
29. Chen, F.-M. (1983) *Nucleic Acids Res.* **11,** 7231–7250.
30. Fasman, G. D., ed. (1975) *Handbook of Biochemistry and Molecular Biology* (CRC, Cleveland, OH), 3rd Ed., Vol. 1, p. 196.
31. Whalen, D. L., Ross, A. M., Dansette, P. M. & Jerina, D. M. (1977) *J. Am. Chem. Soc.* **99,** 5672–5676.
32. Hylarides, M. D., Lyle, T. A., Daub, G. H. & Jagt, D. L. (1979) *J. Org. Chem.* **44,** 4652–4657.
33. Becker, A. R., Janusz, J. M. & Bruice, T. C. (1979) *J. Am. Chem. Soc.* **101,** 5679–5687.
34. Ross, A. M., Pohl, T. M., Piazza, K., Thomas, M., Fox, B. & Whalen, D. L. (1982) *J. Am. Chem. Soc.* **104,** 1658–1665.
35. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1989) *Molecular Biology of the Cell* (Garland, New York), p. 301.
36. Zimmerman, S. B., Cohen, G. H. & Davies, D. R. (1975) *J. Mol. Biol.* **92,** 181–192.
37. Guschlbauer, W., Chantot, J.-F. & Thiele, D. (1990) *J. Biol. Struct. Dyn.* **8,** 491–511.
38. Dewey, T. G. & Turner, D. H. (1979) *Biochemistry* **18,** 5757–5762.
39. Yagi, H., Sayer, J. M., Thakker, D. R., Levin, W. & Jerina, D. M. (1987) *J. Am. Chem. Soc.* **109,** 838–846.
40. Wolfe, A. R. & Meehan, T. (1994) *Chem. Res. Toxicol.*, in press.