Lack of Correlation between Degree of Interference with Transcription and Rate of Strand Specific Repair in the HPRT Gene of Diploid Human Fibroblasts

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The model that transcription-coupled excision repair reflects the interference of DNA damage with the transcription process predicts that the rate of such excision repair will be related to the degree to which a particular type of lesion blocks transcription. We tested this by measuring the rate of excision repair of guanine adducts formed in the HPRT gene of diploid human fibroblasts and in the overall genome by two structurally related polycyclic carcinogens, 1-nitrosopyrene (1-NOP) and N-acetoxy-2-acetylaminofluorene (N-AcO-AAF) and comparing the results with those we found previously using benzo[a]pyrene diol epoxide (BPDE). We also measured the degree of interference with in vitro transcription by these adducts. Our results showed that, although BPDE adducts are four times more effective than 1-NOP adducts in blocking transcription, the preferential and strand-specific repair of 1-NOP adducts was twice as fast as that of BPDE adducts. Excision repair of N-AcO-AAF adducts was significantly slower than that of BPDE adducts and was not strand-specific. The efficiency of blocking of transcription by deacetylated N-AcO-AAF adducts was similar to 1-NOP adducts. Therefore, the extent to which a particular lesion blocks transcription in vitro does not predict its rate of preferential or transcription-coupled excision repair.

Ultraviolet light-induced cyclobutane pyrimidine dimers (CPD) are excised more rapidly from transcriptionally active regions than from inactive regions of the genome of human cells (preferential repair) (1) and more rapidly from the transcribed strand of active genes than from the complementary nontranscribed strand (strand-specific repair). Synchronized populations of diploid human fibroblasts irradiated with 10 J/m² excise 50% of the CPD in the transcribed strand of the hypoxanthine phosphoribosyltransferase (HPRT) gene within 4 h and in the nontranscribed strand within 8 h; 50% are excised from the overall genome in ~12 h (2). In a similarly designed study, Chen et al. (3) showed that excision repair of benzo[a]pyrene diol epoxide (BPDE)-induced adducts is also preferential and strand-specific. However, the rate of excision of BPDE adducts by these cells is 2-fold slower than that of CPD.

Such strand-specific repair, which affects the spectrum of mutations in active genes (4, 5), has been shown to be the result of coupling of transcription and nucleotide excision repair. In Escherichia coli, transcription and repair are coupled by the mfd gene product (6), and mutations in the human homolog have been postulated to be the genetic defect in some patients with Cockayne syndrome, a disorder of development and DNA repair (7, 8). To explain this preferential and transcription-coupled repair, models have been proposed in which RNA polymerase that is stalled at a CPD acts as a signal to the mfd-like gene product, which releases the stalled RNA polymerase, or causes it to back up, thus exposing the damage and targeting the excision repair apparatus to the lesion (9, 10). This model predicts that the rate of preferential excision repair of a specific type of DNA lesion in the transcribed strand of an active gene will be proportional to the extent to which the lesion blocks transcription. To test this hypothesis, and to determine whether structurally related polycyclic adducts are preferentially repaired and exhibit strand-specific repair in diploid human fibroblasts in culture, we measured the rate of repair of adducts formed by 1-nitrosopyrene (1-NOP) and N-acetoxy-2-acetylaminofluorene (N-AcO-AAF) from the individual strands of the HPRT gene and from the overall genome, and compared the results with those we had obtained with BPDE-induced adducts. We also compared the degree of interference with T7 RNA polymerase transcription caused by adducts formed by these carcinogens.

All three agents bind principally to guanine. BPDE, a reactive intermediate of benzo[a]pyrene, binds to the N² position of guanine by a covalent bond between the nitrogen and the carbon at position 10 where the epoxide was located (dG-N²-BP) (11). The adduct is pentacyclic and lies in the minor groove B of the DNA molecule (12). 1-NOP is a partially reduced metabolite of 1-nitropyrene, a major environmental pollutant and a by-product of incomplete diesel combustion (13). 1-NOP must undergo a further intracellular reduction step before it forms an unstable reactive intermediate that binds covalently to the C-8 position of guanine to form the stable tetracyclic adduct N-deoxyguanosin-8-yl-1-aminopyrene (dG-C8-AP) (14) (Fig. 1). N-AcO-AAF is a direct acting compound that spontaneously
Fig. 1. Structures of the adducts induced by N-AcO-AAF and by 1-NOP.

loses the acetoxy ester, generating a reactive electrophilic intermediate that also binds principally (>95%) to the C-8 position of guanine (15). In human cells, N-AcO-AAF is rapidly deacetylated before binding so that its principal adduct is the tricyclic deacetylated residue on the C-8 position of guanine, i.e. N-(deoxyguanosin-8-yl)-2-aminofluorene (dg-C8-AP) (16) (Fig. 1). Both dg-C8-AP and dg-C8-AF lie in the major groove of the DNA molecule (17, 18).

dg-C8-AP was much less effective than dg-N2-BP in blocking T7 RNA polymerase transcription. Repair of dg-C8-AP was preferential, strand-specific, and very rapid, i.e. twice as fast as repair of dg-N2-BP. In contrast dg-C8-AF, which blocked transcription to the same extent as dg-C8-AP, was repaired slowly, and the repair was not strand-specific. The results indicate that the extent to which an adduct arrests transcription in vitro does not predict its rate of transcription-coupled repair.

EXPERIMENTAL PROCEDURES

Cell Culture and Synchronization—Diploid human fibroblasts from the foreskin of a neonate (19) were cultured in Eagle’s minimal essential medium modified as described previously (20) containing 10% supplemented calf serum (Hyclone, Logan, UT). Cells were driven into G0 by density inhibition and nutrient deprivation as described previously (22). The number of covalently bound residues (specific activity, 484 mCi/mmol) was obtained from Chemsyn Science Laboratories (Lenexa, KS) dissolved in tetrahydrofuran. The culture medium on the cells was removed, the cells were rinsed with phosphate-buffered saline, and the membranes were washed as described and exposed to Kodak XAR-5 x-ray films with intensifying screens or to phosphor storage cassettes.

The intensities of the bands were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The intensity of the full-length BamHI band was normalized to the intensity of the parental BamHI band. The average number of UvrABC-sensitive sites/fragment was calculated by the Poisson distribution (26). These calculations took into consideration the very low number of nonspecific incisions produced by the UvrABC endonuclease.

Determining the Degree of Interference With RNA Transcription Induced by ds-C8-AP, dg-C8-AF, or dg-N2-BP—For dg-C8-AP, plasmid pG2pa was dissolved in 10 mM Tris, 1 mM EDTA, pH 8.0, at a concentration of 500 μg/ml and the compound was dissolved in tetrahydrofuran. The culture medium on the cells was removed, the cells were rinsed with phosphate-buffered saline, and the medium was replaced with serum-free medium. The compound diluted in Me2SO was delivered into the individual dishes by micropipette. Preliminary studies showed that, unlike what happens with N-AcO-AAF adducts, with 1-NOP-induced damage at a level of about 0.5 adducts/20 kb (25/106 nucleotides), the onset of S-phase was delayed long enough to allow measurements over a 16-h period. Therefore, for repair of N-OAP adducts from the overall genome, the cells were synchronized and treated for 1 h in early G1 phase. Immediately following treatment or after 5 or 16 h of incubation in fresh culture medium, the cells were harvested, and the DNA was isolated as described previously (22). The number of covalently bound residues (adducts) per 10^9 nucleotides determined from the A_{260} of the DNA solution and the specific radioactivity. To avoid possible interference with repair studies as the result of S-phase DNA replication, for N-AcO-AAF a double-label technique was used. The DNA was asynchronously growing cells was labeled with [3H]thymidine (specific activity, 59 mCi/mmol) for 48 h. Then, the cells were exposed to tritiated N-AcO-AAF for 30 min and harvested immediately or after 18, 40, or 72 h of incubation in fresh culture medium, and the DNA was isolated as described previously (22). The initial number of AF residues bound was determined as described above, and the percentage remaining on parental DNA at the indicated times was calculated from the [3H]/[3C] ratio at these times divided by the ratio at time zero, as described previously (23).

Determining Rates of Repair in the Individual Strands of the HPRT Gene—The methods used are essentially those we employed previously to measure strand-specific repair of BPDE adducts in human fibroblasts (3). Briefly, synchronized populations of cells were treated with 1-NOP or N-AcO-AAF for 1 h in early G1 phase and harvested immediately or after the indicated times for repair. The cells were washed with phosphate-buffered saline and lysed in lysis buffer. The cell lysates were incubated at 56 °C for 8–12 h. DNA was extracted as described previously (22), precipitated, resuspended in TE buffer, and treated with RNase A. The DNA was re-extracted as above, precipitated with ETOH, and dissolved in TE buffer at 0.2 mg/ml. The purified DNA was digested with BamHI (5 units/μg DNA) overnight at 37 °C; the completion of digestion was verified by electrophoresis, and the digested DNA was purified by extraction and precipitation as above.

Purified DNA (12 μg) was used for repair analysis. As an internal standard, 10 pg of the plasmid used to synthesize the riboprobe was added to each sample. The samples were divided in half; one half was exposed to UvrABC endonuclease (17 pmol of each subunit); the other to exonuclease buffer alone. The enzyme subunits were a generous gift from Dr. P. van de Putte (Leiden University, The Netherlands). After 1 h at 37 °C, the reaction was stopped by the addition of proteinase K and SDS, and the DNA was purified by ultrafiltration and precipitated with EtOH.

The DNA samples were denatured in 90% formamide and analyzed by electrophoresis in Tris borate/EDTA buffer as described previously (3). After electrophoresis, the gel was stained with ethidium bromide, acid-depurinated, equilibrated with 0.4 μl NaOH, and transferred for 40–48 h to a Zeta Probe GT membrane (BioRad) under alkaline conditions (3).

Plasmid pG2pa (24), constructed by subcloning a 1.4 kb of EcoRI-XhoI fragment containing sequences from intron 1 of the human HPRT gene into the vector pGEM2, was kindly provided by A. C. Chinault (Baylor College, Houston, TX). It contains the SP6 and T7 promoters on either side of the insert. RNA transcripts hybridizing strand-specifically were generated as described previously (3, 25). Hybridizations were performed in 5 ml of solution as described previously (3), using ~10^7 cpm of [3P]labeled probe at 42 °C for 20–24 h. After hybridization, the membranes were washed as described and exposed to Kodak XAR-5 x-ray films with intensifying screens or to phosphor storage cassettes.

The intensities of the bands were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The intensity of the full-length BamHI band was normalized to the intensity of the parental BamHI band. The average number of UvrABC-sensitive sites/fragment was calculated by the Poisson distribution (26). These calculations took into consideration the very low number of nonspecific incisions produced by the UvrABC endonuclease.

Determining the Degree of Interference With RNA Transcription Induced by ds-C8-AP, dg-C8-AF, or dg-N2-BP—For dg-C8-AP, plasmid pG2pa was dissolved in helium-purged sodium citrate buffer (10 mM, pH 5.0) at a concentration of 250 μg/ml. A 5-μl aliquot of a freshly prepared solution of ascorbic acid (20 mM in H2O) was added to provide needed reduction of 1-NOP, followed by 0–15 μl of a 0.5 mM stock solution of generally tritiated 1-NOP dissolved in Me2SO. The final reaction volume was 200 μl. These samples were mixed and incubated for 3 h at 37 °C. For dg-C8-AP, plasmid pG2pa was treated with generally tritiated N-hydroxy-2-aminofluorene exactly as described for 1-NOP, except ascorbic acid was omitted. The stock concentration was 1.4 mM, and the compound was dissolved in anhydrous ethanol. For BPDE, plasmid pG2pa was dissolved in 10 mM Tris, 1 mM EDTA, pH 8.0, at a concentration of 500 μg/ml (specific activity, 0.885 Ci/mmol), dissolved in tetrahydrofuran, were added to 200 μl of the plasmid solution. The samples were mixed and incubated at room temperature for 2 h.

Unbound carcinogen was removed by phenol-chloroform extraction followed by four successive ethanol precipitations. The mol of [3H]-labeled adduct bound per mol of plasmid was calculated from the A_{260} of the DNA and the specific activity, and the average number of adducts per transcribed strand of the 1.4-kb HPRT fragment was estimated. The plasmid was digested with EcoRI followed by phenol-chloroform
Degree of Transcription Blockage Does Not Predict Repair Rate

### TABLE 1

| Compound   | Dose | Percent survival | Repair time | Adducts/10⁶ Nucleotides of parental DNA | Percent repair |
|------------|------|------------------|-------------|----------------------------------------|---------------|
| 1-NOP      | 0.5  | 30               | h           | 27                                      | 0             |
|            | 5    | 0                | 16          | 6                                      | 56            |
|            | 16   | 12               | 18          | 14                                     | 22            |
|            | 40   | 14               | 72          | 10                                     | 44            |
| N-AcO-AAF  | 0.6  | 91               | 0           | 18                                     | 0             |
|            | 18   | 14               | 40          | 14                                     | 22            |
|            | 72   | 20               | 40          | 20                                     | 0             |
| N-AcO-AAF  | 1.6  | 51               | 72          | 20                                     | 47            |

Determining the Delay in Onset of DNA Replication Induced by Carcinogen Treatment—The rate of repair of carcinogen adducts from DNA is determined from the frequency of adducts initially formed in DNA and that remaining in DNA after particular periods of time. Two methods are used, one measures the frequency of UvrABC excinuclease-sensitive sites in the 5'-half of the HPRT gene and the other measures the frequency of radiolabeled adducts in total DNA. With either method, S-phase replication of DNA by the cells following treatment with carcinogen will dilute pre-existing parental DNA and give the appearance of a decrease in the frequency of adducts. For the majority of the experiments to be discussed, S-phase DNA replication did not occur because the target population was synchronized and exposed to carcinogen in early G1-phase, and the rate of repair was determined during the period prior to the onset of S-phase DNA synthesis. Watanabe et al. (21) in this laboratory showed that diploid human fibroblasts synchronized by being released from the density-inhibited G0-state by being plated at 10⁵ cells/cm² begin DNA replication after ~16 h. Treatment with polycyclic aromatic carcinogens causes a delay in the onset of such replication. To determine the extent of delay caused by the concentrations of 1-NOP or N-AcO-AAF to be used in the majority of the DNA repair studies, i.e., concentrations designed to introduce approximately one adduct/20-kb fragment of the HPRT gene (50 adducts/10⁶ nucleotides), we released cells from the density-inhibited resting state by plating them into a series of dishes at 10⁵ cells/cm², treated them 5 h later (in early G1-phase) with 1-NOP or N-AcO-AAF, and assayed the incorporation of [3H]thymidine into acid-insoluble material at various times after treatment.

As expected, in control populations DNA replication began ~16 h after release. Cells treated with 0.3 µM 1-NOP showed no incorporation of [3H]thymidine whatsoever during the 20-h period examined posttreatment. Cells treated with 1.2 µM N-AcO-AAF began DNA replication approximately 19 h after treatment, i.e. 24 h after release. This is at least an 8-h delay in the scheduled onset of S-phase. The absence of DNA replication during the period of interest, i.e. up to 19 h after exposure to N-AcO-AAF and up to at least 20 h after exposure to 1-NOP, simplified most of the assays of repair reported here because there was no dilution of parental DNA with nascent DNA during those times.

Determining the Rate of Repair of dG-C8-AP and dG-C8-AF from the Overall Genome—Synchronized cells were exposed to 0.5 µM [3H]-labeled 1-NOP in early G1-phase. The cells exhibited a survival of 30% and an initial adduct frequency of 27/10⁶ nucleotides. The rate of repair is shown in Table I. In the case of repair of N-AcO-AAF adducts in the overall genome, a double label technique was used to control for DNA replication. Asynchronously-growing cells, prelabeled with [14C]thymidine to be able to distinguish pre-existing parental DNA, were treated with 0.6 µM or 1.6 µM [3H]-labeled N-AcO-AAF. These cells exhibited an initial adduct frequency of 18 or 38/10⁶ nucleotides, respectively. Their slower rate of repair is also shown in Table I. This difference in rate did not reflect a greater cytotoxic effect (Table I).

Evidence of Strand-Specific Repair of dG-C8-AP But Not dG-C8-AF—Synchronized populations of cells were treated with 0.3 µM 1-NOP or 1.2 µM N-AcO-AAF in early G1-phase (5 h post release from G0). The UvrABC excinuclease-sensitive sites initially induced in the individual strands of the 20-kb BamHI fragment of the human HPRT gene or remaining after various lengths of time for repair were determined using Southern blotting (3). This fragment, which is in the 5'-portion of the gene, is entirely within the transcription unit (3). To avoid detection of pseudogenes, it was probed with a 1.4-kb strand-specific riboprobe that anneals to an intron sequence (27). Representative autoradiograms of such repair studies are presented in Figs. 2 and 3. The intensities of the full-length (20 kb) fragments were determined by densitometric scanning of the autoradiograms and/or by analysis of the image left on storage phosphor cassettes by the blots using a Molecular Dynamics PhosphorImager. The two methods yielded equivalent results.

The results are shown in Table II and are compared with each other and with the rates for overall genome in Fig. 4. In each instance, repair of adducts induced by 1-NOP was rapid and strand-specific; 80% of the dG-C8-AP in the transcribed strand had been removed in 8 h, and repair was virtually complete in 16–20 h. Repair in the nontranscribed strand was slower but was also close to completion (85%) by 16–20 h. The greatest difference in repair of the two strands was found after 4 h, when repair of the transcribed strand was 60–64% complete and of the nontranscribed strand was 30–31% complete. This 2-fold difference is equal to, or greater than, that of CPD in the same gene (2). Although this difference is less striking than in rodent cells, which have minimal repair of the nontranscribed strand, CPD in active genes of human cells are widely recognized to be strand-specifically repaired. In addition, the rate of repair of dG-C8-AP adducts in the HPRT gene was faster than that in the overall genome. In contrast, little if any repair of dG-C8-AF was detected after 5 h of repair. After 15 h,
only 33% of these adducts had been removed, and the rate of repair of adducts in the transcribed strand was not faster than in the nontranscribed strand. This rate was only slightly faster than the rate of repair from the genome overall during the same time period.

Interference of dG-C8-AP, dG-C8-AF, or dG-N2-BP with RNA Transcription—If the degree of interference with transcription is a predictor of the rate at which a particular type of DNA damage is excised from active genes, then dG-C8-AP ought to be an even more effective block to transcription than dG-N2-BP. dG-C8-AF ought to block transcription minimally, if at all. To test this, we treated plasmid pG2pa with various concentrations of tritiated 1-NOP, N-OH-AF, or BPDE and determined the number of adducts per plasmid and the degree of interference with the extent of incorporation of 32p-labeled UTP into nascent transcripts. The results (Fig. 5) showed that the prediction does not hold true. It required an average of 6.8 to 7.0 dG-C8-AP or dG-C8-AF in the transcribed strand to reduce transcription to 37% of the untreated control, whereas with dG-N2-BP, 1.6 was sufficient.

**DISCUSSION**

The basis of the use of Southern blotting as a method to determine rates of repair in specific sequences is the ability of the fragment of interest to remain full-size, i.e. the probability that the fragment of interest does not contain any damage that would cause it to be cut into a smaller sized fragment. If one can assume that such damage is randomly distributed among the population of HPRT genes and is sensitive to enzymatic excision, the Poisson distribution function, i.e. P(0) = e−x, where P(0) is the percentage of fragments with no damage and x is the average number of enzyme-sensitive sites/fragment, yields information on the frequency of adducts. To interpret the results, one must introduce a sufficient level of damage to evaluate the P(0). Under our experimental conditions, 0.3 μM 1-NOP and 1.2 μM N-AcO-AAF resulted in this level of-adduct formation/20-kb strand of the HPRT gene (Table II). This level of damage reduced the colony-forming ability (i.e. the survival) of treated cells to 8% in the case of 1-NOP and 51% in the case of N-AcO-AAF. These results are in excellent agreement with previous results from this laboratory correlating survival of colony-forming ability with the frequency of such adducts induced in the overall genome of excision repair-proficient fibroblasts as determined using labeled carcinogen (28, 29). If the level of adduct formation in the 5′-half of the HPRT gene is similar to that in the overall genome, the data indicate that under the conditions used in the present study, UvrABC recognized and incised the majority of the adducts induced by either agent.

We determined the rate of nucleotide excision repair of adducts induced in DNA by the carcinogens 1-NOP and N-AcO-AAF in the individual strands of the HPRT gene and in the overall genome (global repair). Our results indicate that dG-C8-AP is repaired rapidly and that the excision repair is preferential and strand-specific and twice as rapid as the rate of repair ofadducts induced in DNA by the carcinogens 1-NOP and N-AcO-AAF in the individual strands of the HPRT gene and in the overall genome (global repair). Our results indicate that dG-C8-AP is repaired rapidly and that the excision repair is preferential and strand-specific and twice as rapid as the rate of repair of corresponding dG-N2-BP. In contrast, we found that excision of dG-C8-AP proceeded very slowly, much slower that dG-N2-BP, and was not strand-specific. Yet our data on interference with transcription (Fig. 5), showed that only 1.6 dG-

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**TABLE II**

| Compound      | Percent survival | Repair time | Transcribed strand | Nontranscribed strand |
|---------------|------------------|-------------|--------------------|-----------------------|
|               |                  |             | Incisions/fragment | %                    | Incisions/fragment | %                    |
| 1-NOP         | NDb              | h           | %                  | 0                    | 1.29                | 0                    |
|               | 0                | 8           | 0.38               | 65                   | 0.68                | 47                   |
|               | 0                | 20          | 0.12               | 89                   | 0.28                | 78                   |
|               | 0                | 4           | 1.40               | 0                    | 1.30                | 0                    |
|               | 0                | 8           | 0.50               | 64                   | 0.90                | 31                   |
|               | 0                | 16          | 0.19               | 86                   | 0.55                | 58                   |
|               | 0.02             | 99          | 0.16               | 60                   | 0.44                | 31                   |
| 1-NOP         | 0.08             | 86          | 0.03               | 95                   | 0.11                | 83                   |
|               | 0.08             | 86          | 0.03               | 95                   | 0.11                | 83                   |
| N-AcO-AAF     | 0.90             | 7           | 0.90               | 7                    | 0.97                | 14                   |
|               | 0.90             | 7           | 0.90               | 7                    | 0.97                | 14                   |

a Calculated by image analysis on a PhosphorImager, as described. The nonspecific incisions have been subtracted. The average number of incisions was determined from two separate Southern blots of DNA derived from each repair time point.

b ND, not determined.
N2-BP were required to reduce transcription to 37% of that directed by the unmodified template while 6.8–7.0 dG-C8-AP or dG-C8-AF were required to do so. These data are in good agreement with the degree of interference with elongation by T7 RNA polymerase presented by site specifically placed dG-N2-BP (30), or dG-C8-AF (31). Caution must be exercised in extrapolating from in vitro transcription assays to the behavior of human RNA polymerases in vivo. However, Sorcher and Cordaro-Stone (32) showed that the absence of excision repair, 2.1 dG-N2-BP reduced transcription by human RNA polymerases of a transfected reporter gene to 37% of control. This is in agreement with the value of 1.6 found in the in vitro assay reported in this study for the same adduct. Xenopus RNA polymerase III has been reported to be blocked to a greater extent by site-specifically placed dG-C8-AF than predicted from in vitro assays (33). However, if human RNA polymerase II were also blocked by such lesions to a greater extent than predicted, our conclusions would still hold, namely, that the rate at which cells carry out preferential, strand-specific repair is not determined solely by the degree to which the adducts being excised block transcription.

Comparison of the structure of these adducts (Fig. 1) reveals that they are quite similar, although dG-C8-AP has four aromatic rings, whereas dG-C8-AF has two aromatic rings connected by a cyclopentane ring. NMR analysis of the conformation adopted by duplex DNA containing dG-C8-AF shows that the adduct does not cause substantial helix perturbation (34). The adducted guanine remains in the normal major groove of a relatively undisturbed B-type DNA (34, 35). More recently, Cho et al. (18) reported that the planar arylamine has minor conformers that may represent stacking interactions with neighboring bases, which could cause significant local conformational perturbation. Much less information on the conformation adopted by DNA containing dG-C8-AP is available, but Nolan et al. (17) showed that such adducts also lie in the major groove of DNA and cause little helical distortion but that there may be significant stacking interactions with neighboring bases.

Despite the apparent structural similarity of the two kinds of adducts, previous studies from this laboratory have shown marked differences in their cytotoxic and mutagenic effects. In a shuttle vector system, in which dG-C8-AP (23) or dG-C8-AF (36) were formed in plasmid pZ289 in vitro and the plasmid was allowed to replicate in repair-proficient human cells and then analyzed for mutations induced in a bacterial target gene carried on the plasmid, dG-C8-AP was found to be 4 times as effective in interfering with bacterial transformation than dG-C8-AF. Nevertheless, the frequency of mutations induced in the supF gene during replication of the plasmid in human cells was similar, as were the kinds of mutations seen. The location of base substitutions in the target gene (spectrum) was not identical (23, 36). Earlier studies in this laboratory on the cytotoxic and mutagenic effect of dG-C8-AP and dG-C8-AF in repair-proficient human cells showed that a level of 25 dG-C8-AP/106 nucleotides reduces the surviving fraction of a population of treated cells to 37% (29); dG-C8-AF is much less cytotoxic, i.e. 50 such adducts/106 nucleotides are needed to reduce survival to 37% in these cells (28). What is more, at a level of 25 adducts/106 nucleotides, dG-C8-AP yields 4 times as many HPRT mutants as dG-C8-AF (28, 37).

The molecular basis for the observed differences in cytotoxicity, mutagenicity, and rates of repair of structurally-related adducts may well lie in the local conformational alterations induced by DNA-adduct formation. Although limited data exist on the structure of dG-C8-AP and dG-C8-AF, general structural features that influence the biological response to these adducts can be inferred by comparison to the extensively
studied BPDE adducts. In contrast to dG-C8-AP or dG-C8-AF, BPDE adducts form primarily at the N^2 position of guanine, a site of base pairing, but the reorientation of the added guanine causes the (+)-anti-BPDE adduct to lie in the minor groove, with the pyrenyl ring oriented toward the 5'-end of the modified strand where it causes little helical distortion (12, 38). BPDE adducts (39) are significantly more cytotoxic and more mutagenic than dG-C8-AP (29) or dG-C8-AF (37). Like dG-C8-AP, BPDE-induced adducts are repaired from the human HPRT gene in a strand-specific manner (3), although at a rate 2-fold slower than that of dG-C8-AP. (The half-life of 1-NOP adducts in the transcribed strand was 4 h; in the nontranscribed strand was 8 h; and in the overall genome was 10 h, compared with 7.5, 15, and 24 h, respectively, for BPDE adducts.)

Sancar and colleagues (40) have recently proposed a model to explain the substrate specificity of the human repair excinuclease system in which the recognition subunit forms a stable complex with DNA in the region of adducted bases. Formation of the stable complex includes melting of the DNA in the region of the adduct. Such local unwinding is facilitated by adducts that interfere with base pairing, such as the N^2-guanine adduct formed by BPDE. However, this model does not explain why an adduct such as dG-C8-AP, which lies in the major groove and does not interfere with base pairing, is repaired so efficiently, nor why the structurally related dG-C8-AF is repaired so poorly. Our data indicate that the rate and mode (i.e. global versus transcription-coupled mechanisms) of excision repair are determined by a complex interplay of adduct structure, its accessibility to repair enzymes, its ability to arrest transcription, and local DNA conformation.

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REFERENCES

1. Bohr, V. A., Smith, C. A., Okamoto, D. S., and Hanawalt, P. C. (1985) Cell 40, 359–363.
2. Tung, B. S., McGregor, W. G., Wang, Y.-C., Maher, V. M., and McCormick, J. J. (1995) Mutation Research: DNA Repair, in press.
3. Chen, R.-H., Maher, V. M., Brouwer, J., van de Putte, P., and McCormick, J. J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5413–5419.
4. Vriend, G., van Roosmalen, W. M., Groen, N. A., Zdravkovic, M. Z., Simons, J. W., I. M., Lohman, P. H. M., and van Zeeland, A. A. (1989) Mol. Cell. Biol. 9, 1277–1283.
5. McGregor, W. G., Chen, R.-H., Lukash, L. L., Maher, V. M., and McCormick, J. J. (1991) Mol. Cell. Biol. 11, 1927–1934.
6. Selby, C. P., Witkin, E. M., and Sancar, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11574–11578.
7. Troelstra, C., van Goor, A., de Wit, J., Vermeulen, W., Bootsma, D., and Hoeijmakers, J. H. J. (1992) Cell 71, 939–953.
8. Henning, K. A., Li, L., Iyer, N., McDaniel, L. D., Reagan, M. S., Lerguski, R., Schultz, R. A., Stefanini, M., Lehmann, A. R., Mayne, L. V., and Friedberg, E. C. (1995) Cell 82, 555–564.
9. Selby, C. P., and Sancar, A. (1993) Science 260, 53–58.
10. Donahue, B., Yin, S., Taylor, J.-S., Reines, D., and Hanawalt, P. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8502–8506.
11. Weinstein, I. B., Effrey, A. M., Jensenet, K. W., Blobstein, S. H., Burkhart, R. G., Harris, C., Atupru, H., Oasal, H., and Nakashima, K. (1976) Science 193, 599–595.
12. Cosman, M., Chomski, C., Fiala, R., Hingerty, B. E., Singh, S. B., Ibanez, V., Margulis, L. A., Live, D., Geacintov, N. E., Broyde, S., and Patel, D. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1914–1918.
13. Rosenkranz, H. S. (1982) Mutat. Res. 101, 1–10.
14. Beland, F. A., Ribovich, M., Howard, P. C., Heflich, R. H., Kuriian, P., and Milos, G. E. (1986) Carcinogenesis 7, 1279–1283.
15. Beland, F. A., Broyde, K. L., and Cassandro, D. A. (1979) J. Chromatogr. 174, 177–186.
16. Porrier, M. C., Williams, G. M., and Yuspa, S. H. (1980) Mol. Pharmacol. 18, 501–507.
17. Nolan, S. J., Vyas, R. R., Hingerty, B. E., Ellis, S., and Shapiro, R. (1995) Carcinogenesis, in press.
18. Cho, B. P., Beland, F. A., and Marques, M. M. (1994) Biochemistry 33, 1373–1384.
19. McCormick, J. J., and Maher, V. M. (1981) in DNA Repair, A Laboratory Manual of Research Procedures (Friedberg, E. C., and Hanawalt, P. C., eds) Vol. 3B, pp. 501–523, Marcel Dekker, New York.
20. Wang, Y., Parks, W. C., Wige, J., Maher, V. M., and Battley, J. J. (1986) Mutation Res. 175, 107–114.
21. Vriend, G., Maher, V. M., and McCormick, J. J. (1985) Mutat. Res. 146, 285–294.
22. McCormick, J. J., Yang, D., Maher, V. M., Farber, R. A., Neuman, W., Peterson, W. D., Jr., and Pollack, M. S. (1986) Carcinogenesis 9, 2073–2079.
23. Yang, J.-L., Maher, V. M., and McCormick, J. J. (1988) Mol. Cell. Biol. 8, 196–202.
24. Lin, D., and Chinnault, A. C. (1988) Somat. Cell Mol. Genet. 14, 261–272.
25. Misko, T. P., A., Krieg, P. A., Reddi, G., Maniatis, T., Zinn, K. G., and Green, M. D. (1984) Nucleic Acids Res. 12, 7035–7056.
26. Bohr, V. A., and Okumoto, D. S. (1988) in DNA Repair: A Laboratory Manual of Research Procedures (Friedberg, E. C., and Hanawalt, P. C., eds) Vol. 3, pp. 347–366, Marcel Dekker, New York.
27. Chinnault, A. C., and Caskey, C. T. (1984) Prog. Nucleic Acids Res. 31, 295–313.
28. Heflich, R. H., Hazard, R. M., Lommel, L., Scribner, J. A., Maher, V. M., and McCormick, J. J. (1980) Somat. Cell Mol. Genet. 6, 397–404.
29. Patton, J. D., Maher, V. M., and McCormick, J. J. (1986) Carcinogenesis 7, 89–93.
30. Choi, D.-J., Marino-Alessandri, D. J., Geacintov, N. E., and Scicchitano, D. A. (1994) Biochemistry 33, 780–787.
31. Chen, Y.-H., and Bogenhagen, D. F. (1993) J. Biol. Chem. 268, 5849–5855.
32. Stadler, D. H., and Cordeiro-Stone, M. (1994) Carcinogenesis 15, 1093–1096.
33. Chen, Y.-H., Matsumoto, Y., Shibuata, S., and Bogahagen, D. F. (1993) Proc. Natl. Acad. Sci. U. S. A. 88, 9583–9587.
34. Broyde, S., and Hingerty, B. (1983) Biopolymers 22, 2423–2441.
35. Hingerty, B., and Broyde, S. (1986) J. Biol. Struct. Dyn. 4, 365–372.
36. Mah, M. C.-M., Maher, V. M., Thomas, H., Reid, T. M., King, C. M., and McCormick, J. J. (1989) Carcinogenesis 10, 2321–2328.
37. Aust, G. A., Drinkwater, N. L., Deben, K. C., Maher, V. M., and McCormick, J. J. (1984) Mutat. Res. 125, 95–104.
38. Singh, S., Hingerty, B. E., Singh, U. C., Greenberg, J., Geacintov, N. E., and Broyde, S. (1991) Cancer Res. 51, 3482–3492.
39. Yano, L. M., Maher, V. M., and McCormick, J. J. (1982) Mutat. Res. 94, 435–447.
40. Huang, J.-C., Hsu, D. S., Kazantsev, A., and Sancar, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12213–12217.