Articles

Mutagenic Specificity of (Acetylamino)fluorene-Derived DNA Adducts in Mammalian Cells†

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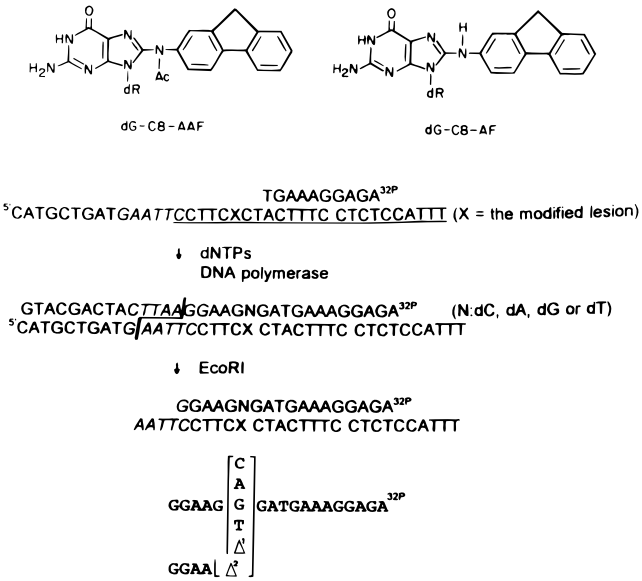
ABSTRACT: Site-specifically modified oligodeoxynucleotides were used to explore the mutagenic potential of dG-AAF and dG-AF adducts in mammalian cells. The miscoding properties of these arylamine adducts were established by analyzing fully extended products of primer extension reactions catalyzed by mammalian DNA polymerases α, *β*, and *δ*. On DNA templates containing dG-AAF, pol α generated two-base deletions and promoted incorporation of small amounts of dCMP, dAMP, and dTMP opposite the lesion. Reactions with pol *β* were associated exclusively with two-base deletions. Primer extension catalyzed by pol *δ* was strongly blocked by the adduct. On DNA templates containing dG-AF, all three DNA polymerases generated full-length products, preferentially incorporating dCMP opposite the lesion. A single-stranded shuttle vector containing 5′TCCTCCTCXCCTCTC (X = dG-AAF, dG-AF, or dG) was used to establish the frequency and specificity of dG-AAF- and dG-AF-induced mutations in simian kidney (COS-7) cells. Vectors containing a single dG-AAF or dG-AF adduct promote significant incorporation of dAMP and lesser amounts of dTMP opposite the lesion. dG-AAF also promoted some incorporation of dGMP and a two-base deletion. dG-AAF was 3.8 times more mutagenic than dG-AF (11% vs 2.9%) in COS cells. We conclude from this study that dG-AAF and dG-AF produce G → T transversions and, to a much lesser degree, G → A transitions in mammalian cells.

(Acetylamino)fluorene (2-AAF[1]) is a prototypic arylamine mutagen and chemical carcinogen. The genotoxicity of this agent and its metabolites has been reviewed by Heflick and Neft (*1*). Administration of activated forms of 2-AAF to animals or cells in culture leads to the formation of covalent DNA adducts, the predominant forms being *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene (dG-AAF) and *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-AF) (Scheme 1). dG-AAF is the principal adduct formed when DNA is treated with *N*-hydroxy-2-(acetylamino)fluorene (N-OH-AAF) (*2*). dG-AF is the major adduct recovered from DNA of rats treated with 2-AAF (*3*, *4*) and from CHO cells treated with *N*-acetoxy-2-(acetylamino)fluorene (N-AAAF) (*5*−*8*).

The mutagenic properties of dG-AAF and dG-AF have been examined extensively in bacteria; surprisingly, the mutational spectra of these adducts have not clearly been established for mammalian cells (*1*). Treatment of an SV40-based shuttle vector containing the *supF* gene with *N*-(trifluoroacetyl)-2-aminofluorene to produce dG-C8-AF adducts, or with *N*-acetoxy-*N*-(trifluoroacetyl)-2-aminofluorene, resulted mainly in point mutations, the majority of which were G → T transversions following transfection of the vector into human embryonic kidney cell lines (*9*). Using a site-

Scheme 1: Structures of dG-AAF and dG-AF and Procedures Used To Determine Miscoding Specificity



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[1] Abbreviations: dG, 2′-deoxyguanosine; dNTP, 2′-deoxynucleoside triphosphate; 2-AAF, 2-(acetylamino)fluorene; N-AAAF, *N*-acetoxy-2-(acetylamino)fluorene; dG-AAF, *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene; dG-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; pol α, DNA polymerase α; pol *β*, DNA polymerase *β*; pol *δ*, DNA polymerase *δ*; PCNA, proliferating cell nuclear antigen; PAGE, polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; t_R, retention time; Δ, deletion; ds, double strand; ss, single strand.

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specifically modified SV40-based shuttle vector that replicates in simian (COS-1) cells, Moriya et al. (*10*) reported that a single dG-AAF adduct generated mainly targeted G → C and G → T transversions. Using the endogenous *dhfr* gene in CHO cells as a mutational target, N-AAAF (*11*) and *N*-hydroxy-2-AF (*12*) produced similar spectra of mutations with G → T transversions being the predominant alteration.

Several studies of arylamine mutagenesis in mammalian cells yielded results that are inconsistent with the foregoing reports. Using the human *hprt* cDNA sequence integrated into chromosomal DNA of mouse VH-12 cells as a mutational target, nearly two-thirds of the mutations induced by N-AAAF were deletions (*13*). Among these deletions were a number of −1 frameshifts that occurred in runs of dAs. Gentil et al. (*14*) reported that reversion of a temperature-sensitive mutation in SV40, produced by N-AAAF treatment of the vector followed by replication in African green monkey kidney cells, was mainly due to base substitutions at A:T base pairs. Finally, Klein et al. (*15*) found that mutations were not associated with a site-specifically positioned dG-AAF adduct in a repair-deficient human cell/shuttle vector system.

In the present paper, single lesions introduced site specifically into oligodeoxynucleotides were used to explore the miscoding potential of dG-AAF and dG-AF in vitro and to establish the mutational specificity and mutagenic frequency of these adducts during extrachromosomal replication in mammalian cells.

EXPERIMENTAL PROCEDURES

Materials and Methods. [γ-^{32}P]ATP (specific activity, >6000 Ci/mmol) was obtained from Amersham Corp. Calf thymus DNA pol α (30 000 units/mg of protein) and human pol β (100 000 units/mg of protein) were purchased from Molecular Biology Resources, Inc. *Eco*RI restriction endonuclease (100 units/μL) was purchased from New England BioLabs. A Waters 990 HPLC instrument, equipped with a photodiode array detector, was used for separation and purification of oligodeoxynucleotides. UV spectra were measured with a Hewlett-Packard 8452A diode array spectrophotometer.

Synthesis of Oligodeoxynucleotides. DNA templates, primers, and standard markers shown in Scheme 1 were prepared by solid-state synthesis, using an automated DNA synthesizer (*16*). A 24-mer oligodeoxynucleotide containing a single dG-AAF or dG-AF (5′CCTTCXCTACTTTCCTCTCCATTT, X = dG-AAF, dG-AF) was prepared postsynthetically, as described previously (*17*). The modified 24-mer was ligated to an unmodified 14-mer (5′CATGCTGATGAATT) to form the 38-mer template (5′CATGCTGATGAATTCCTTCXCTACTTTCCTCTCCATTT) (*18*). Modified and unmodified oligodeoxynucleotides were purified on a reverse-phase μBondapak C_{18} column (0.39 × 30 cm; Waters), using a linear gradient of 0.05 M triethylammonium acetate, pH 7.0, containing 10→20% acetonitrile, an elution time of 60 min, and a flow rate of 1.0 mL/min (*17*). DNA templates and primers were purified by electrophoresis on a 20% polyacrylamide gel in the presence of 7 M urea (35 × 42 × 0.04 cm) (*17*). Bands were detected under ultraviolet light and extracted overnight with 2.0 mL of distilled water at 4 °C. Extracts were concentrated on a Centricon 3 filter (Amicon) by centrifugation at 5000 rpm for 2 h and then subjected to HPLC to remove urea (*17*). Oligonucleotides were labeled at the 5′ terminus by treatment with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (*19*) and subjected to acrylamide gel electrophoresis. The position and homogeneity of oligonucleotides following gel electrophoresis were determined by autoradiography using Kodak X-Omat XAR film.

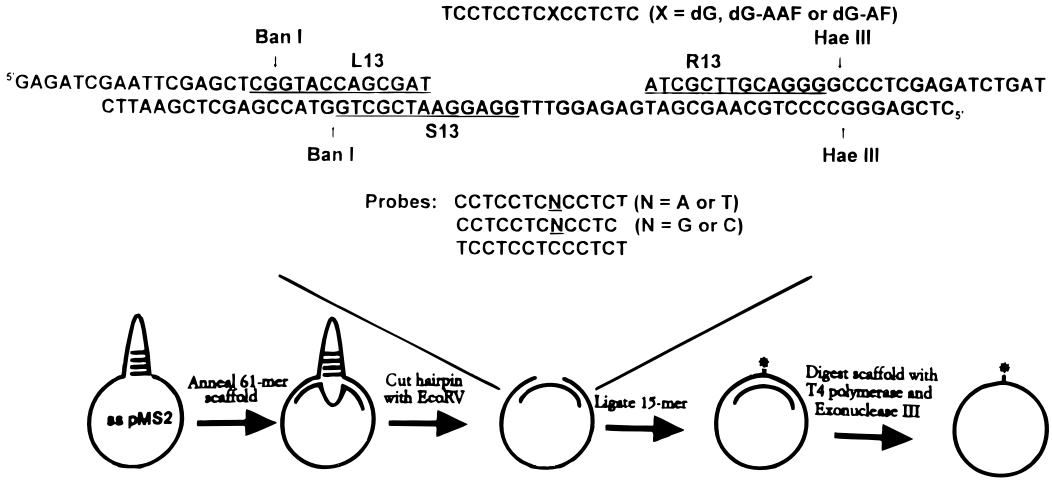
Primer Extension Studies. A 38-mer template (0.75 pmol) (see Scheme 1) annealed to a ^{32}P-labeled 10-mer primer (0.5 pmol of 5′AGAGGAAAGT) was used for primer extension experiments. Reaction mixtures (10 μM) containing pol α, pol β, or pol δ, template DNA, and all four dNTPs (100 μM each) were incubated at 25 °C (*20, 21*). The buffer for reactions with pol α or pol β consisted of 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol (DTT), and BSA (0.5 μg/μL). Pol δ and proliferating cell nuclear antigen (PCNA) prepared from *Xenopus laevis* oocytes (*22*) were a gift from Y. Matsumoto (Fox Chase Cancer Center). For reactions with pol δ, the buffer contained 50 mM Tris-HCl (pH 6.5), 10 mM KCl, 6 mM $MgCl_2$, 2 mM DTT, BSA (0.04 μg/μL), and PCNA (6 ng/μL). Reactions were stopped by adding formamide dye and heating the sample at 95 °C for 3 min. Samples were subjected to electrophoresis on a 20% polyacrylamide gel containing 7 M urea (35 × 42 × 0.04 cm). Bands were detected by autoradiography, and radioactivity was quantified with a Molecular Dynamics β-phosphorimager.

Quantitation of Miscoding Specificity. The procedure used to establish the miscoding properties of dG-AAF and dG-AF is diagramed in Scheme 1. Reactions containing a 38-mer template (0.75 pmol), a ^{32}P-labeled 10-mer primer (0.5 pmol), four dNTPs, and a purified DNA polymerase were incubated for 1 h at 25 °C. The reaction was stopped by heating at 95 °C for 3 min and then subjected to electrophoresis on 20% polyacrylamide gel containing 7 M urea (35 × 42 × 0.04 cm). Fully extended products were recovered from the gel and digested with *Eco*RI after annealing to the unmodified 38-mer (5′CATGCTGATGAATTCCTTCGCTACTTTCCTCTCCATTT). Samples were incubated with *Eco*RI (100 units) for 1 h at 30 °C and then for 1 h at 15 °C to ensure complete digestion of the reaction products; then they were subjected to electrophoresis on two-phase 20% polyacrylamide gels (15 × 72 × 0.04 cm) containing 7 M urea in the upper phase (*18*).

Site-Specific Mutagenesis in COS Cells. A SV40-transformed simian kidney cell line (COS-7) and a single-strand (ss) shuttle vector, pMS2, which confers neomycin (Neo^R) and ampicillin (Amp^R) resistance (*23*), were used to establish mutagenic specificity. A 15-mer oligomer containing a single dG (5′TCCTCCTCXCCTCTC, X = dG) was reacted with N-AAAF (*24*). An oligodeoxynucleotide containing a single dG-AAF at position X was purified by HPLC (*24*). An oligodeoxynucleotide containing dG-AF was prepared by removing the acetyl moiety from the dG-AAF-modified 15-mer under alkaline conditions (*17*). The neighboring bases of dG-AAF and dG-AF, underlined, are the same as the 38-mer that is used for in vitro studies.

A circular ss DNA containing a single DNA adduct was constructed according to procedures established previously in this laboratory (*23*). pMS2 ss DNA (*23*) was purified on a Nucleogen 4000-7 DEAE column (0.6 × 12.5 cm), using

Scheme 2: Construction of a Single-Strand Vector Containing dG-AF or dG-AAF



a linear gradient of 0.02 M potassium phosphate and 5 M urea, pH 6.9 (eluent A), containing 40→100% eluent A and 1.5 M KCl (eluent B), with an elution time of 90 min and a flow rate of 1.0 mL/min. The fraction containing pMS2 (t_R = 33.0 min) was concentrated on Centricon #100 filters, washed three times with distilled water, and subjected to ethanol precipitation. pMS2 DNA was annealed to a 61-mer and then digested with *Eco*RV to create a 15-mer gap (Scheme 2). dG-AAF- or dG-AF-modified 15-mers were ligated to the gapped vector. To establish ligation efficiency, a portion of the vector annealed to the 61-mer scaffold was digested with *Ban*I and *Hae*III, followed by exchange of the terminal phosphate residue using [γ-^{32}P]ATP and T4 polynucleotide kinase, and subjected to 12% denaturing polyacrylamide gel electrophoresis (*23*). The other portion of the ligation mixture was incubated for 2 h with T4 DNA polymerase (1 unit/pmol of DNA) to digest the hybridized 61-mer and then treated with *Eco*RV and *Sal*I to cleave residual ss pMS2. The reaction mixture was extracted twice with phenol/chloroform, 1:1 (v/v), and twice with chloroform. Following ethanol precipitation, the DNA was dissolved in distilled water. A portion of the ligation mixture and known amounts of ss pMS2 were subjected to electrophoresis on a 0.9% agarose gel to separate closed circular and linear ss DNA. DNA was transferred to a nylon membrane and hybridized to a ^{32}P-labeled S13 probe complementary to the DNA containing the 15-mer insert. The absolute amount of closed circular ss DNA was established by comparing the radioactivity in the sample with that in known amounts of ss DNA.

COS-7 cells were transfected over 18 h with ss DNA (100 fmol) using lipofection (*25*), after which the cells were grown for 2 days in Dulbecco's modified Eagle's medium/10% fetal calf serum. Progeny plasmids were recovered by the method described by Hirt (*26*), treated with S1 nuclease to digest input ss DNA, and used to transform *Escherichia coli* DH10B. Transformants were analyzed for mutations by oligodeoxynucleotide hybridization (*10*, *27*). The oligodeoxynucleotide probes used to identify progeny phagemids are shown in Scheme 2. Probes L13 and R13 were used to confirm that the gapped plasmids were constructed correctly. Transformants that failed to react with L13 and R13 were omitted from the analysis. When L13/R13-positive transformants failed to hybridize to probes designed to detect events targeted to the lesion site, double-strand (ds) DNA was prepared and subjected to dideoxynucleotide sequencing analysis (*28*).

RESULTS

Primer Extension Reactions Catalyzed by DNA Polymerases. In reactions catalyzed by pol α, primers annealed to an unmodified template were rapidly extended (Figure 1A). In contrast, primer extension on templates modified with dG-AAF was blocked one base before and opposite to the lesion; translesional synthesis was observed only when higher concentrations (4.8 U) of enzyme were used. On templates containing dG-AF, primer was readily extended to form full length products.

Two 13-mer bands were formed opposite dG-AAF. Using a long denaturing polyacrylamide gel (72 cm), the migration of these bands was compared to that of unmodified 13-mers containing dC, dA, dG, or dT at the 3′ terminus (data not shown). The upper and lower bands represent insertion of dGMP and dAMP, respectively, opposite the lesion.

Fully extended reaction products (Figure 1A) were recovered from the gel, cleaved with *Eco*RI, and subjected to two-phase gel electrophoresis (see Scheme 1 and Experimental Procedures). Standard oligomers containing dC, dA, dG, and dT opposite the lesion or one-base (Δ^1) and two-base (Δ^2) deletions were resolved by this procedure (Figure 1B, lanes 1 and 3). As expected, products synthesized on the unmodified template exclusively incorporated dCMP opposite dG at position 13 (data not shown). The presence of dG-AAF, a blocking lesion, was associated with Δ^2 (6.7% of starting primer) and Δ^1 (0.3%) accompanied by incorporation of small amounts of dCMP (0.5%), dAMP (0.2%), and dTMP (0.2%) opposite the lesion (lane 2). The presence of dG-AF at position 13 was associated with preferential incorporation of the correct base, dCMP (82%), accompanied by small amounts of dTMP (2.1%), dAMP (0.4%), dGMP (0.4%), and Δ^2 deletions (2.3%) (lane 4).

In reactions with pol β (Figure 2), primer extension past dG-AAF was blocked; at high concentrations of enzyme, small amounts of primer were extended to form fully extended products (Figure 2A). Using two-phase gel electrophoresis, these full length products were shown to contain two-base deletions (Figure 2B). For templates containing

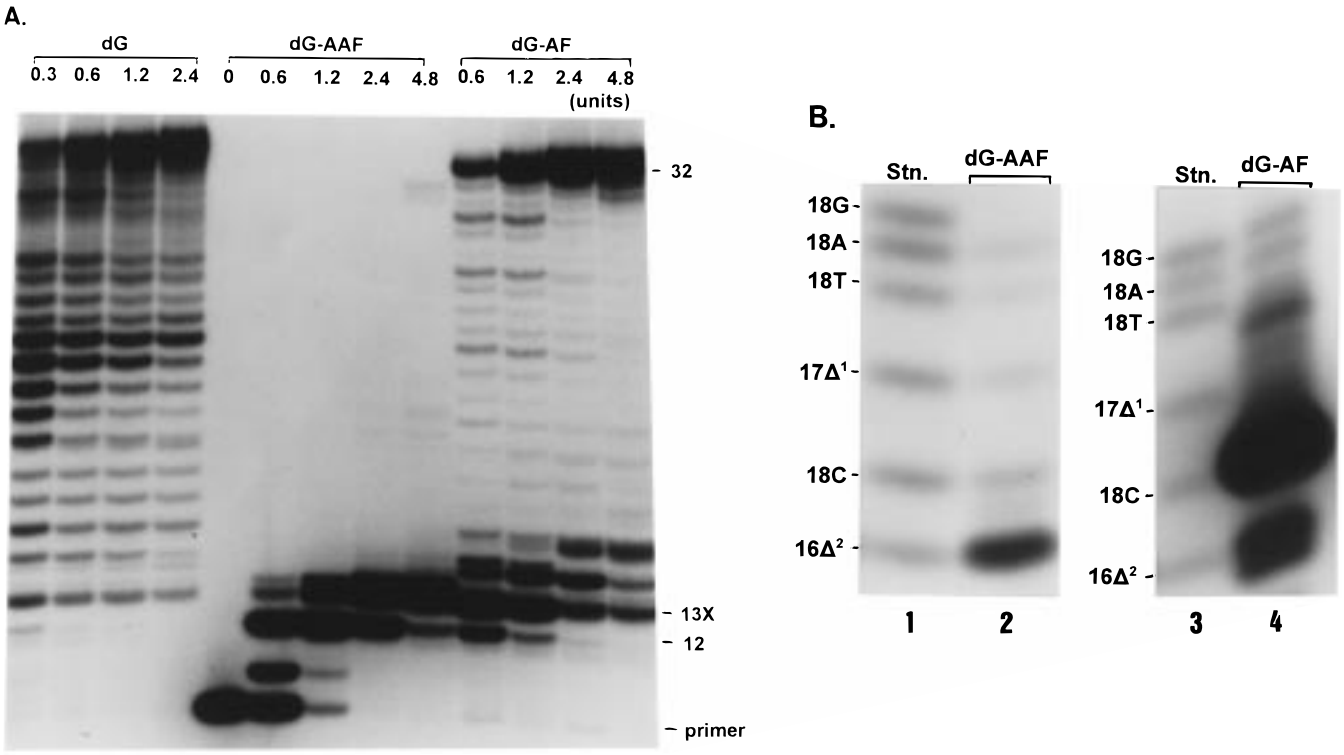


FIGURE 1: Miscoding on DNA templates in reactions catalyzed by pol α. (A) Using an unmodified or dG-AAF- or dG-AF-modified 38-mer template (5′CATGCTGATGAATTCCTTCXCTACTTTCCTCTCCATTT, X = dG, dG-AAF, or dG-AF), primed with a ^{32}P-labeled 10-mer (5′AGAGGAAAGT), primer extension reactions were incubated for 1 h at 25 °C with DNA pol α as described in Experimental Procedures. One-third of the reaction mixture was subjected to electrophoresis on a denaturing 20% polyacrylamide gel (35 × 42 × 0.04 cm). (B) Primer extension reactions incubated at 25 °C for 1 h with 4.8 units of pol α. Fully extended products were recovered from the gel and digested first with 100 units of *Eco*RI for 1 h at 30 °C and, additionally, for 1 h at 15 °C as described under Experimental Procedures. Aliquots were removed from the reaction mixture and subjected to two-phase 20% polyacrylamide gel electrophoresis. Mobilities of reaction products were compared with those of 18-mer standards containing dC, dA, dG, or dT opposite the lesion and 17-mers and 16-mers representing full length products with one-base (Δ^1) and two-base (Δ^2) deletions, respectively (lanes 1 and 3).

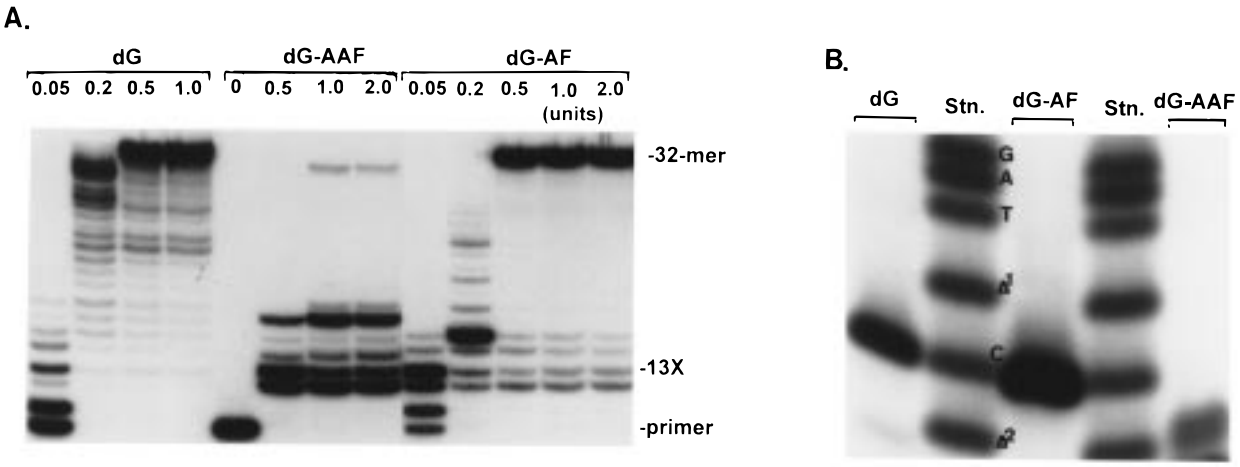


FIGURE 2: Miscoding on DNA templates in reactions catalyzed by pol β. (A) Primer extension reactions catalyzed by pol β were conducted at 25 °C for 1 h, and the products were treated as described in the legend to Figure 1. (B) Primer extension reactions were incubated at 25 °C for 1 h with 1.0 unit of pol β for unmodified oligomer and 2.0 units for modified oligomers, as described under Experimental Procedures. Fully extended products recovered from the gel were digested with *Eco*RI as described under Experimental Procedures and subjected to two-phase 20% polyacrylamide gel electrophoresis.

dG-AF, the rate of DNA synthesis was slightly reduced (Figure 2A) and dCMP, the correct base, was inserted opposite the lesion (Figure 2B).

Primer extension reactions catalyzed by pol δ were strongly blocked at position 12, one base before dG-AAF; fully extended products were not detected in this reaction (Figure 3A, lane 3). In contrast, pol δ catalyzed primer extension past dG-AF to form a full length product (6.0%) (lane 4). The product was analyzed by two-phase gel electrophoresis; only dCMP was incorporated opposite the lesion (Figure 3B).

Mutational Specificity in Vivo. Unmodified, dG-AAF-modified, and dG-AF-modified 15-mer oligonucleotides have the same neighboring bases as those used for in vitro studies. These oligomers were inserted into a gapped single-strand vector, as described under Experimental Procedures. The

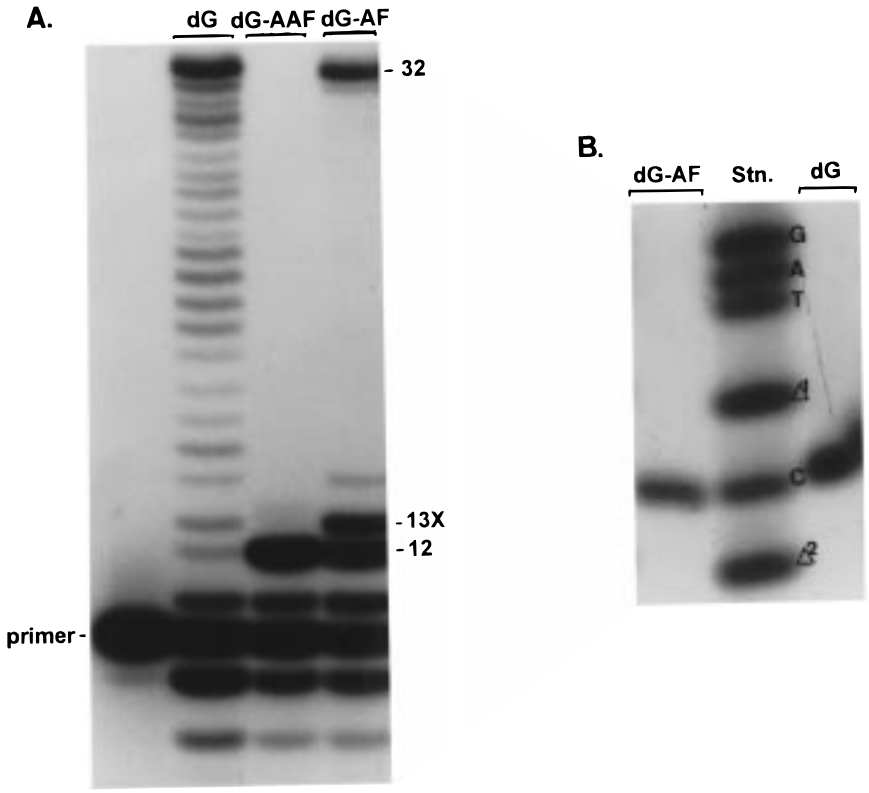


FIGURE 3: Miscoding on DNA templates in reactions catalyzed by pol δ. (A) Primer extension reactions were conducted at 25 °C for 30 min using 0.03 unit of pol δ, as described under Experimental Procedures. One-third of the reaction mixture was subjected to denaturing 20% polyacrylamide gel electrophoresis (35 × 42 × 0.04 cm). (B) Fully extended products generated in the experiment shown in panel A were recovered from the gel and digested with *Eco*RI, as described in Experimental Procedures. Miscoding properties were determined as described in the legend of Figure 1B.

Table 1: Transformation of COS Cells with ss DNA

ss DNA[a]	no. of transformants
pMS2 ($^{5'}$G)	27600 ± 1900 (100%)
pMS2 ($^{5'}$G^{AAF})	19500 ± 2300 (70.6%)
pMS2 ($^{5'}$G^{AF})	18400 ± 4600 (66.7%)

[a] 100 fmol of ss DNA was transfected into COS cells. Progeny phagemid was used to transform *E. coli* DH10B for ampicillin resistance.

construct was cleaved with *Ban*I and *Hae*III, followed by exchange of the terminal phosphate residue using [γ-^{32}P]-ATP and T4 polynucleotide kinase, and then subjected to 12% denaturing polyacrylamide gel electrophoresis (Figure 4). The digestion product containing the unmodified 15-mer migrated identically to the 40-mer standard marker (lane 1); products containing dG-AAF or dG-AF (lanes 2 and 3) migrated more slowly. The 40-mer containing dG-AF migrated slightly less than the 40-mer containing dG-AAF. These results are consistent with the analysis of dG-AAF- and dG-AF-modified oligomers reported previously (*17*).

pMS2 modified with AF or AAF was used to transfect COS-7 cells; the number of transformants recovered was compared to those obtained with an unmodified vector (Table 1). The presence of a single AAF residue reduced transformation efficiency to 71%. dG-AF also reduces transformation efficiency to 67% of control values. No significant difference between dG-AAF and dG-AF was observed.

Vectors modified with dG-AAF and dG-AF preferentially incorporated dCMP (89% and 97%, respectively) opposite the lesion (Table 2). Targeted G → T transversions (5.2%) were detected opposite dG-AAF, along with lesser amounts of G → A transitions (3.9%) and G → C transversions (1.3%). One two-base deletion was detected. dG-AF promoted small amounts of targeted G → T transversions (2.0%) and G → A transitions (1.0%). The overall mutation frequency of dG-AAF was 3.8 times higher than that of dG-AF. Some nontargeted mutations were observed using the dG-AAF- and dG-AF-modified plasmids. No mutations were observed with the unmodified vector.

DISCUSSION

Miscoding Potential of Aminofluorene Adducts. In reactions catalyzed by pol α, dG-AAF promoted formation of two-base deletions, accompanied by incorporation of small amounts of dAMP and dTMP. With pol β, only two-base deletions were detected. Primer extension was blocked in reactions catalyzed by pol δ. Except for misincorporation of dTMP, the pattern of miscoding by pol α on templates containing dG-AAF was similar to that observed in previous studies with the exo$^-$ Klenow fragment of *E. coli* DNA polymerase I (*21*, *24*).

dG-AAF presents a strong block to translesional synthesis, presumably due to distortion of the double-helical structure of DNA (*29*, *30*). The block, which begins at the position 3′ to the lesion, can be avoided when the incoming base can pair to a base 5′ to the adduct. The adduct, itself presumably displaced from the template, forms an extrahelical bulge. This lesion bypass is observed in experiments with pol α and pol β in which the fully extended product contains a two-base deletion. The newly inserted dAMP can pair with dT two

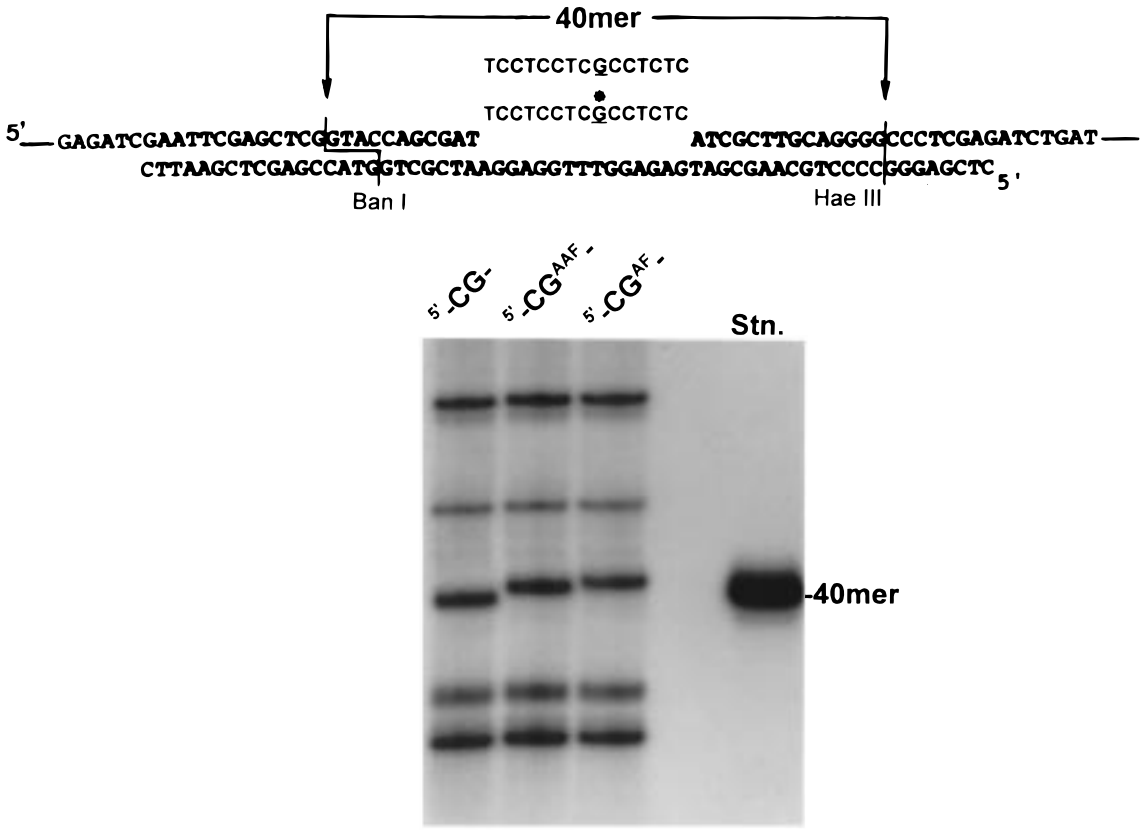


FIGURE 4: Construction and analysis of shuttle vector. A portion of the vector annealed to the 61-mer scaffold was digested with *Ban*I and *Hae*III, followed by exchange of the terminal phosphate residue using [γ-^{32}P]ATP and T4 polynucleotide kinase, and subjected to 12% denaturing polyacrylamide gel electrophoresis, as described in Experimental Procedures.

Table 2: Mutational Specificity of dG-AAF and dG-AF in COS Cells[a]

		targeted mutations (dG, dG-AAF, or dG-AF → X)					
plasmid		G	A	T	C	Δ^1	other[c]
pMS2 (dG)	expt 1[b]	134	0	0	0	0	0
	expt 2	139	0	0	0	0	0
	total	273 (100%)	0 (<0.4%)	0 (<0.4%)	0 (<0.4%)	0 (<0.4%)	0
pMS2 (dG-AAF)	expt 1	77	3	3	1	0	1
	expt 2	61	3	5	1	0	1
	total	138 (89%)	6 (3.9%)	8 (5.2%)	2 (1.3%)	0 (<0.7%)	2
pMS2 (dG-AF)	expt 1	94	2	3	0	0	1
	expt 2	104	0	1	0	0	2
	total	198 (97%)	2 (1.0%)	4 (2.0%)	0 (<0.5%)	0 (<0.5%)	3

[a] Adducted ss DNA (100 fmol) was used to transfect COS-7 cells. Progeny phagemid was used to transform *E. coli* DH10B and analyzed for mutations. [b] Expt 1 and 2 represent independent experiments. [c] Nontargeted mutations associated with a targeted event are listed below:

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5'TCCTCCTCG^AAFCCTCTC                5'TCCTCCTCG^AFCCTCTC

5'--------ΔΔ-------   Δ² deletion     5'-------AA-------   C → A

5'--A----AG---------   two C → A      5'-------TT-------   C → T

                                      5'-------TT-------   C → T
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positions 5′ to the lesion generating an intermediate that leads to a Δ^2 deletion. When dGMP is inserted opposite dG-AAF, it can pair with dC 5′ to the lesion to form a Δ^1 deletion.

The miscoding specificity of dG-AF was different from that observed with dG-AAF. On templates containing dG-AF, all three DNA polymerases preferentially catalyzed incorporation of dCMP opposite the lesion. Only the replicative polymerase, pol α, promotes misincorporation of dTMP, dAMP, and dGMP, together with two-base deletions. Significantly, with pol β and pol α, as in our studies with the Klenow fragment of pol I (*21*, *24*, *31*), dG-AF did not miscode. Thus, despite its bulky nature, dG-C8-AF does not interfere with extension of the base pair at the 3′ terminus when dCMP is positioned opposite the adduct. Structural studies show that dG-AF can adopt interconverting syn and anti alignments with the equilibrium shifting between the conformers depending on the sequence context (*32*, *33*).

Apparently, this structural flexibility permits correct pairing and lesion bypass.

Mutagenic Specificity in Mammalian Cells. A single-strand shuttle plasmid vector was used to establish mutagenic specificities for dG-AAF and dG-AF in simian kidney cells. A single dG-AAF and dG-AF adduct partially blocks DNA replication in this system. Mutational spectra for both adducts are characterized by G → T transversions and a lesser number of G → A transitions. dG-AAF also promotes small amounts of misincorporation of dGMP and generated a single two-base deletion.

The mutational spectra of dG-AAF differ slightly from previously reported shuttle vector experiments in COS cells (*10*). In that study, which employed a double-strand vector, targeted G → T and G → C transversions were produced, along with a few one-base deletions. DNA repair and/or strand switching occur in double-strand vectors which may account for the differences observed.

The primary mutagenic event produced by dG-AAF and dG-AF adducts in COS cells is G → T transversions. The mutagenic spectra observed in our study are similar to those reported for *dhfr* mutations induced by treatment of COS cells with N-OH-AF (*12*) and in the studies of Mah et al. (*34*).

dG-AAF adducts could deacetylate to form dG-AF (*17*); however, on the basis of its electrophoretic migration (Figure 4), the vector containing dG-AAF was not deacetylated prior to the transfection procedure. Using ^{32}P-postlabeling analysis, Mah et al. (*34*) reported that deacetylation of DNA adducts did not occur in CHO cells following transfection. In our experiments, mutational specificity and frequency of dG-AAF did not resemble that observed with dG-AF. Thus, mutational spectra reported here for dG-AAF appear to be an intrinsic property of the adduct and not the result of deacetylation.

Mechanism for Base Deletions. A general model involving template misalignment or incorporation of dNTPs followed by realignment has been proposed (*35*−*37*). With a kinetic approach and several environmental carcinogens, we have also proposed (*24*) a general mechanism for frameshift deletion mutagenesis in which the propensity for template misalignment depends on (a) the sequence context of the lesion, (b) the nature of the base inserted opposite the lesion, and (c) the frequency of translesional synthesis. This mechanism has been used to explain deletions formed by mammalian DNA polymerases (*18*). In vitro, one- and two-base deletions were readily formed. However, in COS cells, only two-base deletion was observed in the same sequence even though pol α readily promotes such deletions in vitro. The 3′→5′ exonuclease function of pol δ (*38*) acts in cells to minimize formation of deletions. Furthermore, formation of mispaired intermediates leading to base substitutions competes with formation of bulged intermediates that promote deletions (*24*). Proteins participating in DNA replication may stabilize the helical structure of DNA, preventing bulge formation. Under these conditions, translesional synthesis is facilitated and the number of base substitutions relative to deletions will increase.

CONCLUSIONS

In reactions catalyzed by purified mammalian DNA polymerases and in simian kidney cells, dG-AF promotes incorporation of dCMP opposite the lesion accompanied by misincorporation of small amounts of dAMP and dTMP. Thus, the mutagenic properties of dG-AF in mammalian cells correlate with the miscoding potential of this adduct. In contrast, dG-AAF is a strong blocking lesion in vitro, while in cells, translesional synthesis with preferential incorporation of dCMP and targeted misincorporation of dAMP and dTMP is observed. Large numbers of deletions were generated by dG-AAF in vitro but not in cells. Both lesions promote G → T transversions, but the mutational frequency of dG-AAF was approximately 4 times higher that of dG-AF. Taken together, these observations suggest that the mutagenic potential of arylamine DNA adducts may be modified by the presence of accessory proteins operating during translesional synthesis in mammalian cells (*38*).

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REFERENCES

1. Heflich, R. H., and Neft, R. H. (1994) *Mutat. Res. 318*, 73−174.
2. Howard, P. C., Casciano, D. A., Beland, F. A., and Shaddock, J. G., Jr. (1981) *Carcinogenesis 2*, 97−102.
3. Kriek, E. (1972) *Cancer Res. 32*, 2042−2048.
4. Beland, F. A., and Kadlubar, F. F. (1985) *Environ. Health Perspect. 62*, 19−30.
5. Carothers, A. M., Urlaub, G., Steigerwalt, R. W., Chasin, L. A., and Grunberger, D. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6519−6523.
6. Arce, G. T., Cline, D. T., Jr., and Mead, J. E. (1987) *Carcinogenesis 8*, 515−520.
7. Heflich, R. H., Morris, S. M., Beranek, D. T., McGarrity, L. J., Chen, J. J., and Beland, F. A. (1986) *Mutagenesis 1*, 201−206.
8. Heflich, R. H., Djurić, Z., Zhou, Fullerton, N. F., Casciano, D. A., and Beland, F. A. (1988) *Environ. Mol. Mutagenesis 11*, 167−181.
9. Mah, M. C.-M., Maher, V. M., Thomas, H., Reid, T. M., King, C. M., and McCormick, J. J. (1989) *Carcinogenesis 10*, 2321−2328.
10. Moriya, M., Takeshita, M., Johnson, K., Peden, K., Will, S., and Grollman, A. P. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1586−1589.
11. Carothers, A. M., Steigerwalt, R. W., Urlaub, G., Chasin, L. A., and Grunberger, D. (1989) *J. Mol. Biol. 208*, 417−428.
12. Carothers, A. M., Urlaub, G., Mucha, J., Yuan, W., Chasin, L. A., and Grunberger, D. (1993) *Carcinogenesis 14*, 2181−2184.
13. Iyehara-Ogawa, H., Kimura, H., Koya, M., Higuchi, H., and Kato, T. (1993) *Carcinogenesis 14*, 2245−2250.
14. Gentil, A., Margot, A., and Sarasin, A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 9556−9560.
15. Klein, J. C., Bleeker, M. J., Saris, C. P., Roelen, H. C. P. F., Brugghe, H. F., van den Elst, H., van der Marel, G. A., van Boom, J. H., Westra, J. G., Kriek, E., and Berns, A. J. M. (1992) *Nucleic Acids Res. 20*, 4437−4443.
16. Takeshita, M., Chang, C.-N., Johnson, F., Will, S., and Grollman, A. P. (1987) *J. Biol. Chem. 262*, 10171−10179.
17. Shibutani, S., Gentles, R. G., Johnson, F., and Grollman, A. P. (1991) *Carcinogenesis 12*, 813−818.
18. Shibutani, S., Suzuki, N., Matsumoto, Y., and Grollman, A. P. (1996) *Biochemistry 35*, 14992−14998.
19. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

20. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) *Nature 349*, 431−434.
21. Shibutani, S. (1993) *Chem. Res. Toxicol. 6*, 625−629.
22. Matsumoto, Y., Kim, K., and Bogenhagen, D. F. (1994) *Mol. Cell. Biol. 14*, 6187−6197.
23. Moriya, M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1122−1126.
24. Shibutani, S., and Grollman, A. P. (1993) *J. Biol. Chem. 268*, 11703−11710.
25. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M., and Danielsen, M. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7413−7417.
26. Hirt, B. (1967) *J. Mol. Biol. 26*, 365−369.
27. Inouye, S., and Inouye, M. (1987) in *Synthesis and Applications of DNA and RNA* (Narang, S., Ed.) pp 181−206, Academic, New York.
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463−5467.
29. Grunberger, D., Nelson, J. H., Cantor, C. R., and Weinstein, I. B. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 488−494.
30. Fuchs, R., and Duane, M. (1972) *Biochemistry 11*, 2659−2666.
31. Michaels, M. L., Reid, T. M., King, C. M., and Romano, L. J. (1991) *Carcinogenesis 12*, 1641−1646.
32. Mao, B., Hingerty, B. E., Broyde, S., and Patel, D. J. (1998) *Biochemistry 37*, 95−106.
33. Mao, B., Hingerty, B. E., Broyde, S., and Patel, D. J. (1998) *Biochemistry 37*, 81−94.
34. Mah, M. C.-M., Boldt, J., Culp, S., Maher, V. M., and McCormick, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 10193−10197.
35. Kunkel, T. A., and Soni, A. (1988) *J. Biol. Chem. 263*, 14784−14789.
36. Kunkel, T. A. (1990) *Biochemistry 29*, 8003−8011.
37. Schaaper, R. M., Koffel-Schwartz, N., and Fuchs, R. P. P. (1990) *Carcinogenesis 7*, 1087−1095.
38. Wang, T. S.-F. (1991) *Annu. Rev. Biochem. 60*, 513−552.

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