Influence of Flanking Sequence Context on the Mutagenicity of Acetylaminofluorene-Derived DNA Adducts in Mammalian Cells[†,‡]

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Received December 4, 2000; Revised Manuscript Received January 22, 2001

ABSTRACT: Site-specifically modified oligodeoxynucleotides were used to explore the influence of neighboring base sequence context on the mutagenic potential of *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF) and *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-AF) in mammalian cells. Oligodeoxynucleotides (5′TCCTCCTNXNCTCTC, where X is dG-AAF, dG-AF, or dG and N is C, A, G, or T) with different bases flanking the lesion were incorporated into a single-strand shuttle plasmid vector and used to establish the mutational frequency and specificity of dG-AAF and dG-AF adducts in simian kidney (COS-7) cells. Vectors containing dG-AAF promote preferential incorporation of dCMP at the site of the lesion; misincorporation of dAMP and dTMP also was observed. Mutational frequencies range from 11 to 23%. High mutational frequencies (18−23%) were observed when G or T was positioned 5′ to dG-AAF and a lower frequency (11%) when C was 5′ to the lesion. dCMP was predominantly incorporated opposite the dG-AF adduct when C, A, or T was 5′ to the lesion; dAMP and dTMP were misincorporated at a frequency of 2−4%. With G 5′ to the lesion, the overall mutational frequency for dG-AF ranged between 11 and 70%; the highest value occurred when C was the 3′ flanking base, and the predominant mutation event was G → T transversion (59%). We conclude from these experiments that dG-AAF and dG-AF promote G → T transversions and G → A transitions in mammalian cells. The mutational frequency and specificity of dG-AF vary significantly, depending on the nature of the bases flanking the lesion.

Acetylaminofluorene (2-AAF)[1] is a prototypic aromatic amide used as a model chemical carcinogen (reviewed in ref *1*). 2-AAF is metabolically activated in animal tissues and cultured cells and reacts with cellular DNA to form *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF) and *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-AF) adducts (Figure 1). dG-AAF is also the principal adduct formed when DNA is reacted in vitro with *N*-hydroxy-2-acetylaminofluorene (*N*-OH-AAF) (*2*); however, dG-AF is the major DNA adduct found in the liver of rats treated with 2-AAF (*3*, *4*) and Chinese hamster ovary (CHO) cells exposed in vitro to *N*-acetoxy-2-acetylaminofluorene (*N*-acetoxy-AAF) (*5*−*8*).

The mutagenic properties of dG-AAF and dG-AF have been examined in various mammalian and human cells (*1*). When an SV40-based shuttle vector carrying the *supF* gene was treated with *N*-acetoxy-*N*-(trifluoroacetyl)-2-aminofluorene to produce dG-AF adducts (*9*), or with *N*-acetoxy-AAF to produce dG-AAF adducts (*10*), and then transfected into human embryonic kidney cell lines, G → T transversions were generated at the lesion site. Using the endogenous *dhfr* gene in CHO cells as a mutational target, *N*-acetoxy-AAF (*11*) and *N*-hydroxy-2-AF (*12*) produced comparable mutational spectra in CHO cells. In contrast, when a human *hprt* cDNA sequence integrated into chromosomal DNA of mouse VH-12 cells was used as a mutational target for *N*-acetoxy-AAF, frame-shift deletions predominated in sequences containing runs of dA (*13*).

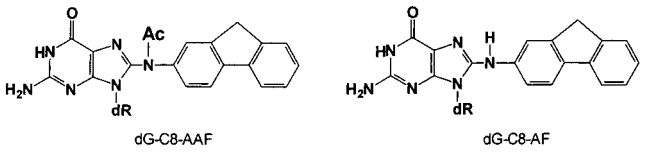

FIGURE 1: Structures of dG-C8-AAF and dG-C8-AF.

Site-specific mutagenesis techniques have been used to explore the mutagenic potential of AAF-derived DNA adducts during extrachromosomal replication in mammalian cells. Using an SV-40-based double-stranded shuttle vector that replicates in simian (COS-1) cells, a single dG-AAF adduct was shown to produce targeted G → C and G → T transversions (*14*). The effect of a single dG-AAF or dG-AF adduct also was tested in a single-stranded shuttle vector (*15*). Both lesions promoted G → T transversions and generated fewer G → A transitions, but in this case, the mutational frequency of dG-AAF was much higher than that of dG-AF. The mutational spectra are consistent with those

[†] Research supported by National Institute of Environmental Health Sciences Grant ES04068.

[‡] We dedicate this paper to the memory of Professor Dezider Grunberger, a pioneer in studies of AAF-derived DNA adducts.

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[1] Abbreviations: dG, 2′-deoxyguanosine; 2-AAF, 2-acetylaminofluorene; *N*-acetoxy-AAF, *N*-acetoxy-2-acetylaminofluorene; dG-AAF, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene; dG-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; PAGE, polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; t_R, retention time; Δ, deletion; ds, double-strand; ss, single-strand.

10.1021/bi0027581 CCC: $20.00
Published on Web 02/27/2001

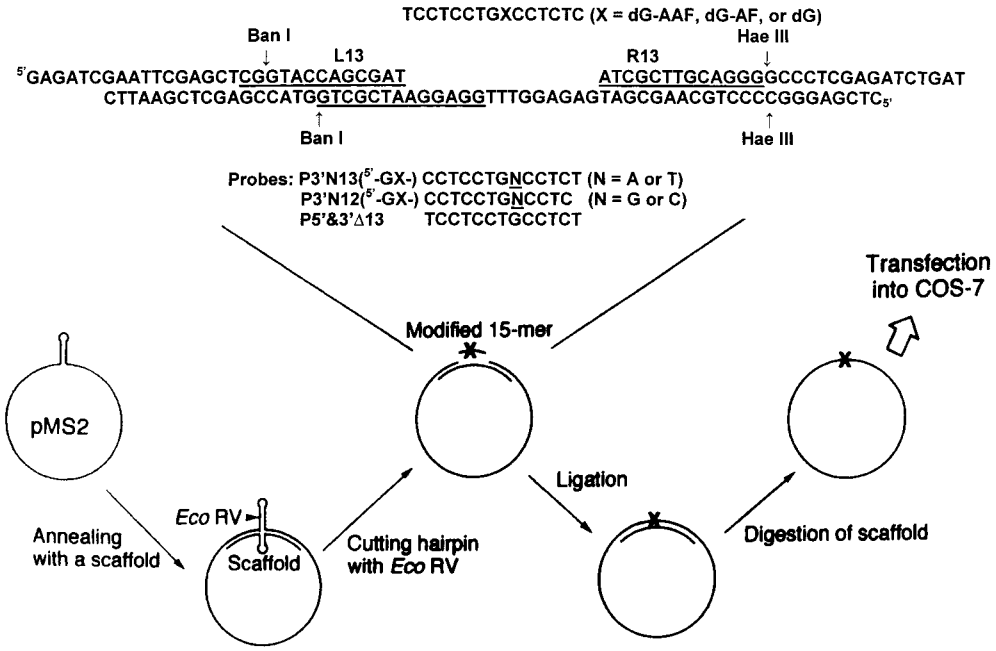

FIGURE 2: Construction of a single-strand vector containing dG-AF or dG-AAF. The upper strand is part of the ss pMS2 sequence, where X represents dG, dG-AAF, or dG-AF. The L13 and R13 probes (underlined) were used to detect correct insertion. The underlined portion of the 61-mer scaffold (S13, bottom strand) was used to determine the concentration of the ss DNA construct. Probes were used with oligodeoxynucleotide hybridization to determine mutation specificity.

observed in mammalian cells exposed to activated forms of 2-AAF (*9*−*12*).

In this paper, the effect of sequence context on the mutagenic specificity and frequency of dG-AAF and dG-AF DNA adducts was studied systematically by comparing the effect of the bases flanking the lesion. We find that the neighboring sequence context dramatically influences the mutagenic potential of these adducts.

EXPERIMENTAL PROCEDURES

Materials and Methods. [γ-^{32}P]ATP (specific activity, >6000 Ci/mmol) was obtained from Amersham Corp. *Eco*RI, *Ban*I, and *Hae*III restriction endonucleases were 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 recorded with a Hewlett-Packard 8452A diode array spectrophotometer.

Synthesis of Oligodeoxynucleotides. Oligodeoxynucleotides were prepared by solid-state synthesis, using an automated DNA synthesizer (*17*). To prepare dG-AAF-modified oligodeoxynucleotides (5′TCCTCCTNG^{AAF}NCTCTC, where N is C, A, G, or T), a 15-mer oligomer containing a single dG (5′TCCTCCTNGNCTCTC, where N is C, A, G, or T) was reacted with *N*-acetoxy-AAF (*18*, *19*). In the 15-mer containing two Gs (5′TCCTCCTGGCCTCTC), the oligomer containing a single dG-AAF adduct was isolated on a reverse-phase μBondapak C_{18} column (0.39 cm × 30 cm, Waters), using a linear gradient composed 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 (*19*), as reported previously (*18*). The position of the dG-AAF modification in the oligomer was determined by treatment with venom phosphodiesterase digestion (*20*). The acetyl group of the dG-AAF-modified 15-mer was cleaved under alkaline conditions to prepare the corresponding dG-AF-modified oligomer (*19*). When a 15-mer containing three Gs was reacted with *N*-acetoxy-AAF, the oligomer containing a single dG-AAF modification (5′TCCTCCTGG^{AAF}GCTCTC) could not be separated from the other modified oligomers. This sequence was not examined. The dG-AF-modified 15-mer (5′TCCTCCTGG^{AF}GCTCTC) was prepared by phosphoramidite base chemical synthesis.[2] Oligomers were purified by electrophoresis on a 20% polyacrylamide gel in the presence of 7 M urea (35 cm × 42 cm × 0.04 cm) (*19*). 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 (*19*). Oligonucleotides were labeled at the 5′ terminus by treatment with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (*21*) and subjected to acrylamide gel electrophoresis (35 cm × 42 cm × 0.04 cm). The position and homogeneity of oligonucleotides following gel electrophoresis were determined by autoradiography using Kodak X-Omat XAR film or a Molecular Dynamics β-phosphorimager.

Site-Specific Mutagenesis in COS-7 Cells. A single-strand (ss) shuttle plasmid vector, pMS2, that confers neomycin (Neo^R) and ampicillin (Amp^R) resistance (*22*) was used to establish the mutagenic specificity of dG-AF and dG-AAF adducts embedded in different sequence contexts in COS-7 cells. Circular ss DNA containing a single DNA adduct was constructed according to procedures established previously (*22*). pMS2 DNA was annealed to a 61-mer oligonucleotide and then digested with *Eco*RV to create a 15-mer gap (Figure 2). A 15-mer containing a single dG-AAF, dG-AF, or dG was 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

[2] F. Johnson et al., unpublished data.

exchange of the terminal phosphate residue using [γ-^{32}P]-ATP and T4 polynucleotide kinase, and subjected to 12% denaturing polyacrylamide gel electrophoresis (*22*). 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 a 1:1 (v/v) phenol/chloroform mixture 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 DNA containing the 15-mer insert. The absolute amount of closed circular ss DNA was established by comparing the amount of radioactivity of the sample with that in known amounts of ss DNA.

COS-7 cells were transfected with ss DNA (100 fmol) for 18 h in the presence of lipofectin (*23*), after which the cells were incubated for 2 days in Dulbecco's modified Eagle's medium and 10% fetal calf serum. Progeny plasmids were recovered by the method described by Hirt (*24*), treated with S1 nuclease to digest input ss DNA, and used to transform *Escherichia coli* DH10B. Transformants were analyzed for mutations by oligodeoxynucleotide hybridization (*14*, *25*). The oligodeoxynucleotide probes used to identify progeny phagemids are shown in Figure 2. Probes L13 ($^{5'}$CGGTAC-CAGCGAT) and R13 ($^{5'}$ATCGCTTGCAGGG) were used to confirm that the gapped plasmids had been 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 (*26*).

RESULTS

Construction of Vectors Containing dG-AAF or dG-AF. A dG-AAF- or dG-AF-modified 15-mer was ligated into a gapped single-strand plasmid, pMS2 (Figure 2). A comparable unmodified oligonucleotide served as a control. The plasmid vector was cleaved with *Ban*I and *Hae*III, followed by phosphorylation of the terminal phosphate residue with [γ-^{32}P]ATP and T4 polynucleotide kinase, and subjected to 12% denaturing polyacrylamide gel electrophoresis. The digestion product containing the unmodified 15-mer comigrated with the 40-mer standard marker; products containing dG-AAF or dG-AF migrated more slowly, as reported previously (*15*).

Mutational Specificity of dG-AAF. Plasmid vectors were transfected into COS-7 cells. Progeny plasmids were recovered and used to transform *E. coli* DH10B. Transformants analyzed by oligodeoxynucleotide hybridization and dideoxy sequence analysis were used to establish the mutational properties of the lesion, as described in Experimental Procedures. Vectors containing the dG-AAF adduct promote preferential incorporation of dCMP opposite the lesion (Table 1). Misincorporation of dAMP and dGMP opposite the lesion also was observed. Six of the 16 possible flanking sequence contexts were examined. With C 3′ to the adduct, we compared the effect of the 5′ flanking base. G → T transversions predominated when C, T, or G was in the 5′ position. With A 5′ to the lesion, the level of incorporation of dTMP was greater than that of dAMP. The highest mutation frequency was observed with a 5′ G (23%) followed by T (21%), A (17%), and C (11%). With G 5′ to the adduct, G → T transversions predominated when C, A, or T was the 3′ flanking base; the comparable sequence with 3′ and 5′ flanking Gs was not tested. The highest mutation frequency was observed in the sequence containing the T 3′ (29%) followed by C (23%) and A (21%).

A few nontargeted mutations were observed with the dG-AAF-modified plasmid. No targeted mutations were detected with unmodified vectors (Table 1). Thus, both the overall mutation frequency and specificity are significantly affected by sequence context. The 5′ position has a more pronounced effect.

Mutational Specificity of dG-AF. dG-AF adducts were used to transfect COS cells; seven of the 16 possible contexts were examined. When C is the 3′ flanking base and C, A, or T is 5′ to the adduct, dCMP was predominantly incorporated opposite the lesion (Table 2). Small numbers of G → T transversions and G → A transitions were detected; mutational frequencies were 2−4%. However, when G was the 5′ flanking base ($^{5'}$-GG^{AF}C-), the overall mutation frequency at the lesion site increased dramatically to 70% with dAMP (59%) incorporated opposite the lesion, accompanied by smaller amounts of dTMP (9.0%) and dCMP (1.5%).

The effect of varying the 3′ flanking base was determined with G positioned 5′ to the lesion. G → T transversions predominated in all cases. The mutation frequencies observed with C, A, T, and G at the 3′ position were 70, 49, 29, and 11%, respectively.

DISCUSSION

Influence of Sequence Context on dG-AF- and dG-AAF-Induced Mutations in Simian Kidney Cells. Single-strand shuttle vectors were used to determine the influence of sequence context on mutagenesis by dG-AAF and dG-AF in COS-7 cells (Figure 3). Striking differences were observed, especially with dG-AF, where the mutation frequency for GG^{AF}C was 50-fold higher than for TG^{AF}C. G positioned 5′ to the lesion appears to play a dominant role as the mutation frequency for C, T, or A in this position is <4% and 70% for GG^{AF}C. The 3′ flanking base also affects mutagenic frequency in the following order: C > A > T > G. Interestingly, codon 60, the predicted target for AF mutagenesis in the noncoding strand of the human cHa-*ras*-protooncogene (*27*), contains the GGC sequence. The effect of sequence context on dG-AAF-induced mutagenesis is less marked with only a 2-fold difference in mutation frequency observed among the six sequences that were tested. The greatest difference is between CG^{AAF}C and GG^{AAF}C, suggesting that the 5′ flanking base is influential.

The primary mutagenic events associated with dG-AAF and dG-AF adducts in COS-7 cells are G → T transversions and G → A transitions. The mutation spectra observed in our study are similar to that reported for *sup*F mutations induced by treatment of human cells with *N*-acetoxy-AAF (*10*). Carothers et al. also found that G → T transversions

Table 1: Mutational Specificity of dG-AAF in COS Cells[a]

plasmid		targeted mutations (dG or dG-AAF → X)					
		G	A	T	C	Δ^1	other
5′-TCGC-	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
5′-TCG^{AAF}C-	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[c]
5′-TAGC-	expt 1	154	0	0	0	0	0
	expt 2	163	0	0	0	0	0
	total	317 (100%)	0 (<0.3%)	0 (<0.3%)	0 (<0.3%)	0 (<0.3%)	0
5′-TAG^{AAF}C-	expt 1	71	12	7	0	0	2
	expt 2	80	6	5	1	0	4
	total	151 (83%)	18 (9.9%)	12 (6.6%)	1 (0.5%)	0 (<0.5%)	6[d]
5′−TTGC-	expt 1	142	0	0	0	0	0
	expt 2	139	0	0	0	0	0
	total	281 (100%)	0 (<0.4%)	0 (<0.4%)	0 (<0.4%)	0 (<0.4%)	0
5′-TTG^{AAF}C-	expt 1	82	7	18	0	0	0
	expt 2	78	5	13	0	0	0
	total	160 (79%)	12 (5.9%)	31 (15%)	0 (<0.5%)	0 (<0.5%)	0
5′-TGGC-	expt 1	92	0	0	0	0	1
	expt 2	108	0	0	0	0	3
	total	200 (100%)	0 (<0.5%)	0 (<0.5%)	0 (<0.5%)	0 (<0.5%)	4[e]
5′-TGG^{AAF}C-	expt 1	56	2	13	0	0	0
	expt 2	50	4	12	0	0	0
	total	106 (77%)	6 (4.4%)	25 (18%)	0 (<0.7%)	0 (<0.7%)	0
5′-TGGA-	expt 1	78	0	0	0	0	0
	expt 2	76	0	0	0	0	1
	total	154 (100%)	0 (<0.6%)	0 (<0.6%)	0 (<0.6%)	0 (<0.6%)	1[f]
5′-TGG^{AAF}A-	expt 1	64	4	13	0	0	0
	expt 2	74	0	16	0	2	1
	total	138 (79%)	4 (2.3%)	29 (17%)	0 (<0.6%)	2 (1.1%)	1[g]
5′-TGGT-	expt 1	77	0	0	1	0	0
	expt 2	69	0	0	0	0	0
	total	146 (99%)	0 (<0.7%)	0 (<0.7%)	1 (0.7%)	0 (<0.7%)	0
5′-TGG^{AAF}T-	expt 1	37	6	17	3	0	3
	expt 2	62	3	10	0	1	4
	total	99 (71%)	9 (6.5%)	27 (19%)	3 (2.2%)	1 (0.7%)	7[h]

[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 expt 2 represent independent experiments. [c–h] Nontargeted mutations associated with a targeted event are listed below:

c) 5′TCCTCCTCG^{AAF}CCTCTC
5′--------ΔΔ------- 1 Δ^2
5′--A----A--------- 1 two C → A

d) 5′TCCTCCTAG^{AAF}CCTCTC
5′-------C--------- 1 A → C
5′----------Δ----- 1 C → Δ^1
5′-----T----------- 2 C → T
5′-Δ--------------- 1 C → Δ^1
5′------Δ---------- 1 T → Δ^1

e) 5′TCCTCCTGGCCTCTC
5′-A------------- 2 C → A
5′---------A----- 1 C → A
5′------------A-- 1 C → A

f) 5′TCCTCCTGGACTCTC
5′-------A------- 1 G → A

g) 5′TCCTCCTGG^{AAF}ACTCTC
5′-----T----------- 1 C → T

h) 5′TCCTCCTGG^{AAF}TCTCTC
5′-------T---------- 1 G → T
5′-------A---C------ 1 G → A & T → C
5′-------T---C------ 1 G → T & T → C
5′------------Δ----- 3 C → Δ^1
5′----------Δ------ 1 T → Δ^1

frequently occurred at the 3′ G of 5′-TGGA-, a sequence that includes codon 350 of the *dhfr* gene in CHO cells treated with *N*-OH-AF (*12*). A high frequency of targeted G → T mutations (34%) were observed in the 5′-TGG^{AF}A- sequence in our study.

Structural studies reveal that the dG-AAF adduct assumes the syn conformation in ds DNA and significantly distorts helical structure (*36*). In contrast, dG-AF can assume either the syn or anti conformation with the aminofluorene moiety residing inside or outside of the DNA helix (*28*, *29*). The ratio of anti to syn conformations is likely to be sensitive to flanking sequence context (*28*). Furthermore, adduct structure in the replication fork may be affected when the primer template is bound by DNA polymerase; this complex also may be affected by the nature of the flanking bases. Thus, due to the inherent conformational flexibility of dG-AF, the mutational frequency and specificity of the adduct may be affected by the flanking sequence context. To prove these several hypotheses, a series of structure studies including the dA·dG-AF and dA·dG-AAF pairs with different flanking sequence contexts are required using NMR or crystallography.

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

plasmid		targeted mutations (dG or dG-AAF → X) G	A	T	C	Δ[1]	other
5′-TCG[AF]C-	expt 1[b]	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[c]
5′-TAG[AF]C-	expt 1	107	0	4	0	0	0
	expt 2	101	2	3	0	0	0
	total	208 (96%)	2 (0.9%)	7 (3.2%)	0 (<0.5%)	0 (<0.5%)	0
5′-TTG[AF]C-	expt 1	127	0	3	0	0	1
	expt 2	127	1	1	0	0	1
	total	254 (98%)	1 (0.4%)	4 (1.5%)	0 (<0.4%)	0 (<0.4%)	2[d]
5′-TGG[AF]C-	expt 1	18	5	42	0	0	4
	expt 2	22	7	37	2	0	1
	total	40 (30%)	12 (9.0%)	79 (59%)	2 (1.5%)	0 (<0.8%)	5[e]
5′-TGG[AF]A-	expt 1	47	18	36	3	0	3
	expt 2	43	2	3	4	0	0
	total	90 (51%)	20 (11%)	59 (34%)	7 (4.0%)	0 (<0.6%)	3[f]
5′-TGG[AF]T-	expt 1	78	6	27	0	0	0
	expt 2	61	7	16	0	1	3
	total	139 (71%)	13 (6.6%)	43 (22%)	0 (<0.5%)	1 (0.5%)	3[g]
5′-TGG[AF]G-	expt 1	96	5	7	0	0	2
	expt 2	92	1	9	0	0	0
	total	188 (89%)	6 (2.9%)	16 (7.6%)	0 (<0.5%)	0 (<0.5%)	2[h]

[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 expt 2 represent independent experiments. [c–h] Nontargeted mutations associated with a targeted event are listed below:

c) 5'TCCTCCTCG[AF]CCTCTC
5'-------A-------- 1 C → A
5'-------T-------- 2 C → T

d) 5'TCCTCCTTG[AF]CCTCTC
5'-------A-------- 1 T → A
5'------AC-------- 1 T → A & T → C

e) 5'TCCTCCTGG[AF]CCTCTC
5'-A-------------- 1 C → A
5'----T---------- 1 C → T
5'-------T-------- 1 G → T
5'------C--------- 1 T → C
5'-------Δ-------- 1 G → Δ[1]

f) 5'TCCTCCGG[AF]ACTCTC
5'------A--C----- 2 G → A & A → C

g) 5'TCCTCCGG[AF]TCTCTC
5'------T--C----- 2 G → T & T → C
5'------A--C----- 1 G → A & T → C

h) 5'TCCTCCGG[AF]GCTCTC
5'---------T----- 2 G → T

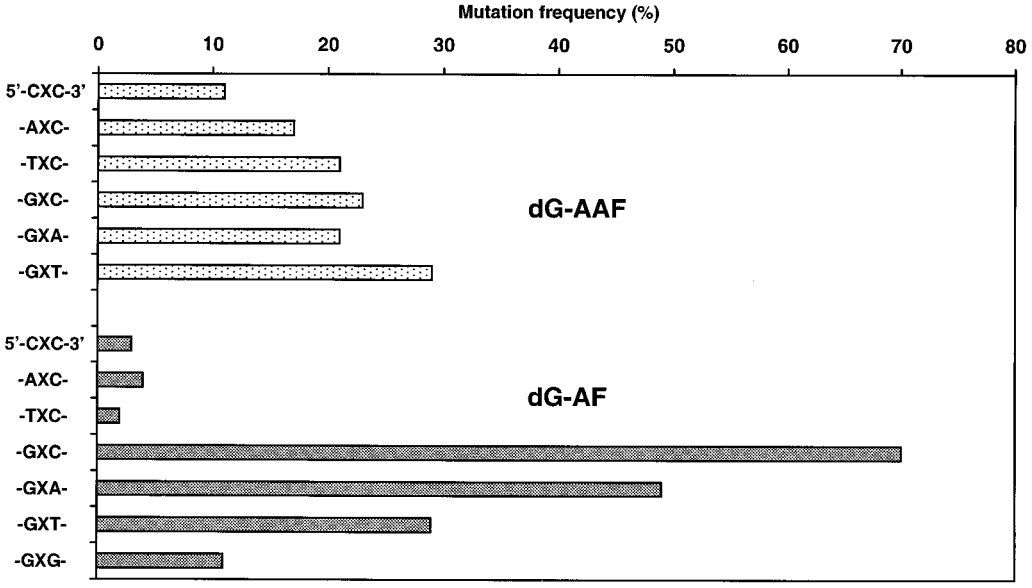

FIGURE 3: Effect of sequence context on mutational frequencies of dG-AAF and dG-AF. Data were taken from Tables 1 and 2.

Comparison of dG-AF with dG-PhIP. Our site-specific mutagenesis system previously has been used to establish the mutation frequency and specificity of *N*-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (dG-PhIP), a mutagenic aromatic amide derived from cooked food (*16*). dCMP was incorporated preferentially opposite dG-PhIP, and targeted G → T transversions, followed by fewer G → A transitions, were observed. The mutational spectra

observed in different sequence contexts were similar to those observed in our study with dG-AF. Computational modeling studies suggest that dG-PhIP occupies the minor grove in B-DNA, with the adduct adopting the syn conformation (*12*). However, with C, A, or T 5′ to the lesion, the mutational frequency of dG-PhIP was 3−11 times higher than that observed with dG-AF. When G was the 5′ flanking base, the mutational frequency of dG-PhIP was 2.3 times lower than that of dG-AF. Thus, the structure of the adduct also influences mutational frequency.

Mechanism for Base Deletions. A general model for frame-shift deletions has been proposed involving either an initial template misalignment or incorporation of dNTPs followed by realignment (*30*−*32*). Via the combination of primer extension studies with steady state kinetic analysis, the propensity for template misalignment was shown to depend critically on (a) the sequence context of the lesion, (b) the nature of the base inserted opposite the lesion, and (c) the relative frequency of translesion synthesis (*18*). This model was used to rationalize the generation of deletions by 3,N^4-etheno-2′-deoxycytidine (*33*) and abasic sites (*34*) in reactions catalyzed by mammalian DNA polymerases α and β. In studies using dG-PhIP and COS-7 cells (*16*), significant numbers of one-base frame-shift deletions were detected when G was 5′ to the lesion ($^{5'}$-G$\underline{\text{G}}$$^{\text{PhIP}}$C-). Apparently, dCMP inserted opposite dG-PhIP pairs preferentially with dG 5′ to the lesion, generating a single-base deletion. This mechanism is consistent with the model proposed for dG-AAF-induced deletions in vitro (*18*); however, significant amounts of single-base deletions were not detected when plasmids containing the dG-AAF or dG-AF adduct were allowed to replicate in COS-7 cells. Only a two-base deletion was observed when plasmids containing dG-AAF in which C was 5′ to dG-AAF were replicated in COS-7 cells (*15*). Pol δ is responsible for translesion synthesis past the dG-AF lesion by incorporating dCMP, the correct base, opposite the lesion; no deletions were detected (*15*). In cells, the 3′ → 5′ exonuclease function of pol δ may, therefore, act to minimize the formation of deletions (*35*). Furthermore, the formation of mispaired intermediates leading to base substitutions competes with the formation of the bulged intermediates producing frame shifts (*18*). Proteins participating in DNA replication may stabilize helical forms of DNA and prevent bulge formation. These functions may depend on the nature of the DNA adduct.

We conclude from this study that dG-AAF and dG-AF DNA adducts can be mutagenic in simian kidney cells, generating G → T transversions and fewer G → A transitions. The relative mutational frequency of dG-AF depends strongly on the nature of the bases flanking the lesion.

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BI0027581